Ultrasensitive Diagnostics for Low-Density Asymptomatic *Plasmodium falciparum* Infections in Low-Transmission Settings

Kayvan Zainabadi^{a,b}

^aCenter for Global Health, Weill Cornell Medicine, New York, New York, USA

^bDepartment of Microbiology & Immunology, Weill Cornell Medicine, New York, New York, USA

ABSTRACT The emergence of multidrug-resistant *Plasmodium falciparum* malaria in Southeast Asia (SEA) has accelerated regional malaria elimination efforts. Most malaria in this and other low-transmission settings exists in asymptomatic individuals, which conventional diagnostic tests lack the sensitivity to detect. This has led to the development of new ultrasensitive diagnostics that are capable of detecting these low-parasitemia infections. This review summarizes the current status of ultrasensitive technologies, including PCR and loop-mediated isothermal amplification (LAMP)-based methods, as well as a newly developed ultrasensitive rapid diagnostic test (uRDT). The sensitivity, specificity, and field performance of these platforms will be examined, as well as their suitability for use in resource-limited settings to aid in malaria elimination efforts. uRDTs, with their improved sensitivity, are now able to detect approximately half of asymptomatic infections, providing a useful point-of-contact tool for malaria surveillance. The increased sensitivity and high-throughput nature of PCR-based tests make them ideal for screening large populations in places where laboratory capacity exists, and the recent commercialization of malaria LAMP kits should facilitate their adoption as a public health tool in places where such infrastructure is lacking. Finally, recent advances with dried blood spots may enable utilization of the extensive laboratory infrastructure of higher-income countries to assist with molecular surveillance in support of malaria elimination. If malaria is to be eliminated in SEA and other low-endemicity regions, then ultrasensitive diagnostics will likely play a key role in identifying and clearing the vast asymptomatic pool of infections that are common to these regions.

KEYWORDS cross-sectional, surveillance, malaria, transmission, elimination, Southeast Asia, drug resistance, RDT, LAMP, PCR, RT-LAMP, *Plasmodium falciparum*, *Plasmodium vivax*, diagnostics, epidemiology, infection control, molecular epidemiology, multidrug resistance, public health, surveillance studies

Over the last few decades, strengthening of malaria control programs based on traditional public health and vector control strategies has led to remarkable gains in lowering the malaria burden in Southeast Asia (SEA) (1). For example, Cambodia, China (Yunnan Province), Laos, Myanmar, Thailand, and Vietnam have collectively seen declines of malaria incidence and deaths by 76% and 95%, respectively, between 2010 and 2018 (1). However, progress has been uneven in the region, with war, conflict, and political instability forestalling and in some instances reversing gains and likely contributing to the emergence of drug resistance.

The rise of multidrug-resistant *Plasmodium falciparum* malaria in the Greater Mekong Subregion poses a grave global threat, especially if drug resistance spreads to Africa, where most malaria deaths occur (1). In response, the WHO has recommended

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Address correspondence to kayvan@alum.mit.edu.

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FIG 1 Ultrasensitive diagnostics display different sensitivities for detection of symptomatic and asymptomatic *Plasmodium falciparum* infections from high- or low-transmission settings.

a policy of regional malaria elimination for SEA countries by 2030 (2). Elimination efforts have been hampered, however, by the dearth of field-deployable diagnostics capable of detecting the low-density malaria infections common to the region.

The low endemicity of *P. falciparum* in SEA and other similar settings means that individuals on average harbor lower parasite densities than those in higher-transmission settings. Symptomatic infections, however, still do generally fall within the sensitivity of conventional rapid diagnostic tests (cRDTs) and light microscopy (LM) (Fig. 1). In contrast, asymptomatic individuals have some of the lowest parasitemia levels ever recorded, as low as one parasite per ml (a mean of thousands of parasites per ml), well below the limit of detection of cRDTs ($\sim 100,000$ parasites/ml) and LM ($\sim 50,000$ parasites/ml) (3–7). Similarly, pregnancy-associated *P. falciparum* malaria also results in low peripheral parasite densities due to sequestration in the placenta (5). These asymptomatic infections typically constitute greater than 90% of the total malaria burden in low-endemicity regions and may represent an important, yet hidden, reservoir for continued transmission (4–7). If malaria is to be eliminated, these asymptomatic infections may need to be identified and cleared.

