World Health Organization

WHO technical consultation on research requirements to support policy recommendations on highly sensitive point-of-care diagnostics for *P. falciparum* malaria

Meeting report, 4–6 June 2018, Geneva, Switzerland

Summary

Recently, next-generation highly sensitive rapid diagnostic tests (hsRDTs) for *Plasmodium falciparum (Pf)* have become commercially available. These tests claim a limit of detection (LOD) that is 10-fold more sensitive than that of conventional rapid diagnostic tests (cRDTs). Similar tests are also under development for the improved detection of pan-species malaria, *P. vivax (Pv)*, and *Pf* parasites with *pfhrp2/3* gene deletions. In May 2017, WHO convened an evidence review group (ERG) on low-density malaria infections that highlighted the scarcity of data on the relative contributions of low-density *Pf* and *Pv* infections to onward transmission in human populations. It was therefore concluded that it would be difficult to determine the impact of identifying and treating these infections in a number of endemic settings through active test-and-treat based interventions. Subsequently, the Malaria Policy Advisory Committee (MPAC) recommended that hsRDTs only be used for research purposes until there is evidence that the detection of low-density infections using these tools will have a significant impact on transmission *(1)*.

In light of these conclusions, the WHO Global Malaria Programme (GMP) convened a technical consultation in June 2018 to identify the evidence required to develop recommendations on the use of highly sensitive point-of-care tests (HSPOCTs) to support surveillance and elimination activities, and prevention of malaria in pregnancy (MiP). The following conclusions and draft recommendations are presented for consideration by the MPAC.

Draft conclusions

1. Determining the role of HSPOCTs in surveillance and elimination strategies, and the prevention or treatment of MiP will require impact studies assessing the public health and clinical benefit of such interventions. This includes evaluating the effects on patient and/or community outcomes, diagnosis and treatment, as well as cost-effectiveness. While impact studies are the most informative for policy decisions, they are also the most complex in design and may not be feasible in many settings. To help address these constraints, modelling-based studies in areas of low and very low transmission may provide insights into potential impact.

2. Any new malaria diagnostic tests, including both HSPOCTs and cRDTs, should ideally meet the ASSURED criteria[1]. Impact studies should follow independent HSPOCT performance

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[1] Affordable by those at risk of infection, Sensitive (few false-negatives), Specific (few false-positives), User-friendly (simple to perform with minimal training), Rapid (to enable treatment at point of care) and Robust (no need for refrigerated storage), Equipment-free, Delivered to those who need it

assessments through i) laboratory studies using well-characterized reference samples of known parasite and antigen concentrations, and ii) a systematic review of field-based accuracy studies across a range of transmission settings.

3. The following studies were identified as priorities in determining the clinical accuracy of HSPOCTs and the impact of their use in surveillance and elimination strategies and in the prevention and treatment of MiP:

   a. To define the sensitivity and specificity of the assay for the detection of malaria in different settings and use case scenarios, studies comparing HSPOCTs to cRDTs using quality-assured methods as reference standards (e.g. quantitative PCR, ELISAs, multiplex bead-based immunoassays) were proposed. Studies should reflect a range of i) transmission intensities and degrees of seasonality; ii) target populations (e.g. high-risk occupations, mobile or migrant populations); and iii) health care system levels (e.g. public and private health facilities, community health workers). These studies will ideally follow standardized protocols and employ reference assays to enable comparability across studies or diagnostic tests and assessment of the impact of HRP2 persistence on test accuracy, where feasible and relevant.

   b. To assess the potential applications of HSPOCTs in accelerating elimination (i.e. "rapid" reduction in transmission of indigenous cases), cluster randomized trials (CRTs) were proposed comparing HSPOCTs to cRDTs when used in mass test-and-treat (MTAT) strategies. These studies should estimate i) the number and proportion of additional cases detected and treated, and ii) the impact on reducing malaria transmission based on trends in passively detected clinical cases (confirmed by cRDTs or microscopy) at health facilities in the same area. Relevant CRTs include stepped-wedge, cross-over and factorial designs. Due to the large sample sizes required for measuring reductions or interruptions in transmission in low to very low transmission settings, indirect evidence can be gathered from trials conducted in moderately endemic settings where changes in transmission (e.g. incidence, prevalence or other relevant measures) can be more easily quantified. Modelling-based studies may also be able to provide insights into potential impact.

   c. To assess the potential role in surveillance for elimination, studies were proposed evaluating the effectiveness of HSPOCTs vs. cRDTs in identifying additional foci of transmission through reactive case detection (RACD) or proactive case detection (PACD) for a targeted response beyond what is possible using cRDTs and microscopy.

   d. To provide preliminary evidence on the impact of first-trimester low-density malaria infections detectable with HSPOCTs on pregnancy outcomes, a retrospective study of samples from a cohort of women, followed from pre-conception through to delivery, is ongoing. High-quality evidence on the potential role of HSPOCTs in testing for MiP will require individually randomized controlled trials (RCTs) on the effectiveness of HSPOCTs vs. cRDTs when used for early detection and treatment in the first trimester of pregnancy in moderate to high transmission settings.

4. In areas of low transmission, there are limited data on the natural history of infection and longitudinal infection dynamics. However, studies are currently being implemented and planned in multiple African settings. These seek to understand the epidemiology of low-density infections in relation to clinical illness, detectability throughout the course of infection, acquisition of protective immunity, and duration of infectiousness. The outcomes of this research should be followed closely to inform how the use of HSPOCTs in the

detection and elimination of all infections (including those with low parasite density) may affect malaria transmission.

5. Several other applications for HSPOCTs were considered but determined to be of lower priority. These include the use of HSPOCTs in border or port-of-entry screening to prevent importation of malaria parasites, in clinical case management, and in intermittent test-and-treat strategies for MiP (including in HIV coinfections).

Abbreviations

ACD	Active case detection		LDH	Lactate dehydrogenase
ACT	Artemisinin-based combination therapy		LOD	Limit of detection
ANC	Antenatal care		MDA	Mass drug administration
API	Annual parasite incidence		MiP	Malaria in pregnancy
CHW	Community health worker		MSAT/MTAT	Mass screening and treatment / Mass test and treat
cRDT	Conventional rapid diagnostic test			
CRT	Cluster-randomized trial		NMCP	National malaria control programme
ELISA	Enzyme-linked immunosorbent assay		PACD	Proactive case detection
ERG	Evidence Review Group		PCD	Passive case detection
FSAT/FTAT	Focal screening and treatment / Focal test and treat		PCR	Polymerase chain reaction
			PDP	Product development partnership
HRP2	Histidine-rich protein 2		*pfhrp*	*P. falciparum* histidine-rich protein gene
HSPOCT	Highly sensitive point-of-care test		qPCR	Quantitative PCR
hsRDT	Highly sensitive rapid diagnostic test		RACD	Reactive case detection
IPTp	Intermittent preventive treatment in pregnancy		SP	Sulfadoxine pyrimethamine
			SSTp	Single screen and treat in pregnancy
ISTp	Intermittent screen and treat in pregnancy			
IVD	In vitro diagnostic			

1. Background

Considerable evidence suggests that, across all transmission settings, a large proportion of low-density malaria infections (<100 parasites/µL) *(1)* are often undetectable using microscopy and cRDTs *(2–4)*. Several population-based studies have shown that mass test-and-treat (MTAT) strategies employing the point-of-care RDTs currently available do not significantly interrupt transmission *(5–7)*. As a result, these methods are not currently recommended for countries pursuing elimination. Until a number of years ago, LODs for the best performing *P. falciparum* cRDTs were around 600–1000 pg/mL HRP2 (roughly equivalent to 100–200 parasites/µL) *(8–10)*. However, a recently developed hsRDT is now commercially available (Alere™ Malaria Ag *Pf* RDT) *(11)*. This test is designed to detect low-density infections (40–125 pg/mL HRP2) with a claimed analytical sensitivity that is 10-fold greater than that of cRDTs *(8,12)*.

In October 2017, the MPAC concluded that additional research was required to determine the public health impact of using HSPOCTs in test-and-treat strategies *(1)*. Following these conclusions, WHO GMP convened a technical consultation in June 2018 to identify the evidence required to develop WHO recommendations on the use of HSPOCTs in surveillance and elimination activities and the prevention of MiP. The technical consultation also aimed to propose a series of study designs through which to generate such evidence.

2. Objectives

The specific objectives of the meeting were to:

1. Review the current landscape of research on the use of HSPOCTs, including recently completed and ongoing studies.

2. Prioritize the research questions needed to conclude that strategies incorporating HSPOCTs for the detection of *Pf* malaria will:

   a. Have a significant impact on malaria transmission in areas working towards elimination when used in passive case detection (PCD), RACD, PACD, MTAT and focal test and treat (FTAT), as defined below (pages 6–7);

   b. Prevent re-establishment of malaria transmission;

   c. Prevent adverse effects of MiP.

3. Outline study methodologies to gather direct or indirect evidence for each research question, including the most appropriate transmission settings (accounting for seasonal variation and recent history of transmission), study outcomes, comparators, covariates and sample size requirements.

