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# Diagnostic performance of loop-mediated isothermal amplification and ultra-sensitive rapid diagnostic tests for malaria screening among pregnant women in Kenya

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## Abstract

**Background:** Screen-and-treat strategies with sensitive diagnostic tests may reduce malaria-associated adverse pregnancy outcomes. We conducted a diagnostic accuracy study to evaluate new point-of-care tests to screen pregnant women for malaria at their first antenatal visit in western Kenya.

**Methods:** Consecutively women were tested for *Plasmodium* infection by expert-microscopy, conventional rapid diagnostic test (cRDT), ultra-sensitive RDT (usRDT), and loop-mediated isothermal amplification (LAMP). Photo-induced electron-transfer polymerase-chain-reaction

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***Author Contributions***. AMS, SK and FtK conceived the study design. AMS, LS, KS, PW, SK and FtK secured funding for the study. OT, BS, TK, KP and MC were involved with the implementation of the field and laboratory work in Kenya. NL and DL conducted the PET-PCR in Atlanta, GA, USA. AMS and RW verified the underlying data and analyzed the results with input from FtK and PW. AMS drafted the paper with input from FtK, PW, RW, LS, KSL and SK. All authors reviewed and approved the final draft.

**Potential Conflicts of Interest** All authors: None of the authors have a commercial or other association that may pose a conflict of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

***Prior presentations***. Part of the results was presented as a poster presentation at the American Society of Tropical Medicine and Hygiene Annual Meeting hosted at the National Harbor, Maryland, United States from November 20–24, 2019 (Abstract #903).

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(PET-PCR) served as the reference standard. Diagnostic performance was calculated and modelled at low parasite densities.

**Results:** Between May-September 2018, 172 out of 482 screened participants (35.7%) were PET-PCR positive. Relative to PET-PCR, expert-microscopy was least sensitive (40.1%, 95% CI 32.7–47.9), followed by cRDT (49.4%, 41.7–57.1), usRDT (54.7%, 46.9–62.2), and LAMP (68.6%, 61.1–75.5). Test sensitivities were comparable in febrile women (N=90). Among afebrile women (N=392), the geometric-mean parasite density was 29 parasites/μL and LAMP (sensitivity=61.9%) and usRDT (43.2%) detected 1.74 (1.31–2.30) and 1.21 (0.88–2.21) more infections than cRDT (35.6%). Per our model, tests performed similarly at densities >200 parasites/μL. At 50 parasites/μL, the sensitivities were 45%, 56%, 62% and 74% with expert-microscopy, cRDT, usRDT, and LAMP, respectively.

**Conclusions:** This first-generation usRDT provided moderate improvement in detecting low-density infections in afebrile pregnant women compared to cRDTs.

## Summary:

Most pregnant women in sub-Saharan Africa have low parasite densities and are asymptomatic when screened for *Plasmodium falciparum* at their first antenatal care visit. The first-generation usRDT provide detect 21% more low-density infections in afebrile pregnant women compared to cRDTs.

### Keywords

Malaria in Pregnancy; Screening at first Antenatal Care clinic visit; Diagnostic sensitivity in malaria in pregnancy; ultra-sensitive rapid diagnostic tests for malaria

## Introduction

Pregnancy increases the risk and severity of *Plasmodium falciparum* infections, which contribute to adverse maternal, fetal, and infant outcomes [1, 2]. Many infections in semi-immune pregnant women remain asymptomatic and are below the level of detection (LOD) of microscopy and conventional RDTs (cRDT) (LOD=100–200 parasites/μL), partly due to placental sequestration of the parasite [1]. They, therefore, remain undetected and untreated. In malaria-endemic areas in Africa, the World Health Organization (WHO) recommends intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP), beginning in the second trimester [3]. However, the efficacy of IPTp-SP to clear existing infections is threatened by SP resistance [4, 5]. There are no specific interventions recommended for the first trimester when falciparum infections are particularly harmful to the developing placenta, but when IPTp-SP is contraindicated [6, 7].

Four recent trials found that intermittent screening with cRDT and subsequent treatment with highly effective artemisinin-based combination therapies (ACTs) in pregnancy (ISTp) is not superior to IPTp-SP for reducing malaria in pregnancy in high SP resistance areas [4]. However, a recent evaluation of screening and treatment of asymptomatic pregnant women [8] suggests combining IPTp-SP with single screening and treatment (SST) at the first antenatal clinic (ANC) visit may offer substantial benefit by ensuring early clearance

of existing patent infections. This hybrid strategy is currently implemented in areas of Tanzania and western Kenya, where *P. falciparum* is highly resistant to SP. Modelling suggests this could substantially improve pregnancy outcomes by reducing the overall exposure to placental infections and their duration [9]. Screening strategies addressing early infections have been buoyed by recent evidence supporting the safety of ACT treatment for uncomplicated malaria in the first trimester [10].

Modelling also suggests that incremental gains could be achieved by using more sensitive point-of-care (POC) tests than cRDT or microscopy. While highly sensitive malaria diagnostic tests such as polymerase chain reaction (PCR) are needed to detect these infections, they cannot be used at POC because they require significant laboratory capacity and resources not readily available in many malaria-endemic settings [11].

Two diagnostic tests with reported high sensitivity that can be used at POC in resource-limited settings include loop-mediated isothermal amplification (LAMP), a molecular test with similar sensitivity to PCR [12], and ultra-sensitive malaria RDT (usRDT). usRDTs are reported to be up to ten times more sensitive than cRDTs.[13] We compared the diagnostic performance of usRDT and LAMP against cRDTs and microscopy among pregnant women attending their first ANC visit in a highly endemic setting for malaria.

## Methods

### Study design and participants

This prospective study was performed in nine facilities providing ANC services in western Kenya [14]. Here, malaria transmission is high year-round, with two seasonal peaks in July and December, following the long and short rainy seasons. In 2015, malaria prevalence in children <5 years of age by smear microscopy was 39.0% [15]. In 2013, 99% of parasite isolates collected from pregnant women enrolled in a study in this area harboured the quintuple gene mutant of *pfdhfr/pfdhps*, which confers high-grade SP resistance [16]. In 2015, attendance to at least one ANC visit from a skilled provider during pregnancy was high (97.3%) [17], and pregnant women are routinely screened for malaria [18].

Following written informed consent, all pregnant women attending their first ANC visit at one of the nine study facilities between May and September, 2018 were consecutively enrolled, and a finger-prick blood sample of 200 μL was collected in BD Vacutainers® Plastic K2 ethylene diamine tetra-acetic acid (EDTA) tube (Franklin Lakes, NJ, USA).. The only exclusion criterion was inability to provide informed consent. Data on gravidity, trimester of pregnancy, axillary temperature, and history of fever in the last 48 hours were prospectively extracted from the Ministry of Health Routine ANC Register and double-entered into a database. Women were classified as febrile if they had a history of fever or an axillary temperature of ≥37.5°C at the clinic.