This review focuses on recent advancements in the development of ultrasensitive technologies capable of detecting these low-density *P. falciparum* malaria infections. Three main platforms will be examined: (i) PCR-based methods that require significant laboratory infrastructure, (ii) loop-mediated isothermal amplification (LAMP) tests that are more field friendly, and (iii) ultrasensitive rapid diagnostic tests (uRDTs) that are point-of-contact tests. This review will examine the strengths and weaknesses of each diagnostic platform, with a particular focus on sensitivity, specificity, and performance in field trials in low-transmission settings. Finally, the scalability of each technology for widespread adoption will be examined, especially for deployment to the low-resource settings where they are needed the most.

PCR-BASED ULTRASENSITIVE TESTS

The first generation of *P. falciparum* ultrasensitive tests were based on nested PCR of multicopy genes (8) (Table 1). These tests achieved a limit of detection of $<1,000$ parasites/ml, which is at least 100-fold more sensitive than cRDTs (9, 10). Nested PCR has the advantage of being compatible with dried blood spots (DBS), requiring relatively simple nucleic acid extraction methods, and having the ability to distinguish multiple malarial species. One of the main drawbacks of nested PCR, however, is its propensity for false-positive results. The opening of tubes from the first PCR to add to the second can release tremendous amounts of contaminating nucleic acids to the

TABLE 1 Examples of different ultrasensitive PCR methods currently in use for surveillance of asymptomatic *Plasmodium falciparum* infections^a

Method	Molecular target	LoD (p/ml)	Other species?	Species ID method	Analyte	Extraction method	Comment
Singh et al., 1996 (10)	18S rDNA nested PCR	<1,000	<i>P. vivax</i> , <i>P. ovale</i> , <i>P. malariae</i>	Secondary nested PCR	20–50 μ l DBS	Chelex method	DBS stable at RT for 12 mo
Steenkiste et al., 2009 (9)	Cytochrome <i>b</i> nested PCR	75	<i>P. vivax</i> , <i>P. ovale</i> , <i>P. malariae</i>	RFLP on PCR product	20 μ l DBS	QIAamp DNA blood kit	Instagene resin extraction method performed similarly to QIAamp
Imwong et al., 2014 (13)	18S rDNA qPCR	22	<i>P. vivax</i> , <i>P. malariae</i>	Secondary nested PCR	2 ml venous blood	QIAamp DNA blood kit	Requires buffy coat removal and cold chain; species ID method fails for lowest-density infections; amenable to automation
Hofmann et al., 2015 (14)	varATS/TARE2 qPCR	<150	No	NA	50–200 μ l blood	Qiagen RNeasy Plus 96 kit	Outperformed 18S rDNA qPCR; amenable to pooling strategy; majority of submicroscopic infections carry gametocytes
Adams et al., 2015 (15)	18S rRNA qRT-PCR	≤ 16	<i>P. vivax</i>	qPCR probes	0.3 ml preserved capillary blood	QIAamp 96 DNA blood kit	Obviates cold chain for sample transport for 2 wk; uses human actin probe as internal control
Zainabadi et al., 2017 (16)	18S rRNA qRT-PCR	20	<i>P. vivax</i>	qPCR probes	50 μ l DBS	Homemade	Recovers both DNA and RNA; DBS stable for ≥ 3 mo at RT; uses human actin probe as internal control

^aLoD, limit of detection; ID, identification; qPCR, quantitative PCR; qRT-PCR, quantitative reverse transcription-PCR; DBS, dried blood spot; RFLP, restriction fragment length polymorphism; p/ml, parasites/ml; rDNA, ribosomal DNA; varATS, var gene acidic terminal sequence; TARE2, telomere-associated repetitive element; NA, not available; RT, room temperature; mo, months; wk, weeks.

environment. Furthermore, the need for two separate PCRs increases its procedural complexity and cost, making it impractical to screen large populations. Nested PCR is also a qualitative assay (i.e., yielding positive or negative results) and as such is unable to provide quantitative information on parasitemia counts.