4. Develop a realistic timeline, based on the findings of ongoing, planned and newly identified study requirements, for generating evidence on the impact of using HSPOCTs for *Pf* malaria in a range of transmission settings and use case scenarios.

3. Process

The WHO technical consultation was planned by three GMP units: Prevention, Diagnostics and Treatment; Elimination; and Surveillance. The consultation included nine independent experts in diagnostics, surveillance, elimination and MiP, as well as experts in applied field research methodology and modelling. Also in attendance were 10 representatives from product development partnerships (PDPs) and research institutions involved in the research and development of highly sensitive malaria diagnostic tests; seven observers from funding agencies, nongovernmental

organizations and academic institutions; and seven members of the WHO Secretariat. The list of participants is given in Annex 1, and the list of meeting pre-reads is presented in Annex 2.

The first day of the meeting involved plenary sessions during which current WHO guidelines on the use of malaria diagnostics for surveillance, in elimination strategies, and in pregnancy were presented, followed by a review of ongoing and planned study designs to evaluate the performance and application of HSPOCTs. General principles and methods for assessing *in vitro* diagnostics (IVDs) were also presented and discussed in order to provide a framework for reviewing the evidence and designing evaluation studies for malaria diagnostics.

A general discussion on the evidence and core research questions took place on the second day, and participants were subsequently split into three working groups: i) surveillance; ii) elimination and prevention of re-establishment; and iii) MiP. The working groups were tasked with defining key research questions on the use of HSPOCTs and ranking them in order of priority based on medium- to short-term needs for national malaria control programmes (NMCPs). For each of the identified research questions, the working groups were asked to propose feasible study designs through which to assess the public health impact of HSPOCTs in different epidemiological settings when used in surveillance and elimination strategies, and in the prevention and treatment of MiP. Rapporteurs presented the findings from each group in a plenary for discussion and consensus-building. The last half day of the meeting was restricted to independent experts and the WHO Secretariat, who discussed and summarized the key conclusions of the meeting.

The meeting report was compiled based on the meeting pre-reads and presentations, as well as discussions held during the technical consultation. All participants were invited to review the report and provide input to be considered for the final report.

4. Current WHO guidelines on surveillance, elimination and malaria in pregnancy in relation to point-of-care diagnostics

4.1. Surveillance for elimination *(13)*

The primary use of point-of-care malaria diagnostics is for case management and surveillance by national NMCPs. Detection of malaria cases among treatment-seeking individuals who present at health facilities or to community health workers (CHWs) forms the foundation of PCD. Diagnosis should be made by quality-assured malaria microscopy or using cRDTs that meet WHO's procurement criteria. In areas of low to very low transmission, active case detection (ACD) by health workers in the community, households or high-risk population groups is recommended.

ACD can be further categorized as i) reactive (RACD), i.e. screening and/or testing of populations potentially linked to confirmed cases or clusters, or ii) proactive (PACD), i.e. screening and/or testing that is not prompted by detection of cases, but undertaken in high-risk groups or during high-risk periods *(14)*. When there is a limited number of cases or transmission foci, RACD is conducted in households or populations linked to index cases detected through PCD at health facilities. The outcomes of PCD case investigations are used to determine the foci of transmission and the target area for response – a defined radius around an index case or cluster of cases that depends on a number of factors, including case classification, extent of clustering, level of receptivity or vulnerability, and resource availability. Unlike RACD, PACD is performed regularly (primarily during transmission season) to confirm active local transmission or to facilitate earlier detection of cases. PACD is recommended in populations with limited access to facilities, inadequate health-seeking behaviour, or increased risk of transmission.

Currently, PCD forms the basis of subnational risk stratification, typically using annual parasite incidence (API), which enables the tracking of overall progress and impact of malaria control programmes. These data are also used to identify foci in areas of very low transmission and can be

supplemented with ACD data over time. While RACD surveillance systems are recommended in elimination areas where caseload is low, they are not considered a substitute for PCD and should only be implemented where PCD is of very high quality and coverage. Due to its focal nature, RACD is used for increasing the coverage of case management, reducing the risk of transmission, classifying and stratifying transmission foci, and as part of surveillance activities to confirm elimination.

4.2. Elimination *(15)*

Enhancing surveillance to target foci and prevent onward transmission is a key focus for countries moving towards elimination. There is still limited understanding of the contribution of low-density infections to onward transmission, particularly in areas that have remained at very low levels of transmission for some time. Currently, more research is needed to determine how the targeting of these infections can reduce transmission more rapidly.

Data are also limited on the effectiveness of intermittent mass screening and treatment (MSAT) (i.e. screening individuals for risk factors or symptoms followed by testing and treatment) or MTAT (i.e. testing an entire population – without initial screening for symptoms – and treating). In April 2015, a WHO ERG reviewed existing data in two low and very low transmission settings, but did not find sufficient evidence to recommend MTAT with current diagnostic tools *(7)*. The ERG concluded that the limited capability of cRDTs to detect low-density infections was one reason as to why studies have not shown an impact on transmission. However, this may also be due to other factors such as population mobility and adherence to treatment.

Variations of test-and-treat strategies include FTAT – a form of MTAT focused on limited geographical areas or following the identification of an index case in high-risk communities – and proactive community treatment, which involves frequent (e.g. weekly) mass screening, testing and treatment in high transmission seasons. While WHO does not recommend MTAT or FTAT as elimination strategies, other test-and-treat strategies, such as PACD and RACD, are recommended for surveillance. More specifically, PACD is recommended to extend surveillance coverage to high-risk populations that are unable to access health facilities for case management and, therefore, are unlikely to be detected through PCD. While there is no operational difference between FTAT and RACD, the objective of RACD is to provide data on case distribution to inform focus investigations, while the aim of FTAT is to reduce transmission.

Testing and treatment of co-travellers is a form of RACD that is triggered specifically by imported cases when the likely source of infection is outside the area of residence, such as among forest-goers. WHO recommends testing and treatment of co-travellers or co-workers as best practice, but to date there have been no evaluations of the impact of this strategy.

Border screening to reduce importation of parasites from other endemic countries has been suggested for the reduction of local transmission and to prevent re-establishment of transmission after elimination. Although no studies have been conducted to evaluate the impact of border screening on importation rates, a small number of studies have investigated the feasibility and yield of border screening. The effectiveness of border screening is generally considered low, and, therefore, WHO does not currently recommend border screening for reducing the importation of malaria parasites.

4.3. Malaria in pregnancy

The rationale for improved test-and-treat strategies for MiP is motivated by the high prevalence of malaria in pregnant women and its impact on adverse maternal and newborn outcomes *(16,17)*. WHO has reviewed multiple test-and-treat strategies applied to pregnant women exposed to

malaria, including intermittent screening[2] and treatment (ISTp) and single screening[2] and treatment (SSTp), typically at the first visit to an antenatal care (ANC) clinic. A hybrid strategy combining SSTp with intermittent preventive treatment (IPTp) is being used in some countries in sub-Saharan Africa, but this approach has not yet been systematically researched or reviewed by WHO.

In 2015 and 2017, two consecutive WHO ERGs on MiP reviewed recommendations on the safety, efficacy and cost-effectiveness of ISTp vs. IPTp, and discussed evidence from several studies conducted in Africa and Asia. Pooled analysis of studies conducted in Africa showed that ISTp was associated with both increased maternal infections at delivery and low birth weight compared to IPTp with sulfadoxine pyrimethamine (SP), even in areas with high SP resistance. Similarly, in India, ISTp was not found to reduce the risk of placental malaria or adverse birth outcomes compared to PCD. The majority of placental infections were also found to be undetectable by cRDTs. In Indonesia, IPTp with DHA-piperaquine was found to halve the risk of malaria in pregnancy and at delivery compared to SSTp in an area of moderate transmission. However, findings were inconsistent between study sites and study outcomes, which included malaria infection during pregnancy, malaria infection at delivery, or adverse pregnancy outcomes.

Based on this evidence, WHO does not currently recommend ISTp or SSTp with cRDTs as an alternative to IPTp with SP for the prevention of MiP, and hybrid strategies combining IPTp and SSTp have yet to be formally studied or reviewed.

5. Evidence review

5.1 Considerations for assessing evidence on *in vitro* diagnostics

In 2004, WHO developed the ASSURED criteria as a set of desirable characteristics for POC tests in resource-limited settings *(18)*, suggesting that new diagnostics should be:

- <u>A</u>ffordable by those at risk of infection
- <u>S</u>ensitive (few false-negatives)
- <u>S</u>pecific (few false-positives)
- <u>U</u>ser-friendly (simple to perform with minimal training)
- <u>R</u>apid (to enable treatment at point-of-care) and <u>R</u>obust (no need for refrigerated storage)
- <u>E</u>quipment-free
- <u>D</u>elivered to those who need it

However, building a strong evidence base for diagnostic use involves several stages. The Standards for Reporting Diagnostic Accuracy (STARD) guidelines, developed in 2003 and updated in 2015 *(19)*, provide core elements that should ideally be included in every study report to assist readers in interpreting diagnostic accuracy studies. For the assessment of evidence on IVDs, analytical validity should first be assessed in controlled laboratory conditions, followed by field-based accuracy studies to determine diagnostic and clinical performance in the settings and populations of intended use.