Ethical approval was obtained from the Kenya Medical Research Institute (KEMRI) and Liverpool School of Tropical Medicine. The institutional review boards of the U.S. Centers for Disease Control and Prevention (CDC) and PATH relied on KEMRI for approval.

### Sample processing and malaria infection detection

Blood sample aliquots were pipetted from the EDTA tubes for malaria testing by cRDT (First Response® Malaria Ag. [pLDH/HRP2] Combo RDT, Premier Medical Corporation Ltd., India), and usRDT (Alere™ Ultra-sensitive Malaria Ag. *P. falciparum* RDT, Waltham, MA, USA now commercially available as *NxTek™ Eliminate malaria pf*, Abbott Diagnostics) at the clinics' laboratory. The manufacturer's recommendations were strictly followed for all testing steps. Five μL of blood were added to the test sample well; two and four drops of buffer solution were added to the cRDT and usRDT buffer well, respectively, per the product insert. A timer was set to 20 minutes, when both RDTs were read. Only tests with a positive control line were considered valid. The same individual read both the cRDT and usRDT results and was not blinded to the result of the other test or the patient from whom the sample was drawn. Those testing positive by cRDT were treated according to national guidelines.

Blood samples were transported at room temperature to a central laboratory in Siaya County, Kenya within 8 hours of collection. All efforts were made to test samples by microscopy and LAMP on the day of collection, but when not possible, they were stored at room temperature for 7 days or at 2–8° C for 14 days before testing as recommended by the manufacturer (LAMP). Thick and thin blood smears were prepared at the laboratory in Siaya, using 9 μL of blood according to WHO research-grade microscopy standards [19]. All smears were independently examined by two microscopists who had passed an external quality assurance program provided by the National Institute of Communicable Diseases, South Africa and certified at the equivalent of WHO competence level 1 or 2 for the accuracy in detection of, species identification, and parasite counts [20]. Microscopists were blinded to each other's results. Parasite densities were calculated as the arithmetic mean of the two reads. A malaria smear was considered negative if no parasites were found in 200 high-power microscopic fields. A third microscopist, blinded to the results of prior examinations, confirmed discordant results (Supplemental Methods).

An aliquot of 50 μL whole blood was tested in the Siaya laboratory using the LAMP assay (Illumigene® Malaria, Meridian Bioscience, Cincinnati, OH, USA) (Supplemental Methods). A second aliquot of 50 μL was pipetted to a Whatman 903 filter paper and dried overnight at room temperature. Each dried filter paper was sealed in a plastic bag with desiccant and a moisture indicator, transported to the KEMRI laboratory in Kisumu, Kenya, and stored at −80 °C until shipment on dry ice to CDC, Atlanta, GA, USA for genus-specific photo-induced electron transfer (PET) PCR (PET-PCR), which was conducted between October-December 2019 (Supplemental Methods) [21]. Staff conducting LAMP and PET-PCR assays were blinded to the results of all other tests. The mean cycle threshold (Ct) values from serially diluted reference samples were used to prepare a standard curve to obtain parasite densities of the field isolates per reference [21].

PET-PCR was selected as the reference standard due to its high sensitivity (as sensitive as many quantitative polymerase chain reaction assays), specificity, and ease of use [21]. Readers of cRDT and usRDT results had access to individual-level clinical information, whereas readers of expert microscopy, LAMP and PET-PCR did not. This study was

conducted according to STARD Statement for Reporting studies of diagnostic accuracy (Supplemental Table 1).

### Sample size

The study was designed to test a non-inferiority hypothesis that the sensitivity of LAMP was within 10% of PCR and required 179 positive individuals (power=80%, alpha=0.05).

### Statistical analyses

Data from women with incomplete clinical, diagnostic, or invalid test results were excluded. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), accuracy (defined as percent concordant with referent test), and respective Clopper-Pearson confidence limits were calculated. The relative diagnostic sensitivity for detecting *P. falciparum* infection within subgroups (fever status, gravidity, and trimester of pregnancy) was calculated using univariable robust Poisson regression and expressed as a Sensitivity-Ratio (SR) [22]. Sensitivity-ratios were also calculated using generalized estimating equations accounting for multiple observations per participant to compare the sensitivity between tests by subgroup. Models of estimated diagnostic sensitivity by $\log_{10}$-transformed parasite density from samples with densities <500 parasites/μL (where most diagnostic performance variability occurred) were created using logistic regression models. Analyses were performed in SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA) and R version 4.0.1 (Comprehensive R Archive Network, Vienna, Austria).

## Results

Between May 28 and September 11, 2018, 489 women attending their first ANC visits were enrolled at nine clinics. Complete diagnostic and clinical data were available for 482 (98.6%) (Supplemental Figure 1). Among these, 25.5%, 25.9%, and 48.6% were primi-, secundi-, and multigravidae, and 26.4%, 57.1%, and 16.6% were in their first, second and third trimesters of pregnancy, respectively. Ninety (18.7%) had a recent history or documented fever (Table 1).

Overall, 172 (35.7%) women were positive for *P. falciparum* by PET-PCR. Most infections (135, 78.5%), were of low density (<200 parasites/μL), only 8 (4.7%) had densities >2000 parasites/μL. The geometric mean parasite density (GMPD) was 43 parasites/μL (95% CI 33–58) and higher among febrile than afebrile women (108 parasites/μL (60–194) vs 29 parasites/μL (21–38), respectively). The GMPD decreased with increasing gravidity but not by trimester (Table 1, Figure 1).

### Diagnostic accuracy

Of the 482 women, 69 (14.3%), 97 (20.1%), 107 (22.2%), and 173 (35.9%) were positive for malaria by expert microscopy, cRDT, usRDT, and LAMP respectively (Table 2, Figure 2). Relative to PET-PCR, expert microscopy was the least sensitive test (40.1%; 95% CI 32.7–47.9), followed by cRDT (49.4%; 41.7–57.1), usRDT (54.7%; 46.9–62.2), and LAMP (68.6%; 61.1–75.5). LAMP was the least specific (82.3%; 95% CI 77.5–86.4) and had the lowest PPV (68.2%; 60.7–75.1). The specificity and PPV of usRDT, cRDT, and microscopy

were each above 95% and 85%, respectively. The NPV and diagnostic accuracy were similar for all four tests (Table 2).

The modelled sensitivity of tests at densities between 200–500 parasites/μL was high and similar across the tests (Figure 3, Figure 2B, Table 3). At 50 parasites/μL, differences between modelled test sensitivities were pronounced; a parasite density value higher than the GMPD of the subgroup of afebrile pregnant women and those in their first and second trimesters. At 10 parasites/μL the modelled sensitivities were 7% (95% CI 5–14), 26% (18–36), 32% (24–43), and 55% (45–64), for microscopy, cRDT, usRDT, and LAMP, respectively.