The second generation of ultrasensitive tests replaced nested PCR with quantitative PCR (qPCR) (11, 12). The qPCR assay is a closed system: PCR products do not require any manipulation following amplification, thereby reducing potential routes of contamination. qPCR products are detected in real time with the use of DNA-binding fluorescent dyes or target-specific molecular probes. The use of molecular probes provides a second layer of specificity and allows for multiplexed reactions to be performed, enabling the concurrent detection of multiple *Plasmodium* species from one reaction, as well as the incorporation of internal controls to gauge sample quality and/or qPCR efficiency. The quantitative nature of the assay also allows for parasite densities to be accurately determined and for cycle threshold cutoff values to be incorporated to more accurately distinguish false- from true-positive results.

To achieve higher sensitivity, these qPCR assays used either higher blood volumes (up to 2 ml) or targeted multicopy genes present in higher copy numbers (13, 14) (Table 1). This helped to improve sensitivity by an order of magnitude (<100 parasites/ml) in comparison to nested PCR methods. This, however, came at a cost, requiring more complex sample collection, storage, and transport methods.

The third generation of ultrasensitive PCR tests attempted to overcome some of these limitations (15, 16). Adams et al. in 2015 developed an ultrasensitive method that used capillary blood (i.e., from a fingerstick) coupled with a preservative that obviated the need for a cold chain for 2 weeks (15). Zainabadi et al. in 2017 further improved upon this method by adapting it to work with standard dried blood spots, helping to significantly cut the cost and complexity of sample collection (16). Both methods achieved a sensitivity of ≤ 20 parasites/ml by targeting the highly expressed 18S rRNA (which exists in thousands of copies per parasite versus 4 to 8 copies of ribosomal DNA [rDNA]) with quantitative reverse transcription-PCR (qRT-PCR). In fact, the sensitivity of the DBS method reached one parasite per DBS (16).

The main drawback of qPCR-based methods is their sensitivity to PCR inhibitors and thus requirement for rather complex nucleic acid purification procedures. In fact, all of the aforementioned PCR methods require significant laboratory infrastructure and thus can only realistically be performed in a well-equipped laboratory. Such tests, however, are able to take advantage of high-throughput methods that allow for testing of hundreds of samples at a time, thereby reducing the overall cost and effort per test, making them ideal for screening large populations. This was recently demonstrated in Myanmar, where scientists at the Department of Medical Research, Ministry of Health and Sports, analyzed over 14,000 DBS as part of the U.S. President's Malaria Initiative-sponsored national Malaria Indicator Survey (16).

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

The significant laboratory infrastructure required for PCR-based tests is not achievable in all settings (Fig. 2). Accordingly, attempts have been made to develop a more field-friendly molecular testing method. This has led to the development of loop-mediated isothermal amplification (LAMP) (17).

LAMP utilizes the same core principle of PCR, but with slight modifications in primer design and DNA polymerase to allow for isothermal (constant temperature) amplification (typically 60 to 65°C) (17). This means that LAMP can be performed even in the absence of electricity (such as with the use of chemical heat packs) (18). The *Bst* DNA polymerase used in the reaction is also more resistant to PCR inhibitors, making it compatible with minimal sample preparation techniques. In fact, sample preparation usually consists of a simple boil and spin procedure. The test is typically completed in under an hour, and test results can be read visually. (One of the by-products of the reaction, magnesium pyrophosphate, precipitates and causes the normally clear solution to turn turbid.) Specialized dyes such as SYBR green or hydroxynaphthol blue can

FIG 2 The capacity for field deployment of different ultrasensitive diagnostic methods for *Plasmodium falciparum* malaria surveillance. While uRDTs are completely field deployable, PCR-based methods require substantial laboratory infrastructure and are thus more suitable for central laboratories. LAMP is field deployable, although likely not to the most remote settings, whereas RT-LAMP is best suited for health clinics that have some laboratory infrastructure.

also be incorporated to help further improve sensitivity and specificity, and the use of a portable turbidimeter or spectrophotometer even allows for quantitation (19, 20).