The most challenging evaluations to conduct are impact studies, which assess the potential usefulness and role of diagnostics as a part of specific health interventions and evaluate the effects on patient or community outcomes. The impact depends critically on a number of intermediate

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[2] The term "screening" is commonly used in these strategies, while the most appropriate term should be "testing", as pregnant women are tested for malaria by microscopy or cRDTs, regardless of the presence of fever or other malaria symptoms

factors, including the effect on diagnostic and treatment decisions by the health care provider and the effectiveness of treatment delivery itself *(20)*. To be of practical use, these assessments need to detect statistically significant effects on patient case management or community outcomes as a direct or indirect result of the test or to statistically confirm their non-inferiority. Additionally, the impact of the positive and negative predictive value of the test will depend on the prevalence of the disease in the population and the effect of misdiagnosis on appropriate treatment. In low transmission settings, impact studies often require prohibitively large sample sizes, particularly for trials designed to compare more than two tests *(20)*.

Despite these challenges, impact studies may still be needed if the adequate evaluation of diagnostic accuracy is not feasible in the absence of a well-established reference standard. They may also be required when the link between the test result and the treatment/intervention is unclear or if the impact of the test on public health outcomes can occur through multiple routes. In the context of clinical outcomes, trials may also be useful if a test is likely to have a significant impact by detecting infection earlier in disease progression.

5.2 Laboratory-based assessment of currently available highly sensitive RDTs

5.2.1 Reference assays

There are a number of assays currently available or in development that can serve as reference standards for HSPOCTs. Molecular methods such as quantitative reverse-transcription PCR (qRT-PCR) enable laboratory-based detection and quantification of low-level parasite nucleic acids (LODs <1 parasite/uL) that are below the detection capability of microscopy and cRDTs. For the assessment of RDT-positive/PCR-negative test results, reference assays are required that have lower LODs for the target antigen than the RDT under evaluation. For HRP2, several recently developed assays can serve as suitable references. Quantitative HRP2-based ELISAs launched by Cellabs (Quantimal™ Ultra-sensitive PfHRP2 Malaria CELISA, LOD of 20 pg/mL HRP2), and the Standard Diagnostics/Alere™/Abbott Malaria Ag *Pf* HRP2 ELISA (LOD of 25 pg/mL HRP2) have claimed LODs that can facilitate the validation of studies using HRP2-based RDTs.

Recently developed HRP2 Q-Plex array ELISA kits also have an established laboratory LOD of 5 pg/mL HRP2 and are multiplexed to enable the simultaneous quantification of HRP2, C-reactive protein (CRP), pan-*Plasmodium* LDH, and *Pv* LDH. Development is underway to expand this technology to a 5-Plex platform for the additional quantification of *Pf*-specific LDH. This would enable *Pf* infections with *pfhrp2/3* gene deletions to be differentiated from *Pv* infections. Similarly, research-based quantitative suspension array technologies (qSATs), such as the Luminex™ platform, have shown high sensitivity for detecting HRP2 in serum derived from dried blood spots *(21)*. There is a need to review these technologies to determine the best reference assays with the capacity to quantify the analytes.

In the short to medium term, the technologies described are only applicable as reference standards in laboratory settings and not for large-scale field use due to their cost, the need for highly trained personnel, and long processing times. However, these technologies may have significant utility in measuring biomarker concentrations in asymptomatic infections. In such cases, the evaluation of persistent antigenaemia, particularly in relation to concurrent levels of gametocytaemia, will inform the LOD needed to detect a suitable proportion of the infectious reservoir.

5.2.2 Diagnostic accuracy

Although a review of HSPOCT accuracy studies was not an objective of this meeting, the results of two published studies were presented as background (8,37), and a landscape of ongoing studies or recently completed studies was compiled (Annex 3). These are expected to be included in a future systematic review of HSPOCT accuracy.

Das et al. compared the Alere™ Malaria Ag *Pf* test (Reference number: 05FK140) and the Standard Diagnostics Bioline Malaria Ag *Pf* test (Reference number: 05FK50) in terms of their diagnostic accuracy, using whole blood specimens from asymptomatic study participants in endemic clinical research sites and malaria-naïve volunteers from human challenge studies *(8)*:

1. In Myanmar, in a low transmission area with parasitaemia ranging from 0.2 to 136.9 parasites/uL and HRP2 from 31.2 to 265.6 pg/mL, samples were obtained from cross-sectional village-based surveillance in adults over 18 years of age.

2. In Uganda, in a high transmission area with parasitaemia ranging from 0.01 to 235 095 parasites/uL and HRP2 from 6.1 to 14 600 pg/mL, samples were collected from routine health facility surveillance in three epidemiological settings in children aged 6 months to 11 years.

3. *Pf*-induced blood-stage malaria (IBSM) studies performed in Queensland, Australia included malaria-naïve individuals monitored up to 7 days after inoculation. Pre-treatment samples were used to assess the impact of parasitaemia and HRP2 concentration at the time of detection by the Alere™ Malaria Ag *Pf* test and the Standard Diagnostics Bioline Malaria Ag *Pf* test.

When compared to qRT-PCR, the Alere™ Malaria Ag *Pf* test sensitivity/specificity was 44%/99.9% in Myanmar and 84%/92% in Uganda. Based on the Q-Plex quantitative HRP2 (qHRP2) ELISA as the reference standard, the sensitivity/specificity of the Alere™ Malaria Ag *Pf* test was 80%/99.8% in Myanmar and 91%/99% in Uganda. By comparison, in Uganda, the sensitivity/specificity of the Standard Diagnostics Bioline Malaria Ag *Pf* test was 62%/95% compared to qRT-PCR, and 61%/96% with qHRP2 ELISA as the reference standard. In Myanmar, the Standard Diagnostics Bioline Malaria Ag *Pf* test did not detect any infections that were positive by either qRT-PCR or qHRP2 ELISA. However, the Alere™ Malaria Ag *Pf* tests were performed in laboratory conditions by expert laboratory technicians, which may not reflect performance under field conditions. In the IBSM studies, the sensitivity of the Alere™ Malaria Ag *Pf* test was 2-fold higher than that of the Standard Diagnostics Bioline Malaria Ag *Pf* test (sensitivity of 47%/68% versus 19%/25%, when compared to qRT-PCR/HRP2-ELISA, respectively), while specificity of both tests was high 100%/97% versus 100%/94% when compared to the same reference tests. In the same studies, the Alere™ Malaria Ag *Pf* test detected new infections in study participants 1.5 days earlier than the Standard Diagnostics Bioline Malaria Ag *Pf* test. A recently published cross-sectional study in eastern Myanmar also tested the accuracy of the Alere™ Malaria Ag *Pf* test compared to that of the Standard Diagnostics Bioline Malaria Ag *Pf Pv* test in both laboratory and field conditions (37), as described in more detail on pages 12–13 below.