## Diagnostic sensitivity by fever status, gravidity, and trimester of pregnancy

Diagnostic sensitivity is primarily associated with parasite density. Thus, test sensitivity by subgroup followed their respective GMPDs. cRDT, usRDT, and LAMP had similar, relatively high sensitivity among febrile women (GMPD=108; Sensitivities=79.6%, 79.6%, and 83.3%, respectively) and relatively low sensitivity among afebrile women (GMPD=29; Sensitivities=35.6%, 43.2% and 61.9%) (Table 2). Test sensitivity decreased by increasing gravidity. The modelled sensitivity at low densities corroborated these findings (Table 3, Figure 3). By contrast, the diagnostic sensitivity by trimester of pregnancy did not follow a consistent pattern, consistent with the lack of a clear pattern in the distribution of parasite densities by trimester (Figure 1).

The differences in modelled sensitivities between tests increased among afebrile women, primi- and secundigravidae, and those in the first trimester at densities below 100 parasites/μL (Table 3). LAMP was more sensitive than usRDT and cRDT across all gravidities and women in the first and second trimesters. usRDTs were slightly more sensitive than cRDTs in afebrile women, primigravidae, and first and second trimesters.

## Comparison of diagnostic test sensitivity among afebrile women in early pregnancy and by gravidity

When afebrile women were further stratified by trimester, only LAMP had a sensitivity greater than 50% in any trimester (Figure 4). Among afebrile women in their first (n=28) and second trimester (n=70), LAMP detected 71.4% (51.3–86.8) and 61.4% (49.0–72.8) of the infections, respectively. usRDT detected 46.4% (27.5–66.1) and 41.4% (29.8–53.8) of the infections in afebrile women in their first and second trimester, respectively. usRDT detected >60% more infections than cRDT (sensitivity ratio [SR] 1.63, 0.80–3.30) and microscopy (SR=1.62, 0.80–3.30) in the first trimester, and 16% (SR=1.16, 0.76–1.77) and >60% (SR=1.61, 0.99–2.66) more infections than cRDT and microscopy, respectively, in the second trimester. The sensitivity of each test among afebrile pregnant women in their third trimester (n=20) was low (Figure 4).

When afebrile women were stratified by gravidity, only LAMP and usRDT had a sensitivity >50% among primigravid and secundigravid women. Among afebrile primigravid women (n=32), LAMP detected 71.9% (53.3–86.3) and usRDT detected 59.4% (40.6–76.3) of all infections; usRDT detected >25% (SR=1.27, 0.79–2.02) more infections than cRDT and microscopy. The sensitivity of LAMP and usRDT among afebrile secundigravidae (n=37)

was similar to afebrile primigravidae, but the difference in sensitivity between usRDT and microscopy increased (SR=1.58, 0.90–2.77). Among afebrile multigravidae, the sensitivity of each test was below 50%.

When evaluating primigravid women in their first trimester of pregnancy, LAMP identified 83.3% (51.6–97.9), and usRDT identified 66.7% (34.9–90.1), while cRDT and microscopy identified just 50.0% (21.1–78.9 for both) of the malaria infections (Supplemental Figure 2). Among secundigravid women in their second trimester of pregnancy, LAMP identified 80.0% (63.1–91.6) of the infections while usRDT, cRDT, and microscopy identified 53.3% (26.6–78.7), 40.0% (16.3–67.7), and 40.0% (16.3–67.7), respectively. Sample sizes for this group were very small and results should be interpreted with caution as indicated by the wide confidence limits around sensitivity estimates. The sensitivity of each test was slightly lower, but the observations remained similar among primi- and secundigravid women in their second trimesters of pregnancy.

## Discussion

In this population of pregnant women attending their first ANC visit, the majority of whom were asymptomatic, the PET-PCR estimated GMPD was 44 parasites/μL, well below the generally accepted LOD of microscopy and cRDT. When using PET-PCR as the reference, the diagnostic sensitivity of microscopy (40.1%) and cRDT (49.4%) was low, and the sensitivity of usRDT, which is reported to detect parasites at densities ten times lower than cRDT, was 54.7% and only detected 11% more infections than cRDTs (sensitivity ratio 1.11). Our results are similar to a recent meta-analysis that found the sensitivity of usRDT and cRDT among pregnant women to be 52.5% and 44.9%, respectively [23].

Our models of test sensitivity at low parasite densities found that the differences between test performance became more pronounced at and below 50 parasites/μL. For example, the models predicted that among women with densities of 10 parasites/uL, usRDT would detect about 23% more infections than conventional RDTs, compared to 11% more infections at 50 parasites/μL and only 2.5% more at 200 parasites/μL. These models suggested that LAMP performed best at these lower densities and would detect twice as many infections as cRDTs at 10 parasites/μL and 1.5 times as many at 50 parasites/μL.

While the overall added value of usRDT over cRDT was marginal, analyses of subgroups with lower GMPD, corroborated the model findings, suggesting usRDTs may have more utility over cRDTs in these sub-populations. For example, among afebrile women (GMPD 29 parasites/μL), usRDTs detected about 21% more infections than cRDTs (43.2 vs 35.6%, SR 1.21) and LAMP 74% more. Our findings are consistent with four similar screening studies in afebrile pregnant women [24–27], and suggest that LAMP and usRDT are likely to detect more infections than cRDTs and microscopy when screening afebrile pregnant women attending their first ANC.

A recent model estimated that a diagnostic test with 75% sensitivity would substantially reduce placental infections and low birthweight when used as a screening test for malaria in the first trimester [9]. Only LAMP approached this threshold with a 68.6% sensitivity

overall, 75.0% in the first trimester, and 71.4% among afebrile women in their first trimester. By contrast, usRDT detected 54.7% overall, 52.5% in the first trimester and 46.4% among afebrile women in the first trimester.

Our study found that the sensitivity of usRDT does not vary significantly by pregnancy trimester among women attending their first ANC visit, consistent with findings from previous studies in Benin and Colombia [27, 28]. This reflected the lack of a clear relationship between parasite density and trimester of presentation in our study. However, we did find that among afebrile women in their first trimester (GMPD 34 parasites/μL), LAMP and usRDT detected 250% and 63% more infections than cRDTs, respectively. This latter subgroup may be predicted to benefit most from screen-and-treat strategies because they do not benefit from IPTp with SP, which is contraindicated in early pregnancy, and being afebrile, they would not otherwise be tested. Screening these women with sensitive diagnostic tests would allow the detection of patent infections that could be successfully treated with ACT, even during the first trimester of. This would contribute to better protecting these women and their fetus from any adverse effects of malaria infections in early pregnancy.