Due to its simplicity and minimal reliance on laboratory equipment, LAMP is gaining traction as a more field-friendly substitute for PCR for malaria surveillance (21–23). Field evaluation of LAMP-based tests in low-endemicity regions has shown considerable promise, boasting a sensitivity and specificity of >91% in six different field trials in comparison to PCR-based methods (Table 2) (24–27). One notable large cross-sectional study, consisting of 3,650 asymptomatic individuals in Thailand, found that 18S rDNA amplification with LAMP (performed in the field) had a sensitivity of 100% and specificity of 96% in comparison to nested PCR of the same target performed in a central laboratory (27).

LAMP so far has been primarily limited to research use, but with the commercialization of three different LAMP malaria kits (Meridian Illumigene Malaria assay, and Eiken Chemical Loopamp Malaria Pan and Pf Detection kits) this may soon change. These kits contain all of the necessary reagents in stable lyophilized formats, requiring the investigator to only add the analyte being tested and incubate. The Foundation for Innovative New Diagnostics (FIND) recently endorsed one of these kits (Loopamp Malaria Pf Detection kit) and developed standardized procedures for its use (28). Loopamp is compatible with both whole blood and DBS, and requires a simple boil method for sample preparation (24–26). The target is the mitochondrial genome of *P. falciparum* (the exact target is proprietary), with limits of detection of <1,000 parasites/ml for blood and <5,000 parasites/ml for DBS—comparable to other existing LAMP methods in terms of both sensitivity and specificity (Table 2).

Second-generation LAMP tests incorporate a reverse transcription step to enable amplification of more abundant RNA targets (RT-LAMP). These tests show up to a 50-fold increase in sensitivity compared to traditional LAMP, though at the cost of increased procedural complexity (29, 30) (Table 2). For one thing, RT-LAMP is no longer truly isothermal since it requires two different temperatures: one for reverse transcription and one for DNA amplification. Furthermore, most RT-LAMP tests require an RNA purification step prior to testing, which limits their use in the field (although Kemleu et al. in 2016 demonstrated that minimal sample preparation is possible with RT-LAMP [31]). Encouragingly, two independent field trials demonstrated a sensitivity of 100% and specificity of ≥98% for RT-LAMP in comparison to RT-PCR, highlighting its utility for malaria surveillance (Table 2) (29, 30).

Given its increased sensitivity and simplicity, RT-LAMP represents a promising

TABLE 2 Field evaluation of LAMP- and RT-LAMP-based tests for detection of asymptomatic *Plasmodium falciparum* infections in different transmission settings in comparison to PCR^a