5.2.3 Distribution of parasite and HRP2 density

Increases in the clinical sensitivity of the Alere™ Malaria Ag *Pf* test in these studies were highly dependent on the distribution of parasite and HRP2 densities in the sampled population, which varied by transmission setting (Figs. 1 and 2).
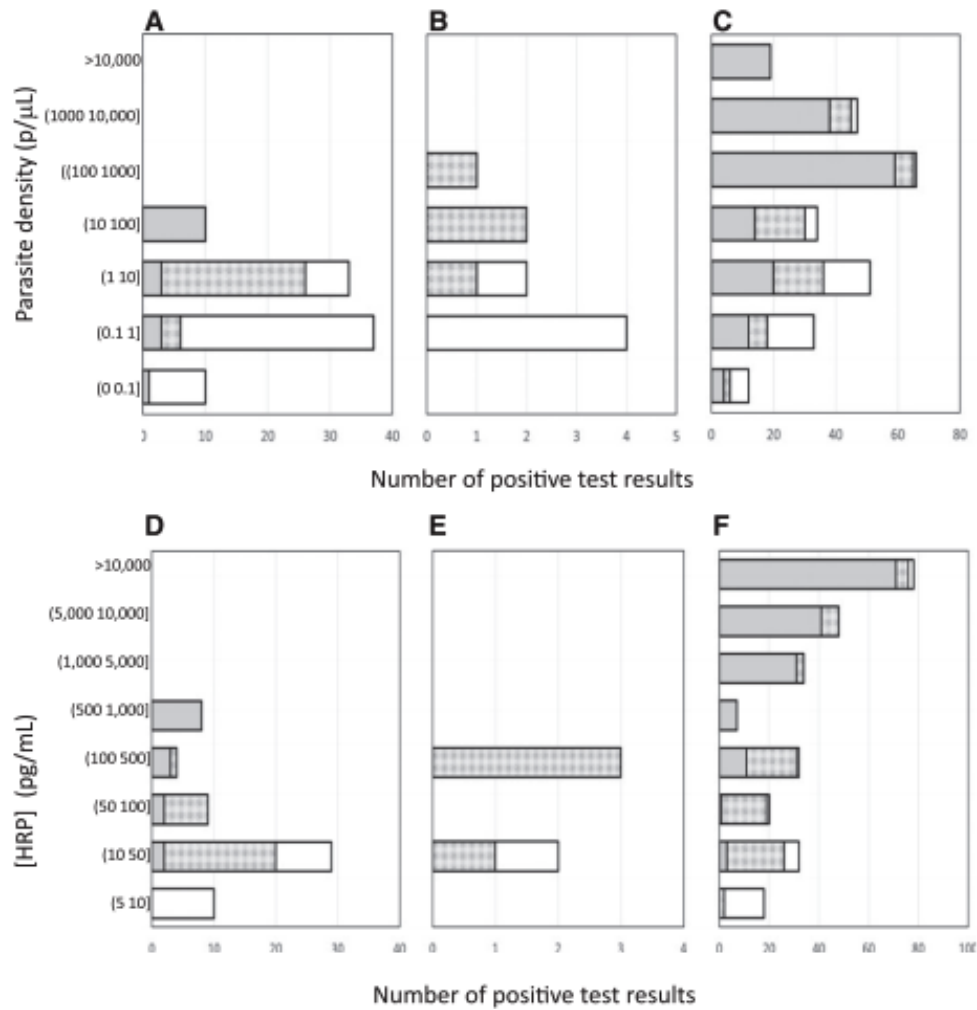
Fig. 1. Distribution of Alere™ Malaria Ag *Pf* test samples by parasite density and HRP2 concentration from blood-stage malaria challenge studies (A, D), Myanmar study (B, E) and Uganda study (C, F). Outer bars are specimens positive by qRT-PCR only, checkered bars are positive test results by qRT-PCR and hsRDT, and grey bars are positive by qRT-PCR, hsRDT and cRDT.
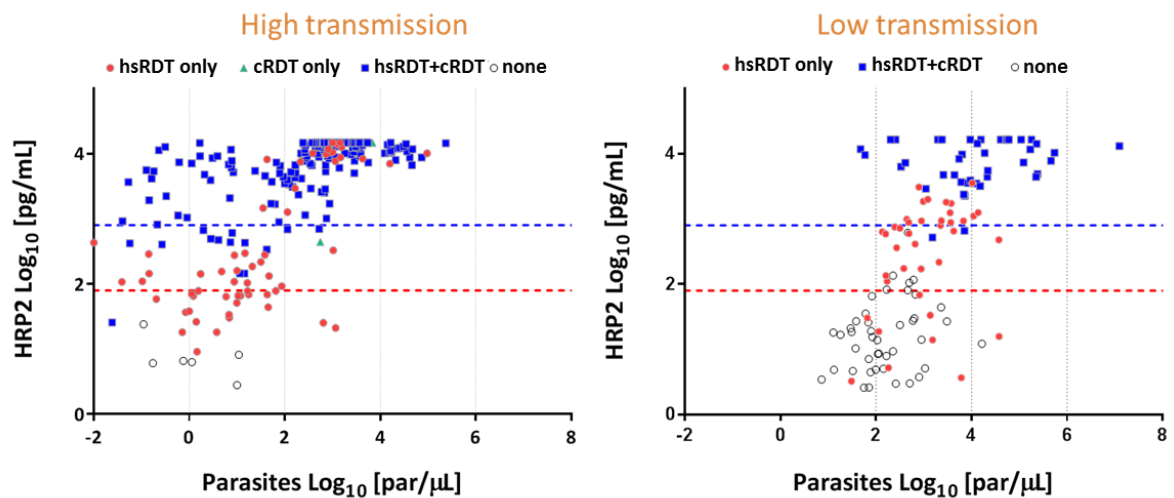

Fig. 2. Distribution of Alere™ Malaria Ag *Pf* test samples by parasite density and HRP2 concentration from high transmission (Uganda study) and low transmission (Myanmar study) settings

A number of studies have found a poor correlation between peripheral parasitaemia and circulating HRP2 concentration *(22,23)*, which may be due to parasite sequestration *(24,25)*, HRP2 persistence *(10,25–29)*, clonal variation in HRP2 expression, or the presence of *pfhrp2/3* gene deletions. Models for predicting HRP2 distributions at given parasite density distributions are being updated by the Queensland Institute for Medical Research in order to better inform future analyses. However, these models are based on cross-sectional data and do not capture the dynamics of HRP2 density over time, the latter of which is the subject of planned field studies (described in more detail on page 12).

There has been some limited evaluation of the Alere™ Malaria Ag *Pf* test and the Standard Diagnostics Bioline Malaria Ag *Pf* test using different HRP2-expressing *P. falciparum* culture strains, with the Alere™ Malaria Ag *Pf* test showing an LOD of at least 10-fold lower antigenaemia than that of the Standard Diagnostics Bioline Malaria Ag *Pf* test *(12)*. Both RDTs also showed the same threshold of cross-reactivity with HRP3, as reported previously with different HRP2-specific monoclonal antibodies and distinct parasite isolates in other studies *(30)*.

Key conclusions

- The evaluation of HSPOCTs will require laboratory-based technical evaluations of analytical validity, field-based accuracy studies of diagnostic and clinical performance, and impact studies assessing downstream effects on patient case management and community outcomes. The impact studies may require prohibitively large sample sizes in low transmission settings, but may still be needed to demonstrate public health impact.

- Laboratory studies based on samples from Uganda and Myanmar have shown increased sensitivity of the Alere™ Malaria Ag *Pf* test compared to the Standard Diagnostics Bioline Malaria Ag *Pf* test. However, clinical sensitivity of the Alere™ Malaria Ag *Pf* test was highly dependent on the distribution of parasite and HRP2 densities in the sampled population, which varied by transmission setting.

- An evaluation of the Alere™ Malaria Ag *Pf* test against HRP2-expressing *P. falciparum* culture isolates, HRP2 recombinants and whole blood archived samples has shown a 10-fold lower antigenaemia LOD compared to the Standard Diagnostics Bioline Malaria Ag *Pf* test.

5.3 Surveillance for elimination

5.3.1 Prevalence and natural history of low-density malaria infections

The proportion of infections detectable by HSPOCTs will depend significantly on the fluctuation of parasite densities and HRP2 concentration in peripheral blood over the course of an infection. Similarly, evidence suggests that the parasite persistence or rate of clearance varies across settings. In Ethiopia, longitudinal data following asymptomatic PCR-positive individuals found that 16.3% of infections were cleared within 28 days, while 17% became detectable by microscopy within the same time period *(31)*. In a study in Viet Nam, a region of very low transmission and risk of re-infection, 20% of individuals still harboured detectable parasitaemia in the absence of treatment after 4 months follow-up, as measured by ultra-sensitive PCR (uPCR) (lower limit of quantification of 22 parasites/mL whole blood) *(32)*.

Currently, there are limited data with which to quantify the threshold of parasite density at which infections are no longer transmissible. As with asexual parasitaemia, gametocyte densities fluctuate periodically. However, they typically comprise less than 5% of total *Pf* parasite biomass *(33)*, and transmission to mosquitoes is believed to decrease once densities fall below 1 gametocyte/µL *(34,35)*. Gametocyte carriage relative to total parasite biomass may also vary considerably between

individuals *(34,36)*. Therefore, more research is needed on the association between gametocytaemia and HRP2 persistence in order to determine the relative contribution of low-density infections to onward transmission.

### 5.3.2 Field studies assessing diagnostic performance of HSPOCTs

Duration of detectability and transmissibility
As part of an ongoing phase I transmission-blocking vaccine trial, a study in Mali is evaluating the association between detection by HSPOCT and mosquito infectiousness. Outcomes measured include parasite density as well as the quantification of HRP2 and LDH concentrations. Two studies set to begin in Burkina Faso in 2018 and Gambia in 2019 will also assess the detectability of infections by Alere™ Malaria Ag *Pf* test and other HSPOCTs with respect to HRP2 concentration, duration of infection and parasite density by molecular diagnostics. These studies are being conducted in areas ranging from low to high malaria intensity (Annex 3, Table 1). Additional transmissibility studies, which may or may not include testing specifically with HSPOCTs, are planned in Ethiopia and Zambia as well as in India and South-East Asia at a number of International Center for Excellence in Malaria Research (ICEMR) study sites. The time to negativity of HSPOCTs is being assessed in five ongoing or planned studies (Annex 3, Table 2). All are based in African settings (Senegal, Namibia, Burkina Faso and Zambia), with the exception of one study in malaria-naïve individuals (NIAID/NIH).

Diagnostic accuracy in cross-sectional surveys

The large majority of field studies currently assessing the diagnostic accuracy of HSPOCTs are village-level cross-sectional surveys via proactive or reactive sampling. These include study sites in Africa (Uganda, Zambia, Namibia, Madagascar and Mozambique), South-East Asia (Myanmar and Cambodia), and Hispaniola (Haiti) (Annex 3, Table 3). All nine studies are evaluating diagnostic accuracy against PCR as the reference standard, with three studies also including quantitative HRP2 ELISA (Zambia, Myanmar and Namibia).