Among febrile pregnant women, we found that LAMP (83.3%), usRDT (79.6%), and cRDT (79.6%) performed similarly to one another, which is consistent with three previous studies comparing the sensitivity of LAMP (100%) [24], usRDT [25] (range 95.2–100%) or both [27] to cRDTs (range: 80.0–95.2%) or microscopy (range: 95.2–100%). In a fourth study, conducted in a high transmission setting in Benin, the sensitivity of usRDT and cRDT among febrile women was 66.7% and 50.0%, respectively, relative to quantitative PCR [26, 28]. In this latter study, the GMPD in this population was not presented, but may have been lower, as 85% of the women had received at least one dose of IPTp, which is known to suppress parasite densities [29]. Together, these findings suggest that cRDTs may be sufficient for screening pregnant women attending their first ANC visit who are febrile [27, 28].

The main limitation of this study was the small sample size in the modelled subgroup strata, which resulted in limited precision around the point estimates and the interpretability of the findings. An individual participant data meta-analysis pooling data from multiple studies may better quantify the sensitivity of these diagnostic tests among sub-groups and the benefit of such a strategy in different settings. Another limitation was the use of PET-PCR as a reference test. There was only a small difference in the LOD of LAMP (2 parasites/uL) and the LOD of PET-PCR (3.2 parasites/uL). LAMP identified some samples as test positive that were test negative by PET-PCR, resulting in the observed lower specificity and PPV of LAMP relative to the other tests. It is uncertain if these are true false positives or if this reflects the limitations of PET-PCR. Additionally, both PET-PCR and LAMP are genus-specific tests whereas usRDT is a *P. falciparum* specific test. While PET-PCR may have identified *Plasmodium spp*. infections other than *P. falciparum* that would have been considered false negatives by usRDT, thus decreasing the calculated sensitivity of usRDT, the proportion of *Plasmodium spp*. infections in this area that are not *P. falciparum* mono- or mixed-infections is 5%[30]. Thus, the expected difference in sensitivity would be minimal and biased towards the null. Finally, the same reader interpreted the cRDT and usRDT

results, and they were not blinded to participant presentation. This may have introduced bias, likely to the null.

In conclusion, LAMP was the most sensitive point-of-care diagnostic test and approached the 75% diagnostic sensitivity estimated to substantially reduce adverse pregnancy outcomes when used in screening and treatment strategies in the first trimester. However, most pregnant women in endemic countries seek ANC care in rural facilities. LAMP may not be a viable solution in these settings due to the training requirements, cost, and need for basic infrastructure, including electricity. However, usRDTs detected 1.21 fold more infections in afebrile women and 63% more in afebrile women in the first trimester; the sub-group most likely to benefit from screen-and-treat strategies at the first antenatal clinic visit. Although it may be tempting to conclude that in rural settings without basic infrastructure, usRDTs should be the preferred choice for screening pregnant women, a thorough assessment of their cost, storage and shelf-life will need to be conducted. Second-generation usRDTs are being developed, which may address some of the limitations of first-generation usRDTs, such as the storage temperature and shelf-life, and may have further increased sensitivity. Studies with the second generation of usRDTs are urgently needed when they become commercially available.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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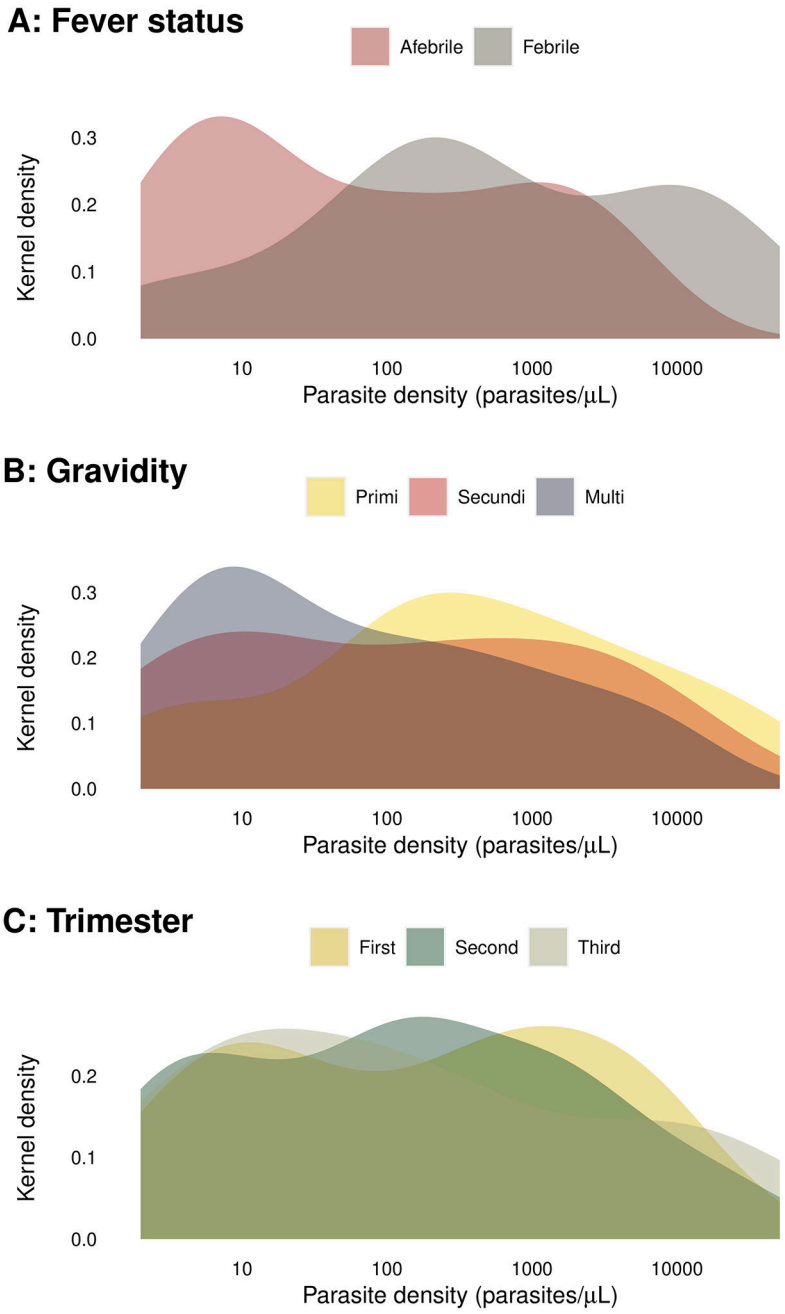


**Figure 1.**

Distribution of PET-PCR positive samples by parasite density stratified by fever status, gravidity and trimester of pregnancy

Samples are plotted as the kernel density by $\log_{10}$-transformed parasites/µL according to (A) fever status, (B) gravidity, and (C) trimester of pregnancy. Abbreviation: PET-PCR, photo-induced electron-transfer polymerase-chain-reaction.