Method	LoD (p/ml)	LAMP target	Analyte	Extraction method	Sample size	Region (endemicity)	sen/spec to PCR (%)	PCR comparator	Comment
Sattabongkot et al., 2018 (27)	>1,500	18S rDNA	50 μ l blood	Boil and spin	3,650 cross-sec	Thailand (low)	100/96	18S rDNA nested PCR	LAMP performed in the field; identified 9-fold more <i>P. falciparum</i> infections than microscopy
Vásquez et al., 2018 (26)	<1,000	Mitochondrial DNA ^b	60 μ l blood	Boil and spin	531 pregnant women (cross-sec)	Colombia (low)	100/100	18S rDNA nested PCR	30% more sensitive than cRDT (2-fold more in afebrile subjects); placental blood showed lower sensitivity
Serra-Casas et al., 2017 (25)	<1,000	Mitochondrial DNA ^b	60 μ l blood	Boil and spin	503 cross-sec	Amazon (low)	91.8/91.9	18S rDNA qPCR	Accuracy declined significantly when used in remote field settings
Aydin-Schmidt et al., 2014 (24)	<5,000	Mitochondrial qDNA ^b	3–5 μ l DBS	Chelex method	465 cross-sec	Zanzibar (moderate)	92.7/100	Cytochrome <i>b</i> qPCR	Identified ~3-fold more <i>P. falciparum</i> infections than cRDT
Buates et al., 2010 (29)	2	Pfs16/25 RNA	2 ml blood	Trizol	32 clinical, 52 asymp	Thailand (low)	100/>98	Pfs16/25 nested RT-PCR	Specific for <i>P. falciparum</i> gametocytes; extraction method not field friendly
Mohon et al., 2019 (30)	Blood, 25; DBS, <100	18S rRNA	250 μ l blood/ 50 μ l DBS	For blood, Trizol; for DBS, see reference	494 asymp	Bangladesh (low), Ethiopia (high)	100/99.8	18S rRNA qRT-PCR	DBS stable at RT for >5 mo; extraction methods not field friendly

^aLoD, limit of detection; sen, sensitivity; spec, specificity; cross-sec, cross-sectional; asymp, asymptomatic; cRDT, conventional rapid diagnostic test; p/ml, parasites/ml.^bLoopamp Ag Pf Detection kit (Elken Chemical), exact target proprietary.

ultrasensitive diagnostic technology. For instance, a recent study found that RT-LAMP for 28S rRNA was four times more sensitive than the Loopamp kit for detecting *P. falciparum* infections during controlled human malaria infections (32). The current weakness of RT-LAMP is the lack of commercially available kits for its greater adoption as a public health tool for malaria surveillance. There has been some progress in this area, however: three generic RT-LAMP kits are now available (New England Biolabs Warmstart, OptiGene ISO-004, and Lucigen LavalAMP), which only require addition of the primers and analyte being tested (29, 30, 32). Importantly, these kits also allow for reverse transcription and DNA amplification to occur at the same temperature, making them truly isothermal. However, kit components are not shelf-stable and require a cold chain, limiting their use in the field. These tests are therefore more appropriate for use in health facilities where rudimentary infrastructure, such as electricity and refrigeration, exists (Fig. 2). Commercialization may not be far off though: RT-LAMP reagents have been shown to be amenable to lyophilization and prolonged stability at ambient temperature (33).

ULTRASENSITIVE RAPID DIAGNOSTIC TESTS (uRDTs)

The sensitivity of conventional RDTs (cRDTs) is 100,000 parasites/ml, making them virtually useless for detecting asymptomatic infections in low-endemicity regions (Fig. 1). To fill this gap, a new ultrasensitive RDT (uRDT) that detects *P. falciparum* histidine-rich protein II (PfHRP2) was recently developed that displays ~10-fold improved analytical sensitivity compared to cRDTs (34). It was hoped that with this increased sensitivity, uRDTs would be able to detect enough asymptomatic infections to extrapolate total infection rates based on statistical modeling. The Alere Ultrasensitive Ag Pf RDT is now commercially available and has been tested in a number of field trials in different transmission settings (35–43) (Table 3).