Recently published results from the aforementioned survey conducted in eastern Myanmar reported a 2-fold higher sensitivity by Alere™ Malaria Ag *Pf* RDT compared to the Standard Diagnostics Bioline Malaria Ag *Pf Pv* test and better performance at low parasitaemias (based on a combined reference of uPCR and ELISA) (Fig. 4). The sensitivity of the Alere™ Malaria Ag *Pf* RDT was 2-fold higher than that of the Standard Diagnostics Bioline Malaria Ag *Pf Pv* test (51.4% vs. 25.2%), while both tests had high specificity (99.9% and 99.5%, respectively) against the combined reference (ultrasensitive qPCR plus ELISA). However, the sensitivity of the Alere™ Malaria Ag *Pf* test was lower in matched tests performed in the field compared to those performed in the laboratory (34.6% vs. 51.4%). This difference may highlight operational challenges, such as the need for test results to be read within a small window of time.
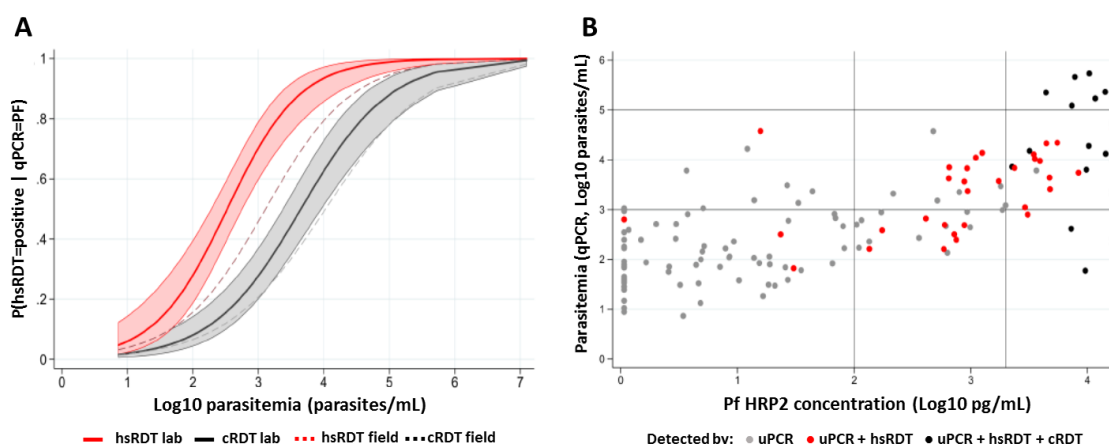
(A) Probability of a *P. falciparum* positive test result by Alere™ Malaria Ag *Pf* test (hsRDT, red) compared to the Standard Diagnostics Bioline Malaria Ag *Pf Pv* test (cRDT, black) in the laboratory (continuous lines) and field (dotted lines) according to the parasitaemia of *P. falciparum* mono-infections, measured by ultra-sensitive PCR (uPCR).

(B) Increased range of *Pf*HRP2 concentration (measured by Quansys ELISA) detected by Alere™ Malaria Ag *Pf* test compared to the Standard Diagnostics Bioline Malaria Ag *Pf Pv* test performed in the field. Vertical lines indicate *Pf*HRP2 concentrations of 100 pg/mL and 2000 pg/mL, while horizontal lines correspond to 1000 parasites/mL and 100 000 parasites/mL.

Fig. 4. Diagnostic accuracy of Alere™ Malaria Ag *Pf* test in Eastern Myanmar *(37)*

Risk stratification and foci detection

Assuming an improved detection capability, HSPOCTs may have utility in risk stratification by measuring a broader range of parasite prevalence. There are only three studies assessing the use of HSPOCTs in foci detection or risk stratification, two of which are located in Myanmar and one in Haiti. These studies aim to evaluate the use of HSPOCTs in risk stratification or identification of transmission foci that meet the minimum criteria to trigger focal mass drug administration (MDA). In Myanmar, cross-sectional surveys will compare prevalence as measured by the HSPOCTs vs. qPCR, while in Haiti, the Alere™ Malaria Ag *Pf* test will be compared to cRDT and microscopy.

Key conclusions

- The proportion of infections detectable by HSPOCTs, similar to cRDTs, will depend on the fluctuation of parasite densities and antigen concentrations over the course of an infection. Parasite persistence and rate of clearance will also vary across settings.

- There are limited data with which to quantify the parasite threshold at which infections are no longer transmissible, also taking into account fluctuations in gametocytaemia. More research is needed on the association between gametocytaemia and HRP2 persistence in order to determine the relative contribution of low-density infections to onward malaria transmission.

- A number of transmissibility studies are investigating the detectability of infection by HSPOCTs in relation to HRP2 concentration, duration of infection and parasite density. However, most of these studies are based in moderate to high transmission settings in Africa.

- The large majority of field studies have assessed the diagnostic accuracy of HSPOCTs compared to PCR through cross-sectional surveys. Recently published results from eastern Myanmar have reported that the Alere™ Malaria Ag *Pf* test has a 2-fold greater sensitivity compared to cRDT.

- There are currently only three studies assessing the use of HSPOCTs in risk stratification or foci detection.

5.4 Elimination

5.4.1 Field studies assessing the use of HSPOCTs in interventions for elimination

While there have been a number of studies assessing test-and-treat strategies to reduce malaria transmission (15 MSAT studies and 31 focal RACD studies), only four field studies have included the use of HSPOCTs specifically. These include two studies nearing completion in Zambia and Cambodia, and additional studies in Lao People's Democratic Republic (expected completion in early 2019) and Myanmar (expected completion in late 2020) – see Annex 3, Table 4.

In Zambia, HSPOCTs are being used to identify additional low-density infections as part of a CRT evaluating RACD vs. reactive focal MDA. In Cambodia, the identification of infections by HSPOCT will be compared to molecular diagnostics (conventional real-time PCR and loop-mediated isothermal amplification [LAMP]) in a trial evaluating PACD vs. RACD. Similarly, in Lao People's Democratic Republic, HSPOCTs will be deployed in a randomized trial of targeted test-and-treat strategies based on proactive sampling to reduce transmission in village residents, mobile and migrant populations, and other high-risk groups. Finally, in Myanmar, HSPOCTs will be used as part of an observational cross-sectional survey to detect pockets of residual subclinical malaria cases in areas with isolated hotspots of high prevalence.

5.4.2 Model-based studies on interventions for elimination

Given the challenges in conducting large field studies, mathematical and simulation models have been used in recent years to estimate the effectiveness of various elimination strategies. The large majority of these have modelled multi-site or country-specific data from African settings (e.g. Zambia, South Africa and United Republic of Tanzania), with additional studies based on data from Myanmar *(38)* and Peru *(39)* as well as *Pv* in Papua New Guinea *(40)*.

Most modelling studies have suggested that MSAT is unlikely to result in elimination except at very low transmission levels and when employed specifically with HSPOCTs *(41,42)* instead of cRDTs. However, MSAT has been shown to reduce disease burden when combined with high-coverage vector control *(43,44)*. Modelling studies of FSAT and RACD have come to similar conclusions as

those modelling MSAT *(45–47)*. In addition, increasing the radius of detection for RACD has not been shown to substantially increase infection detection *(48,49)*.

Key conclusions

- Limited field studies are being conducted to assess the use of HSPOCTs in test-and-treat strategies to reduce malaria transmission. These include CRTs and cross-sectional surveys assessing RACD, reactive focal MDA and/or PACD. HSPOCTs are being used to identify additional low-density infections, detect additional foci in areas of high prevalence, and for risk stratification to target delivery of interventions.

- Modelling studies have suggested that MSAT is unlikely to result in elimination except at very low transmission levels and when employed specifically with HSPOCTs instead of cRDTs.

5.5 Malaria in pregnancy

5.5.1 Test-and-treat strategies for MiP

WHO does not recommend test-and-treat strategies for MiP as an alternative to IPTp in areas of moderate to high transmission in sub-Saharan Africa. Nearly all countries in the Asia-Pacific region currently use PCD in pregnant women with microscopy or cRDTs, while some African countries are employing test-and-treat strategies, either in the absence of IPTp in very low transmission settings or as part of a hybrid strategy combining SSTp and IPTp-SP.

Model-based estimates suggest that testing and treating asymptomatic pregnant women in the first trimester has the potential to prevent onward development of persistent or patent infections that may adversely affect birth outcomes. There is also the potential for up to several weeks of post-treatment prophylaxis for the treated individual. ANC-based test-and-treat strategies may also have population-level benefits and applications. These include the prevention of transmission from pregnant women in low transmission areas, sentinel surveillance through RDT screening at first ANC visit, and form part of RACD strategies in which cRDT-positive pregnant women are the index cases.

The clinical and public health impact of these approaches will depend on the performance of the test and the drug used for treatment. Diagnostic accuracy for MiP has been shown to be highest in primigravid women and at first testing (median 23 weeks of gestation). In this population, test sensitivity declines with transmission, mirroring trends in the general population. However, the dynamics of parasite density in the first trimester are not well understood and are the subject of ongoing studies.