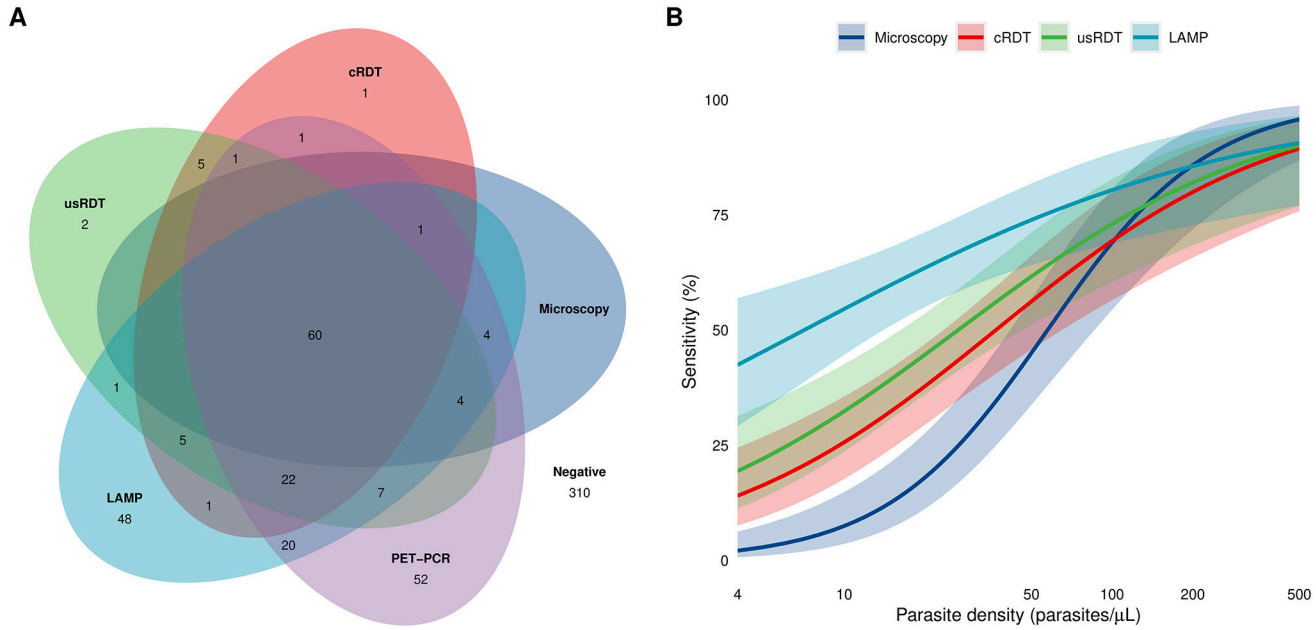


**Figure 2.**

Distribution of positive samples by diagnostic test and modelled sensitivity to PET-PCR at densities below 500 parasites/µL

(A) Venn diagram of *P. falciparum* positivity by PET-PCR, microscopy, RDT, us-RDT, and LAMP. PET-PCR was the reference test. (B) Logistic modelled probability of test sensitivity and 95% credible intervals (shaded area) by $\log_{10}$-transformed parasite density calculated by PET-PCR for each diagnostic. Only samples with calculated densities below 500 parasites/µL are considered in the model. Abbreviations: PET-PCR, photo-induced electron-transfer polymerase-chain-reaction; cRDT, conventional RDT; usRDT, ultra-sensitive RDT; LAMP, loop-mediated isothermal amplification.

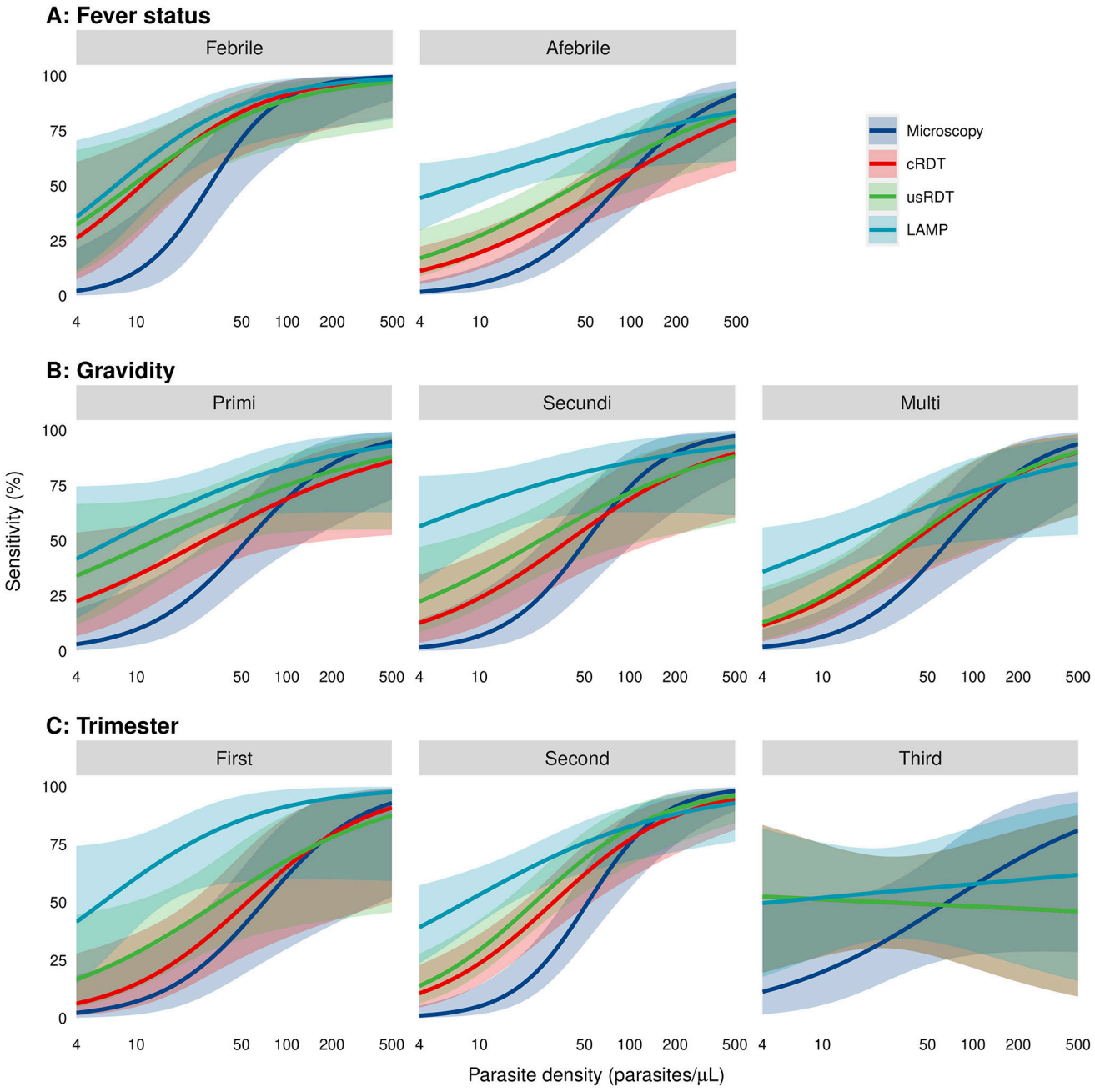


**Figure 3.**

Curves of modelled test sensitivity at low parasite density with PET-PCR as the reference Sensitivities of diagnostic tests at low density derived from logistic models using PET-PCR positive samples with parasite densities below 500 parasites/µL. The vertical axis represents the modelled sensitivity of the test. Models and sensitivity outputs are stratified by (A) fever status, (B) gravidity, and (C) trimester of pregnancy. Abbreviations: 95% CI, 95% confidence interval; parasites/µL, parasites per microliter; PET-PCR, photo-induced electron-transfer polymerase-chain-reaction; cRDT, conventional RDT; usRDT, ultra-sensitive RDT; LAMP, loop-mediated isothermal amplification