Unsurprisingly, this test was found to perform better in high-endemicity settings, detecting between 50 and 84% of asymptomatic infections (mean, 61.3%; median, 50.4%; $n = 4$ studies) in comparison to PCR-based methods (35–38) (Table 3). Specificity was also high ($\geq 95\%$), except in Gambia, where specificity was only 76%. In medium- and low-endemicity settings, sensitivity dropped: uRDTs detected between 19.6 and 71.4% of asymptomatic infections (mean, 45.3%; median, 48.9%; $n = 8$ studies), with specificity of $>96\%$ (except in Gambia, where specificity was 89%) (39–43). Notably, three large cross-sectional studies (consisting of 1,500 to 2,000 individuals each) from Myanmar and Cambodia found that uRDTs were able to detect between 54 and 60% of asymptomatic infections, with high specificity ($>96\%$) (41–43). However, and somewhat surprisingly, in only one of these three trials did uRDTs significantly outperform cRDTs (with a ~2-fold higher sensitivity). Nonetheless, a smaller study from Myanmar (493 subjects) during the dry season—when parasitemias tend to be the lowest—found that uRDTs were able to detect 44% of asymptomatic infections, whereas cRDTs found zero (38). These findings suggest that it may now be possible to identify nearly half of all asymptomatic infections in low-endemicity regions with the use of a simple point-of-contact test. Additional field testing (particularly in other preelimination settings) should help to determine the true potential of these tests for malaria surveillance.

LESSONS LEARNED AND A PATH FORWARD

The last decade has seen an explosion in the development of ultrasensitive technologies for malaria surveillance, partly in response to the rise of drug resistance in Southeast Asia. These tools have been developed by researchers in high-income countries, and as such are often not well suited for use in low-resource settings. The next challenge in this journey will not be improving sensitivities even further (some tests go below 1 parasite/ml) (31, 44), but to make these tests more field friendly and deployable to the regions of the world where they are needed the most (45, 46).

If malaria surveillance is to be scaled up dramatically, then tests will need to be as simple as possible. For instance, sample collection will likely need to be limited to capillary blood, which can either be tested immediately by uRDTs or stored for later use

TABLE 3 Field evaluation of the Alere Ultrasensitive Ag *P. falciparum* rapid diagnostic test in comparison to conventional RDT and PCR-based ultrasensitive techniques in different transmission settings^a

Method	Region (endemicity)	Sample size	sen/spec to PCR (%)		PCR comparator	Comment
			uRDT	cRDT		
Briand et al., 2020 (35)	Benin (high)	942 pregnant women (88% asymp)	60.5/93.6	44.2/95.7	18S rDNA qPCR	uRDT outperformed cRDT during 1st trimester and in multigravidae and asymp women
Girma et al., 2019 (36) ^b	Ethiopia (high)	523 cross-sec	50/98.4	7.3/100	18S rRNA qRT-PCR	uRDT performance dropped for infections with <1,000 p/ml
Mwesigwa et al., 2019 (37)	Gambia (various)	4,060 cross-sec	38.4/88.5	NA	varATS qPCR	uRDT sensitivity higher/specificity lower in high-transmission villages; uRDT sensitivity lower in children
Das et al., 2017 (38)	Uganda (high), Myanmar (low)	Uganda, 607 cross-sec; Myanmar, 493 cross-sec	Uganda, 84/92; Myanmar, 44/99.8	Uganda, 62/95; Myanmar, 0/100	18S rRNA qRT-PCR	uRDT detected infections in CHMI trials 1.5 days earlier than cRDT
Unwin et al., 2020 (40)	Indonesia (medium)	270 pregnant women (asymp)	19.6/98.2	22.8/95.5	18S rDNA nested PCR	Concordance between uRDT and cRDT poor
Hofmann et al., 2018 (39)	Papua New Guinea (medium)	300 cross-sec	27/NA	15/NA	varATS qPCR	95% of cRDT and 75% of uRDT positive infections had detectable gametocytes
Landier et al., 2018 (42)	Myanmar (low)	1,509 cross-sec	53.8/98.9	27.4/99.9	18S rDNA qPCR	RDT sensitivity dropped ~40% when performed in the field
Vásquez et al., 2018 (26)	Colombia (low)	649 pregnant women (asymp)	71.4/99.5	64.3/100	18S rDNA nested PCR	RDTs detected ~2-fold less <i>P. falciparum</i> using placental blood
Liu et al., 2019 (43)	Myanmar (low)	1,991 cross-sec	55.4/96.9	43.1/97.7	18S rDNA nested PCR	uRDT detected 5-fold more <i>P. falciparum</i> than LM
Yeung et al., 2020 (41)	Cambodia (low)	Active, 678; cross-sec, 2,051	Active, 53.8/96.8; cross-sec, 60.0/99.8	Active, 46.2/98.2; cross-sec, 60.0/99.8	Cytochrome <i>b</i> DNA nested PCR	RDTs were performed in the field