5.5.3 MiP studies assessing the performance of highly sensitive diagnostics

There are currently seven completed or ongoing MiP studies assessing the use of HSPOCTs. The majority of these studies are assessing diagnostic accuracy through cross-sectional retrospective testing of samples in controlled laboratory settings. These are based on peripheral or placental blood samples collected in ANC clinics in Colombia, Indonesia, Malawi and Kenya. One potential limitation facing the interpretation of retrospective studies is the effect of sample preparation on study outcomes. For example, the study in Indonesia used plasma reconstituted with packed red blood cells. Two cross-sectional studies are investigating diagnostic test performance at point-of-care under intended clinical settings in Colombia *(50)* (completed and recently published) and Papua New Guinea (study beginning July 2018 with expected completion in mid-2019). There is also an ongoing retrospective study based on samples from a longitudinal cohort study (with follow-up from pre-conception through to delivery) in Benin (RECIPAL) that is evaluating the association between

malarial infections detected by Alere™ Malaria Ag *Pf* test and maternal and birth outcomes. There are no prospective longitudinal studies or trials assessing the clinical impact of HSPOCTs as part of intervention strategies for the prevention of MiP.

Key conclusions

- WHO does not recommend test-and-treat strategies for MiP as an alternative to IPTp in areas of moderate to high transmission in sub-Saharan Africa. Nearly all countries in the Asia-Pacific region are currently using PCD, while some African countries are employing test-and-treat in combination with or as an alternative to IPTp.

- Model-based estimates suggest that testing asymptomatic pregnant women in the first trimester has the potential to prevent onward development of persistent or patent infection that may adversely affect birth outcomes through early detection and treatment.

- ANC-based test-and-treat strategies may have the potential to prevent transmission from pregnant women in low transmission areas, serve as sentinel surveillance through cRDT screening at first ANC visit, and form part of RACD strategies in which cRDT-positive women are the index cases.

- The majority of existing studies assessing the diagnostic accuracy of HSPOCTs have been based on cross-sectional retrospective testing of samples in controlled laboratory settings.

- There are no prospective longitudinal studies or trials assessing the impact of HSPOCTs as part of intervention strategies for prevention of MiP.

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6. Proposed study designs

6.1 Diagnostic accuracy studies

Additional studies to establish the diagnostic accuracy of HSPOCTs vs. cRDTs are considered to be high priority. These would provide important data on the LODs, sensitivity and specificity of HSPOCTs in target populations, and on the biodynamics of HRP2 in relation to detectability. One method would be to use archived biobank samples to determine sensitivity and specificity across a range of settings, but future field studies (in the form of cross-sectional or longitudinal surveys as well as intervention trials) could also include standardized accuracy testing of HSPOCTs.

Therefore, field-based accuracy studies were proposed to define test sensitivity and specificity in different scenarios. Given that test accuracy will vary by population and setting, studies should reflect a range of:

- Transmission intensities and seasonality;

- Target populations (e.g. high-risk occupations, and mobile or migrant populations). Samples should also ideally be representative of age, parasite density, clinical symptoms, immunity or other determinant factors;

- Health care system levels and capacities (e.g. public and private health facilities, CHWs).

To enable comparability across studies and full assessment of the impact of HRP2 persistence on test accuracy, these studies will require standardized protocols and reference assays. Ideally, qPCR should be considered a required reference, with potential use of ELISAs or bead-based multiplex immunoassays depending on the test biomarker. The need to test samples in both field and laboratory settings was emphasized, along with the evaluation of the impact of different kinds of blood samples (e.g. peripheral vs. venous blood, stored samples vs. fresh blood at point-of-care).

Complete harmonization of assays and protocols across research settings will likely be challenging, but a set of recommended criteria could be developed to facilitate optimal comparability. Additionally, laboratories should be required to participate in a malaria molecular External Quality Assessment (EQA) scheme in order to validate the performance of research teams.

The need for longitudinal or cohort studies to understand the prevalence and natural history of low-density infections was also discussed. In addition to ongoing research, studies in non-African settings or at low transmission intensities were suggested with the aim of understanding i) whether infections can be detected by HSPOCTs before they become symptomatic or infectious, ii) the proportion of these infections that lead to malaria symptoms such as anaemia or fever, and iii) the transmissibility of persistent low-density parasitaemia.

> **Key conclusions**
>
> - Diagnostic accuracy studies assessing HSPOCTs vs. cRDTs and applying common reference standards were considered to be high priority for providing data on LODs, sensitivity and specificity in target populations, and on the biodynamics of HRP2 in relation to detectability.
>
> - Archived biobank samples are one potential method for determining sensitivity and specificity across a range of settings, but additional field studies are also needed.
>
> - Field-based accuracy studies were proposed to define test sensitivity and specificity in different scenarios. These studies should reflect a range of transmission intensities and seasonality, target populations, and health care system levels and capacities.
>
> - Accuracy studies will require standardized protocols and reference assays. Recommended criteria for harmonized assays and protocols can be developed to facilitate optimal comparability across studies.
>
> - Longitudinal or cohort studies are needed to understand the prevalence and natural history of low-density infections, particularly in non-African and low transmission settings.

6.2 Studies to evaluate the use of HSPOCTs for malaria elimination

Studies to determine the public health impact of using HSPOCTs to detect and treat infections in elimination strategies were considered to be high priority.

Gold-standard CRTs were proposed for comparing HSPOCTs to cRDTs when used in MTAT strategies (see Annex 4). Relevant trial designs include stepped-wedge, cross-over and factorial designs.

These studies should estimate:

1. The number and proportion of additional infections detected and treated;

2. The impact on malaria transmission based on trends in passively detected clinical cases (confirmed by cRDTs or microscopy) at health facilities in the same area and, where feasible, additional community prevalence surveys.

Intervention trials will need to reflect a variety of transmission settings that are working towards elimination, including areas that have undergone recent reductions in transmission and areas that have had low transmission for an extended period.

In addition, CRTs comparing MTAT to MDA can assess whether the use of HSPOCTs can increase treatment adherence and coverage, while limiting concerns over the development of drug resistance. In all study designs, health-facility-based PCD should be used to monitor impact on

transmission as a proxy for malaria incidence. Therefore, optimal study sites will have health facilities with a strong, well-established PCD surveillance system.

Based on both empirical and modelling studies, the impact of MTAT is likely to depend not only on persistent high coverage, but also on repeated rounds of treatment with artemisinin-based combination therapies (ACTs) that have a long prophylactic period. Therefore, intervention trials with HSPOCTs should ideally include multiple rounds and be of adequate duration to assess the impact on transmission. In effect, the study should include an intervention period long enough to have an effect and a suitable follow-up period to measure the number and proportion of additional infections detected and treated, while ideally minimizing the impact of year-to-year fluctuations. Due to the large sample sizes required for measuring reductions or interruptions in transmission in low to very low transmission settings, indirect evidence can be gathered from trials conducted in moderate transmission settings where changes in incidence can be more easily quantified. Modelling-based studies may be able to provide insights into potential impact.

> ### Key conclusions
>
> - Assessing the public health impact of HSPOCTs used to detect and treat infections in elimination strategies was considered to be high priority.
>
> - Studies were proposed comparing HSPOCTs to cRDTs in estimating the number and proportion of additional infections detected and treated and the impact on malaria transmission as assessed through PCD in health facilities.
>
> - These studies should reflect a range of transmission settings, and interventions should include multiple rounds of adequate study duration to enable effective assessment of the impact on transmission.
>
> - CRTs comparing MTAT to MDA can assess whether the use of HSPOCTs can increase treatment adherence and coverage, while limiting concerns over development of drug resistance.
>
> - Due to the potentially large sample sizes required in low to very low transmission settings, indirect evidence can be gathered from moderate transmission settings or from modelling studies.

6.3 CRTs to evaluate the role of HSPOCTs for surveillance in elimination

To assess the potential role of HSPOCTs in surveillance, CRTs were proposed to evaluate the effectiveness of HSPOCTs compared to conventional RDTs when used in RACD or PACD for identifying additional transmission foci for targeted response (see Annex 4).

It may be easier to assess the use of HSPOCTs in RACD, given that this strategy is already employed by a number of countries. However, designing studies based on PACD may be operationally more feasible due to its routine and continuous implementation, unlike RACD, which is based on the sporadic detection of cases at health facilities. There is currently no evidence on the impact of RACD on the reduction of malaria transmission. Therefore, in the absence of evidence that low-density infections contribute to transmission, studies on surveillance in elimination should focus on measuring improvements in the surveillance system's capability to detect additional foci of infection and map their extension as indirect evidence of the benefits of HSPOCTs for malaria surveillance.

> **Key conclusions**
>
> - Studies were proposed to evaluate the effectiveness of HSPOCTs vs. cRDTs when used in RACD or PACD to identify additional foci or transmission for targeted response.
>
> - As there is currently no evidence on the impact of RACD on the reduction of malaria transmission, studies on surveillance in elimination should focus on the impact of HSPOCTs on improvements in the surveillance system's capability to detect foci of transmission.