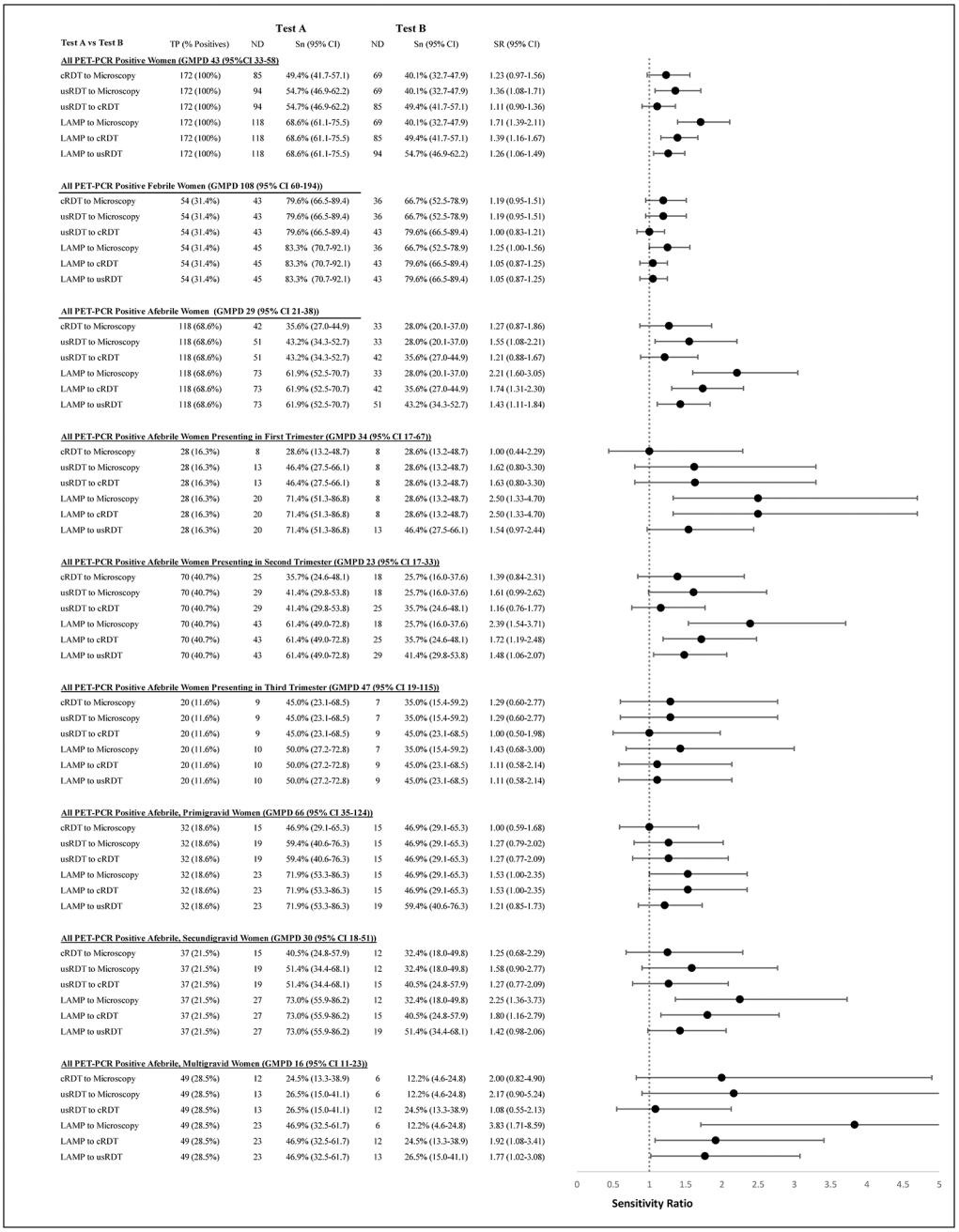


**Figure 4.**

Relative test diagnostic sensitivity to PET-PCR by febrile status and among afebrile women by trimester of pregnancy and gravidity

Sensitivities of tests were calculated using PET-PCR as the reference test. Sensitivity ratios were modelled using Poisson regression. RRs greater than 1 indicate that test A is more sensitive than test B for the given criteria. Calculations are stratified by all PET-PCR positives, all febrile women, all afebrile women, and afebrile women in the first, second, or third trimester of pregnancy, respectively.. Abbreviations: TP, true positives within sub-group by PET-PCR and percent of total positive population; ND, number

of true positives detected by the given test; Sn (95% CI), sensitivity (95% confidence interval); SR, sensitivity ratio; GMPD, geometric mean parasite density. PET-PCR, photo-induced electron-transfer polymerase-chain-reaction; cRDT, conventional RDT; usRDT, ultra-sensitive RDT; LAMP, loop-mediated isothermal amplification.

**Table 1.**

Study population characteristics

| Characteristic | All (N=482; 100%) | Primigravid (n=123; 25.5%) | Secundigravid (n=125; 25.9%) | Multigravid (n=234; 48.6%) |
|---|---|---|---|---|
| **Population characteristics** | | | | |
| Age (years; median (IQR)) | 23 (20–28) | 19 (18–21) | 22 (20–24) | 28 (24–32) |
| Mean gestational age (weeks; mean (SD)) | 19 (7.6) | 19 (7.8) | 18 (7.7) | 20 (7.4) |
| **Trimester (n=476) (n (%))** | | | | |
| First | 127 (26.4) | 33 (26.8) | 41 (32.8) | 53 (22.7) |
| Second | 275 (57.1) | 72 (58.5) | 64 (51.2) | 139 (59.4) |
| Third | 80 (16.6) | 18 (14.6) | 20 (16.0) | 42 (18.0) |
| **Fever (n (%))** | 90 (18.7) | 36 (29.3) | 20 (16.0) | 34 (14.5) |
| **Diagnostic characteristics of PET-PCR positive women (n=172)** | | **Parasite density (parasites/μL)** | | |
| | **GMPD (95%CI) 43 (33–58)** | **<200 (n=135; 78.5%)** | **200 to <2000 (n=29; 16.9%)** | **2000 to <20,000 (n=8; 4.7%)** |
| **Febrile status** | | | | |
| Febrile | 108 (60–194) | 35 (25.9) | 12 (41.4) | 7 (87.5) |
| Afebrile | 29 (21–38) | 100 (74.1) | 17 (58.6) | 1 (12.5) |
| **Gravidity** | | | | |
| Primigravid | 82 (49–138) | 39 (28.9) | 14 (48.3) | 4 (50.0) |
| Secundigravid | 44 (25–77) | 38 (28.2) | 7 (24.1) | 3 (37.5) |
| Multigravid | 25 (17–37) | 58 (43.0) | 8 (27.6) | 1 (12.5) |
| **Trimester** | | | | |
| First | 55 (29–103) | 28 (20.7) | 11 (37.9) | 1 (12.5) |
| Second | 36 (26–51) | 85 (63.0) | 16 (55.2) | 4 (50.0) |
| Third | 62 (26–146) | 22 (16.3) | 2 (6.9) | 3 (37.5) |