^auRDT, Alere Ultrasensitive Ag *P. falciparum* rapid diagnostic test; cRDT, conventional RDT; sen, sensitivity; spec, specificity; cross-sec, cross-sectional; asymp, asymptomatic; Active, active case detection; NA, not available; LM, light microscopy; CHMI, controlled human malaria infection.

^bThis study also performed LAMP with a sensitivity/specificity of 91.5%/97.1% in comparison to the PCR method.

with preservatives or in the form of DBS. The latter format is particularly attractive, as DBS are stable at ambient temperatures, less infectious than whole blood, and easy to transport (thousands can fit inside a simple backpack). Previous work has shown that DBS samples are stable at tropical conditions for months, giving field collectors plenty of time to collect samples from even the most remote villages (16, 20). Importantly, since DBS are considered nonregulated and non-biohazardous agents, this dramatically simplifies their shipment to central laboratories, whether located in-country or abroad. In fact, this quality may make it easier to utilize the vast laboratory infrastructure of more developed nations (where high-throughput methods and automation are more common) to enable testing of the tens of thousands of samples that may be required to guide elimination efforts (46). In this regard, it is also noteworthy that DBS was recently shown to be suitable for amplification of *P. falciparum* drug resistance markers, including *kelch13*, from low-density infections (47). These qualities make DBS a potential game changer for expanding the reach of molecular surveillance to better understand the epidemiology of both asymptomatic and drug resistant malaria in SEA.

How and where samples are analyzed will depend on the laboratory infrastructure and desired turnaround time (Fig. 2). If results are required quickly (i.e., for screening and treatment or for remote villages where a return visit may be impractical), then sample analysis can be performed on-site with the use of commercial LAMP kits. Alternatively, in the case of targeted mass drug administration (where everyone in a village with a high malaria burden is treated), uRDTs may prove more useful, especially if certain positivity rates can be correlated with high endemicity. The low sensitivity of uRDTs, along with the presence of HRP2 deletions in many parts of the world, makes these tests an imperfect tool currently, particularly for use on an individual basis (48). However, these tests should still prove useful when used on a population-wide scale to inform public health decisions.

If turnaround time is not an issue and proper laboratory infrastructure exists, then DBS samples can be transported back to central facilities to take advantage of the aforementioned high-throughput methods (16, 46). However, if only rudimentary laboratory space exists, then samples can be batched and analyzed in a semi-high-throughput fashion with LAMP (19, 49). It cannot be overstated: it will be essential to adopt high-throughput methods if large populations are to be screened. In this regard, molecular methods with their increased sensitivity may provide an added advantage, allowing for pooling of samples to decrease the overall workload (14, 49, 50).

CONCLUDING REMARKS

We currently have a substantial toolbox for tracking asymptomatic malaria in low-transmission settings. Ultrasensitive PCR methods are able to detect virtually the entire asymptomatic reservoir, with more field-friendly LAMP methods showing a high level of concordance. Perhaps most excitingly, uRDTs also show tremendous promise, detecting approximately half of the asymptomatic reservoir, even during the dry season. New studies developing statistical models that use multivariable analysis to incorporate climate, geography, and demographics with uRDT results may help to expand the utility of these tests even further. Importantly, these different ultrasensitive methods need not be mutually exclusive: they will likely need to be used in conjunction with one another (based on the specific setting) to provide a better understanding of the true malaria burden prior to elimination (Fig. 2). As such, understanding how these disparate tests perform in comparison with one another will be important for integrating epidemiological data arising from these varied methodologies (51). These data together will prove invaluable for helping to guide and measure the success of elimination strategies in SEA and other low-transmission settings.

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