6.4 Individual RCTs to evaluate the use of HSPOCTs for first-trimester testing and treatment to prevent MiP

To assess the potential role of HSPOCTs in testing for MiP, individual RCTs were proposed to compare the effectiveness of HSPOCTs vs. cRDTs when used in SSTp of low-density infections, specifically in the first trimester of pregnancy to prevent placental malaria at delivery (see Annex 4). These studies should be implemented in moderate to high transmission settings, where the burden of MiP is highest. Primary outcomes should ideally include placental and peripheral infection at delivery and may also include adverse pregnancy outcomes. Adverse pregnancy outcomes may include low birth weight, pre-term birth, small-for-gestational-age or fetal loss/neonatal mortality. Maternal malaria infection can be defined as peripheral or placental infection based on multiple diagnostics (PCR, RDT, microscopy or placental histology). Secondary outcomes also discussed were an evaluation of the cost-effectiveness of HSPOCT introduction at different levels of transmission, and the impact on ANC attendance in the first trimester.

Ongoing studies are evaluating samples retrospectively from a cohort of women followed from pre-conception through to delivery. These can provide preliminary evidence on the impact of first-trimester low-density malaria infections detectable with HSPOCTs on pregnancy outcomes in order to guide future trial designs with regard to target population, sample size and study outcomes.

> **Key conclusions**
>
> - Individual RCTs were proposed to compare the effectiveness of HSPOCTs vs. cRDTs when used in SSTp in the first trimester of pregnancy.
>
> - These studies should be implemented in moderate to high transmission settings where the burden of MiP is highest, and measure the impact on placental and peripheral infection at delivery and on adverse pregnancy outcomes. Potential secondary outcomes include cost-effectiveness of HSPOCT introduction in different transmission settings, and the impact on ANC attendance in the first trimester.
>
> - Ongoing studies investigating the impact of first-trimester infections detected by HSPOCTs on pregnancy outcomes can provide preliminary evidence to guide future trial designs with regard to target population, sample size and study outcomes.

6.5 Other study designs considered

6.5.1 Border screening to prevent the contribution of imported parasites to local transmission and the re-establishment of transmission

Border screening of individuals entering eliminating areas/countries from areas of higher transmission has been suggested as a means to reduce parasite importation and prevent the re-establishment of malaria transmission in eliminated areas. Border screening has been implemented between several countries in the Elimination 8 partnership (Angola, Botswana, Kingdom of Eswatini, Mozambique, Namibia, South Africa, Zambia and Zimbabwe), the Greater Mekong Subregion (Thailand, Myanmar and Cambodia) and the Arabian Peninsula. These existing programmes may provide opportunities to evaluate impact based on surveillance data, either by comparing programme regions to regions with no border screening or by implementing interrupted time-series study designs.

Currently, there is no evidence on the cost-effectiveness of border screening in reducing the contribution of imported parasites to local transmission and preventing re-establishment of malaria. However, cost-effectiveness is likely to be low given the high resource requirements and the porous nature of borders. Furthermore, due to issues unrelated to test sensitivity, the cost-effectiveness of border screening is unlikely to be improved with the use of HSPOCTs. Therefore, these studies were considered to be low priority.

6.5.2 Clinical case management

The overall scope of the technical consultation was to identify the research requirements to develop recommendations on the use of HSPOCTs in strengthening surveillance, accelerating elimination and preventing MiP. However, the role of HSPOCTs in clinical case management was also briefly discussed. In countries where HSPOCTs will be introduced for clinical diagnosis, it was suggested that studies should focus on the impact of HSPOCTs on provider behaviour and adherence to clinical protocols for fever management. Studies should evaluate the potential risk of missed diagnosis and treatment of serious illness following the identification of low-density malaria infections, and the potential benefits of detection and treatment of low-density infections. To minimize risks, the introduction of any HSPOCT will need to be accompanied by strong provider training, especially on integrated clinical management of febrile illnesses and the monitoring of prescription rates for antimalarials and other treatments.

6.5.3 Surveillance or ISTp for pregnant women

Studies assessing ISTp with HRP2-only HSPOCTs vs. cRDTs were not considered high priority for several reasons: There are limited settings in Africa where ISTp is likely to be more advantageous than IPTp with SP or with DHA-PPQ. While ISTp with HSPOCTs may have a role to play in low to moderate transmission settings, given the high prevalence of *Pv* in these areas, future studies should be planned that include next-generation HSPOCTs for detection of *Pv*, which are expected to be available in 2020.

The use of HSPOCTs in ANC has also been suggested for including pregnant women in focal MDA and MTAT, since they are presently excluded from these strategies. However, it is not yet clear what the impact of including pregnant women in these interventions would be. Not only is the transmissibility of infections in pregnant women not understood, their contribution to the overall infectious reservoir may be relatively small given that pregnant women typically comprise less than 4% of the population. Finally, the use of HSPOCTs in ANC as the basis for malaria sentinel surveillance was considered to be low priority, as there is still limited evidence on the utility of monitoring transmission based on positivity among pregnant women, regardless of test sensitivity.

Key conclusions

- Studies on the use of HSPOCTs in border screening were considered to be low priority given the lack of evidence on the effectiveness of this strategy in reducing the importation of parasites and preventing re-establishment of malaria, and given the potentially high implementation cost.

- In countries where HSPOCTs are to be considered for clinical case management on the basis of a claimed or published increase in sensitivity, studies should first assess the impact of HSPOCTs on provider behaviour, adherence to clinical management protocols and patient outcomes. The introduction of any HSPOCT will need to be accompanied by strong provider training on integrated management of febrile illnesses.

- Studies assessing ISTp with HRP2-only HSPOCTs vs. cRDTs were not considered to be high priority due to the limited settings where this strategy is likely to be more cost-effective than IPTp and due to the preference for including next-generation HSPOCTs designed to detect *Pv*.

- The use of HSPOCTs in ANC for surveillance and focal MDA or MTAT was not considered to be high priority due to the limited evidence on the impact of the detection and/or treatment of pregnant women in these strategies, irrespective of test sensitivity.

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Annex 2. List of pre-reads for the meeting

Unpublished documents

1. The density, temporal dynamics and infectiousness of subpatent *P. falciparum* infections. Hannah Slater, Amanda Ross, Ingrid Felger, Nataile Hofmann, Leanne Robinson, Jackie Cook, Bronner Goncalves, Andre Lin Ouedraogo, Christian Koepfli, Ivo Mueller, Fitsum Tadesse, Smita Das, Gonzalo Domingo, Anders Björkman, Ulrika Morris, Cécile Nabet, Ogobara Doumbo, Renaud Piarroux, Naomi Lucchi, Venkatachalam Udhayakumar, Jacklin Mosha, Daniel Chandramohan, Roly Gosling, Felista Mwingira, Robert Sauerwein, Eleanor Riley, Nick White, Francois Nosten, Malika Imwong, Teun Bousema, Chris Drakeley, Lucy Okell. Unpublished paper – confidential

2. Highly-sensitive rapid diagnostic tests: landscape of field research. MESA Track, Maria Tusell, Sherman Kong. Unpublished paper – confidential

3. Screen and treat strategies for malaria elimination: a review of evidence. Gretchen Newby, Kelly Harvard, Roly Gosling, Chris Cotter, Michelle Roh, Adam Bennett, Ingrid Chen, Jimee Hwang, Nakul Chitnis, Michelle Hsiang. Prepared for the Bill & Melinda Gates Foundation, May 2018. Unpublished paper – confidential

Published papers

4. Ferrante di Ruffano L, Hyde CJ, McCaffery KJ, Bossuyt PM, Deeks JJ. Assessing the value of diagnostic tests: a framework for designing and evaluating trials. BMJ. 2012;344:e686. doi:10.1136/bmj.686

5. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ. 2015;351:h5527. doi:10.1136/bmj.h5527

6. Das S, Peck RB, Barney R, Jang IK, Kahn M, Zhu M, et al. Performance of an ultra-sensitive Plasmodium falciparum HRP2-based rapid diagnostic test with recombinant HRP2, culture parasites, and archived whole blood samples. Malar J. 2018;17(1):118. doi:10.1186/s12936-018-2268-7

7. Desai M, Hill J, Fernandes S, Walker P, Pell C, Gutman J, et al. Prevention of malaria in pregnancy. Lancet Infect Dis. 2018;18(4):e119–32. doi:10.1016/S1473-3099(18)30064-1.

Relevant WHO documents

8. Malaria surveillance, monitoring & evaluation: a reference manual. Geneva: World Health Organization; 2018 (http://www.who.int/malaria/publications/atoz/9789241565578/en/).

9. Meeting report of the WHO evidence review group on low-density malaria infections, Malaria Policy Advisory Committee Meeting, 15–16 May 2017. Geneva: World Health Organization; 2017 (http://www.who.int/malaria/mpac/mpac-oct2017-erg-malaria-low-density-infections-session2.pdf).

10. How to design vector control efficacy trials: guidance on phase III vector control field trial design provided by the Vector Control Advisory Group. Geneva: World Health Organization; 2017 (http://www.who.int/neglected_diseases/vector_ecology/resources/WHO_HTM_NTD_VEM_2017.03/en/).

11. A framework for malaria elimination. Geneva: World Health Organization; 2017 (http://www.who.int/malaria/publications/atoz/9789241511988/en/).