Demographic and presenting characteristics of all women who presented to study facilities between May 28 and September 11, 2018 for their first antenatal care visits. Abbreviations: IQR, interquartile range; SD, standard deviation; GMPD, geometric mean parasite density; 95% CI, 95% confidence interval

**Table 2.**

Diagnostic test performance overall and by fever status, gravidity, and gestational age

| Diagnostic | Number positive (%) | TP | FP | FN | Sensitivity (95%CI) | Specificity (95%CI) | PPV (95%CI) | NPV (95%CI) | Accuracy (95%CI) | SR (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Overall (N=482)** | | | | | | | | | | |
| PET–PCR | 172 (35.7) | | | | | | Reference | | | |
| Microscopy | 69 (14.3) | 69 | 0 | 103 | 40.1% (32.7–47.9) | 100% (98.8–100) | 100% (94.8–100) | 75.1% (70.6–79.2) | 78.6% (74.7–82.2) | |
| cRDT | 97 (20.1) | 85 | 12 | 87 | 49.4% (41.7–57.1) | 96.1% (93.3–98.0) | 87.6% (79.4–93.4) | 77.4% (72.9–81.5) | 79.5% (75.6–83.0) | |
| usRDT | 107 (22.2) | 94 | 13 | 78 | 54.7% (46.9–62.2) | 95.8% (92.9–97.8) | 87.9% (80.1–93.4) | 79.2% (74.7–83.2) | 81.1% (77.3–84.5) | |
| LAMP | 173 (35.9) | 118 | 55 | 54 | 68.6% (61.1–75.5) | 82.3% (77.5–86.4) | 68.2% (60.7–75.1) | 82.5% (77.8–86.6) | 77.4% (73.4–81.1) | |
| **Fever status** | | | | | | | | | | |
| **Febrile (n=90; PET-PCR+=54)** | | | | | | | | | | |
| Microscopy | 36 (40.0) | 36 | 0 | 18 | 66.7% (52.5–78.9) | 100% (90.3–100) | 100% (90.3–100) | 66.7% (52.5–78.9) | 80.0% (70.3–87.7) | Reference |
| cRDT | 47 (52.2) | 43 | 4 | 11 | 79.6% (66.5–89.4) | 88.9% (73.9–96.9) | 91.5% (79.6–97.6) | 74.4% (58.8–86.5) | 83.3% (74.0–90.4) | Reference |
| usRDT | 47 (52.2) | 43 | 4 | 11 | 79.6% (66.5–89.4) | 88.9% (73.9–96.9) | 91.5% (79.6–97.6) | 74.4% (58.8–86.5) | 83.3% (74.0–90.4) | Reference |
| LAMP | 49 (54.4) | 45 | 4 | 9 | 83.3% (70.7–92.1) | 88.9% (73.9–96.9) | 91.8% (80.4–97.7) | 78.1% (62.4–89.4) | 85.6% (76.6–92.1) | Reference |
| **Afebrile (n=392; PET-PCR+ =118)** | | | | | | | | | | |
| Microscopy | 33 (8.4) | 33 | 0 | 85 | 28.0% (20.1–37.0) | 100% (98.7–100) | 100% (89.4–100) | 76.3% (71.6–80.6) | 78.3% (73.9–82.3) | 0.42 (0.30–0.59) |
| cRDT | 50 (12.8) | 42 | 8 | 76 | 35.6% (27.0–44.9) | 97.1% (94.3–98.7) | 84.0% (70.9–92.8) | 77.8% (73.0–82.1) | 78.6% (74.2–82.5) | 0.45 (0.34–0.59) |
| usRDT | 60 (15.3) | 51 | 9 | 67 | 43.2% (34.3–52.7) | 96.7% (93.9–98.5) | 85.0% (73.4–92.9) | 79.8% (75.1–84.0) | 80.6% (76.4–84.4) | 0.54 (0.42–0.69) |
| LAMP | 124 (31.6) | 73 | 51 | 45 | 61.9% (52.5–70.7) | 81.4% (76.3–85.8) | 58.9% (49.7–67.6) | 83.2% (78.2–87.5) | 75.5% (70.9–79.7) | 0.74 (0.62–0.89) |
| **Gravidity** | | | | | | | | | | |
| **Primigravid (n= 123; PET-PCR+=57)** | | | | | | | | | | |
| Microscopy | 30 (24.4) | 30 | 0 | 27 | 52.6% (39.0–66.0) | 100% (94.6–100) | 100% (88.4–100) | 71.0% (60.6–79.9) | 78.1% (69.7–85.0) | Reference |
| cRDT | 42 (34.2) | 35 | 7 | 22 | 61.4% (47.6–74.0) | 89.4% (79.4–95.6) | 83.3% (68.6–93.0) | 75.4% (63.5–85.0) | 76.4% (67.9–83.6) | Reference |
| usRDT | 49 (39.8) | 40 | 9 | 17 | 70.2% (56.6–81.6) | 86.4% (75.7–93.6) | 81.6% (68.0–91.2) | 77.0% (65.8–86.0) | 78.9% (70.6–85.7) | Reference |
| LAMP | 58 (47.2) | 44 | 14 | 13 | 77.2% (64.1–87.3) | 78.8% (67.0–87.9) | 75.9% (62.8–86.1) | 82.4% (69.1–91.6) | 78.1% (69.7–85.0) | Reference |
| **Secundigravid (n=125; PET-PCR+=48)** | | | | | | | | | | |
| Microscopy | 20 (16.0) | 20 | 0 | 28 | 41.7% (27.6–56.8) | 100% (95.3–100) | 100% (83.2–100) | 73.3% (63.8–81.5) | 77.6% (69.3–84.6) | 0.79 (0.52–1.20) |
| cRDT | 25 (20.0) | 23 | 2 | 25 | 47.9% (33.3–62.8) | 97.4% (90.9–99.7) | 92.0% (74.0–99.0) | 75.0% (64.6–83.6) | 78.4% (70.2–85.3) | 0.78 (0.54–1.12) |
| usRDT | 29 (23.2) | 26 | 3 | 22 | 54.2% (39.2–68.6) | 96.1% (89.0–99.2) | 89.7% (72.7–97.8) | 77.1% (67.4–85.1) | 80.0% (71.9–87.0) | 0.77 (0.57–1.05) |
| LAMP | 48 (38.4) | 36 | 12 | 12 | 75.0% (60.4–86.4) | 84.4% (74.4–91.7) | 75.0% (60.4–86.4) | 85.1% (74.3–92.6) | 80.8% (72.8–87.3) | 0.97 (0.78–1.21) |
| **Multigravid (n=234; PET-PCR+=67)** | | | | | | | | | | |