Annex 3. List of ongoing or planned research on highly sensitive point-of-care diagnostics tests for *P. falciparum*

Table 1. Infectivity studies assessing detectability by highly sensitive diagnostic tests

Site(s)	Transmission intensity	Institution	Outcomes	Study completion
Mali	Moderate and high	US National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIAID, NIH), University of Bamako, Mali	Part of a transmission-blocking vaccine trial. Detectability of infection by Alere™ Malaria Ag *Pf* test, parasite density, HRP2 concentration and infectiousness	April 2018
Burkina Faso, Gambia	Moderate and high	London School of Hygiene & Tropical Medicine (LSHTM), Radboud University Medical Center (UMC), National Center for Research and Training for Malaria (CNRFP) Burkina Faso, MRC Unit The Gambia at London School of Hygiene & Tropical Medicine	Detectability of infection by HSPOCT, parasite density, HRP2 concentration and infectiousness	September 2020

Table 2. Studies evaluating time to negativity of highly sensitive diagnostic test results

Site	Transmission intensity	Institution(s)	Study completion
United States	Malaria-naïve volunteers	US National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIAID, NIH), Sanaria	July 2017
Senegal	Low	Foundation for Innovative New Diagnostics (FIND), Cheikh Anta Diop University	January 2018
Zambia	Low	Malaria Control and Elimination Partnership in Africa (MACEPA) / PATH, Ministry of Health Zambia, Zambia Ministry of Community Development, Mother and Child Health	June 2018
Namibia	Low	University of California, San Francisco (UCSF), University of Texas, Southwestern (UTSW), University of Namibia, Ministry of Health and Social Services (MOHSS) Namibia	December 2018
Burkina Faso, Gambia	Moderate and high	London School of Hygiene & Tropical Medicine (LSHTM), Radboud University Medical Center (UMC), National Center for Research and Training for Malaria (CNRFP) Burkina Faso, MRC Unit The Gambia at London School of Hygiene & Tropical Medicine	September 2020

Table 3. Unpublished cross-sectional studies assessing highly sensitive diagnostic test accuracy

Site	Sampling	Transmission intensity	Institution	Reference test (s)	Other outcomes	Study completion
Mozambique	Proactive	Low and moderate	Barcelona Institute for Global Health (ISGlobal), Manhiça Health Research Centre (CISM)	qPCR	Post-intervention evaluation	June 2017
Haiti	Proactive	Low	US Centers for Disease Control and Prevention (CDC), Ministry of Health Haiti, Tulane University	qPCR	Foci detection	December 2017
United Republic of Tanzania	Retrospective testing of samples from primary care attendees	Moderate to high	Swiss Tropical and Public Health Institute (Swiss TPH), Foundation for Innovative New Diagnostics (FIND)	PCR	Child morbidity and mortality in cases detected by hsRDT; rate of viral and bacterial coinfections among hsRDT vs. cRDT detected cases (Management of Fevers Project)	December 2017
Myanmar	Proactive	Low	Shoklo Malaria Research Unit (SMRU), Mahidol Oxford Tropical Medicine Research Unit (MORU)	qPCR, qHRP2 ELISA	Foci detection	January 2018
Cambodia	Proactive (risk groups only)	Low	Médecins San Frontières (MSF), National Center for Parasitology, Entomology and Malaria Control, Cambodia (CNM), Institut Pasteur du Cambodia (IPC)	qPCR		March 2018
Myanmar	Reactive	Low	Ministry of Health Myanmar, US Centers for Disease Control and Prevention (CDC)	qPCR		May 2018
Zambia	Reactive	Low	Malaria Control and Elimination Partnership in Africa (MACEPA) / PATH, Ministry of Health Zambia, Zambia Ministry of Community Development, Mother and Child Health	qPCR, qHRP2 ELISA		June 2018
Namibia	Proactive	Very low	University of California, San Francisco (UCSF), University of Texas, Southwestern (UTSW), University of Namibia, Ministry of Health and Social Services	qPCR, qHRP2 ELISA	Post-intervention evaluation	December 2018

Site	Sampling	Transmission intensity	Institution	Reference test (s)	Other outcomes	Study completion
			(MOHSS) Namibia, London School of Hygiene & Tropical Medicine, Clinton Health Access Initiative (CHAI), PATH			
Myanmar	Proactive	Low	Duke University	qPCR	Foci detection	December 2021
Madagascar	Proactive	Low, moderate and high	US Centers for Disease Control and Prevention (CDC), Institut Pasteur Madagascar (IPM)	qPCR		Cancelled

Table 4. Ongoing or planned test-and-treat intervention studies assessing the use of highly sensitive diagnostic tests

Site	Test and treat intervention(s)	Trial design	Transmission intensity	Institution(s)	Outcomes	Feasibility outcomes	Study completion
Cambodia	FSAT	Individual RCT	Low	London School of Hygiene & Tropical Medicine (LSHTM); National Center for Parasitology Entomology and Malaria Control (CNM) Cambodia	Number of high-risk individuals screened; number of infections detected	Adherence; Health-seeking	December 2017
Zambia	RACD vs. rfMDA	Cluster RCT	Low	Malaria Control and Elimination Partnership in Africa (MACEPA) / PATH, Ministry of Health Zambia, Zambia Ministry of Community Development, Mother and Child Health	Additional infections; prevalence; sero-positivity		March 2018
Lao People's Democratic Republic	RACD	Cluster RCT	Low	University of California, San Francisco (UCSF), Centre for Malaria Parasitology and Entomology (CMPE) Lao PDR	Village-level prevalence	Cost-effectiveness; acceptability	January 2019
Myanmar	MSAT	Pre/post intervention	Low	Shoklo Malaria Research Unit (SMRU)	Prevalence; incidence	Coverage	November 2020

Annex 4. Proposed study designs

Study 1: Evaluate the use of HSPOCTs in elimination strategies

Study objective and design	Index test(s)	Reference test(s)
Cluster RCTs comparing the use of HSPOCTs to that of cRDTs in MTAT Other potential comparator arms include MDA (to assess impact on treatment adherence and coverage)	HSPOCTs	qPCR or validated highly sensitive antigen detection assays (e.g. ELISA), depending on test biomarker
	Study procedures and outcomes	
	- Number and proportion of additional infections detected and treated - Impact on malaria transmission based on trends in passively detected clinical cases (detected by conventional RDT or microscopy) at health facilities in the same area	

Study population, sample size, timeline	Study status
Population in an area targeted for elimination of malaria, preferably with low population mobility and low parasite importation rates Assumes high coverage with testing and treatment of those positive Due to the large sample sizes required for measuring cases in low transmission settings, modelling studies and indirect evidence from moderate transmission settings may also guide and inform evaluations.	Existing studies: Zambia, Myanmar to determine number of additional infections detected and treated No existing or planned studies to assess impact on transmission

Study 2: Evaluate the role of HSPOCTs in surveillance for elimination

Study objective and design	Index test(s)	Reference test(s)
Cross-sectional or longitudinal studies evaluating the effectiveness of HSPOCTs compared to that of cRDTs when used in RACD or PACD to: 1. Identify additional foci of transmission for targeted response	HSPOCTs	qPCR or validated highly sensitive antigen detection assays (e.g. ELISA), depending on test biomarker
	Study procedures and outcomes	
	• Number of additional transmission foci identified	
Study population, sample size, timeline	**Study status**	
Study sites should be selected from a range of: • Transmission intensities and seasonal patterns • Target populations, accounting for particular at-risk groups such as pregnant women, high-risk occupations, and mobile or migrant populations • Health surveillance system capacities Population within a nationally defined RACD radius Sample size should reflect underlying prevalence Potential study sites – Senegal, southern Zambia	Existing studies: Myanmar, Haiti	

Study 3: Evaluate the use of HSPOCTs for first-trimester test-and-treat to prevent MiP

Study objective and design	Index test(s)	Reference test(s)
Individual RCTs to compare the effectiveness of HSPOCTs to that of cRDTs for the prevention of placental malaria at delivery, specifically via early detection and treatment in the first trimester of pregnancy	HSPOCTs	qPCR or validated highly sensitive antigen detection assays (e.g. ELISA), depending on test biomarker
	Study procedures and outcomes	
	Primary outcomes can include placental and peripheral infection at delivery and should also include adverse pregnancy outcomes. Secondary outcomes could include an evaluation of the cost-effectiveness of HSPOCT introduction at different levels of transmission, and the impact on ANC attendance in the first trimester.	

Study population, sample size, timeline	Study status
Studies can be conducted in moderate to high transmission settings where burden of MiP is higher. Composite outcomes could be considered: • Adverse pregnancy outcomes – low birth weight, pre-term birth, small-for-gestational-age, or fetal loss/neonatal mortality • Malaria infection – peripheral or placental infection based on multiple diagnostics (PCR, RDT, microscopy or placental histology)	Existing studies: RECIPAL, retrospective evaluation of samples from cohort of women followed from pre-conception through to delivery can provide evidence on impact of first-trimester low-density infection detectable with HSPOCTs on pregnancy outcomes. No ongoing or planned prospective longitudinal studies or trials assessing the clinical impact of HSPOCTs as part of interventions to prevent MiP