*J Infect Dis*. Author manuscript; available in PMC 2023 September 04.

| Diagnostic | Number positive (%) | TP | FP | FN | Sensitivity (95%CI) | Specificity (95%CI) | PPV (95%CI) | NPV (95%CI) | Accuracy (95%CI) | SR (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| Microscopy | 19 (8.1) | 19 | 0 | 48 | 28.4% (18.0–40.7) | 100% (97.8–100) | 100% (82.4–100) | 77.7% (71.5–83.1) | 79.5% (73.7–84.5) | 0.54 (0.34–0.85) |
| cRDT | 30 (12.8) | 27 | 3 | 40 | 40.3% (28.5–53.0) | 98.2% (94.8–99.6) | 90.0% (73.5–97.9) | 80.0% (73.5–85.5) | 81.6% (76.1–86.4) | 0.66 (0.46–0.94) |
| usRDT | 29 (12.4) | 28 | 1 | 39 | 41.8% (29.9–54.5) | 99.4% (96.7–100) | 96.6% (82.2–99.9) | 81.0% (74.9–86.1) | 82.9% (77.5–87.5) | 0.60 (0.43–0.83) |
| LAMP | 67 (28.6) | 38 | 29 | 29 | 56.7% (44.0–68.8) | 82.6% (76.0–88.1) | 56.7% (44.0–68.8) | 82.7% (75.6–88.4) | 75.2% (69.2–80.6) | 0.73 (0.57–0.95) |
| **Gestational Age** | | | | | | | | | | |
| **First Trimester (n=127; PET-PCR+=40)** | | | | | | | | | | |
| Microscopy | 16 (12.6) | 16 | 0 | 24 | 40.0% (24.9–56.7) | 100% (95.9–100) | 100% (79.4–100) | 78.4% (69.6–85.6) | 81.1% (73.2–87.5) | 0.83 (0.48–1.43) |
| cRDT | 18 (14.2) | 17 | 1 | 23 | 42.5% (27.0–59.1) | 98.9% (93.8–100) | 94.4% (72.7–99.9) | 79.6% (70.3–87.1) | 81.1% (73.2–87.5) | 0.72 (0.45–1.16) |
| usRDT | 23 (18.1) | 21 | 2 | 19 | 52.5% (36.1–68.5) | 97.7% (91.9–99.7) | 91.3% (72.0–98.9) | 81.7% (73.0–88.6) | 83.5% (75.8–89.5) | 0.89 (0.58–1.36) |
| LAMP | 46 (36.2) | 30 | 16 | 10 | 75.0% (58.8–87.3) | 81.6% (71.9–89.1) | 65.2% (49.8–78.7) | 88.9% (79.3–95.1) | 79.5% (71.5–86.2) | 1.19 (0.85–1.67) |
| **Second Trimester (n=275; PET-PCR+=105)** | | | | | | | | | | |
| Microscopy | 40 (14.6) | 40 | 0 | 65 | 38.1% (28.8–48.1) | 100% (97.9–100) | 100% (91.2–100) | 72.3% (66.2–78.0) | 76.4% (70.9–81.3) | 0.79 (0.50–1.25) |
| cRDT | 60 (21.8) | 52 | 8 | 53 | 49.5% (39.6–59.5) | 95.3% (90.1–98.0) | 86.7% (75.4–94.1) | 75.9% (69.2–81.9) | 77.8% (72.4–82.6) | 0.84 (0.58–1.21) |
| usRDT | 65 (23.6) | 57 | 8 | 48 | 54.3% (44.3–64.0) | 95.3% (90.9–98.0) | 87.7% (77.2–94.5) | 77.1% (70.9–82.6) | 79.6% (74.4–84.2) | 0.92 (0.64–1.31) |
| LAMP | 103 (37.5) | 71 | 32 | 34 | 67.6% (57.8–76.4) | 81.2% (74.5–86.8) | 68.9% (59.1–77.7) | 81.5% (74.3–87.4) | 76.0% (70.5–80.9) | 1.07 (0.78–1.48) |
| **Third Trimester (n=80; PET-PCR+=27)** | | | | | | | | | | |
| Microscopy | 13 (16.3) | 13 | 0 | 14 | 48.2% (28.7–68.1) | 100% (93.3–100) | 100% (75.3–100) | 79.1% (67.4–88.1) | 82.5% (72.4–90.1) | Reference |
| cRDT | 19 (23.8) | 16 | 3 | 11 | 59.3% (38.8–77.6) | 94.3% (84.3–98.8) | 84.2% (60.4–96.6) | 80.7% (68.1–90.0) | 82.5% (72.4–90.1) | Reference |
| usRDT | 19 (23.8) | 16 | 3 | 11 | 59.3% (38.8–77.6) | 94.3% (84.3–98.8) | 84.2% (60.4–96.6) | 82.0% (70.0–90.6) | 82.5% (72.4–90.1) | Reference |
| LAMP | 24 (30.0) | 17 | 7 | 10 | 63.0% (42.4–80.6) | 86.8% (74.7–94.5) | 70.8% (48.9–87.4) | 80.0% (66.3–90.0) | 78.8% (68.2–87.1) | Reference |

Diagnost performance of each test is presented overall and by sub-group of fever status, gravidity, and gestational age. Percent positive for each test was calculated using sub-group denominator (n) for each subset category in the Diagnostic column. Clopper-Pearson 95% confidence intervals were calculated for test diagnostic performance results. Accuracy for a given test is defined as the percentage of results concordant with PET-PCR. The risk ratio (RR) represents the sensitivity of a test to detect *P. falciparum* infection in a sub-group compared to the sensitivity of the same test to the reference sub-group. Abbreviations: TP, true positive by PET-PCR; FP, false positive; FN, false negative; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value; SR, sensitivity ratio; PET-PCR, photo-induced electron-transfer polymerase-chain-reaction; cRDT, conventional RDT; usRDT, ultra-sensitive RDT; LAMP, loop-mediated isothermal amplification.

**Table 3.**

Modelled sensitivity of diagnostic tests

| Diagnostic Test | Sensitivity (95% CI) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 p/μL | 50 p/μL | 100 p/μL | 200 p/μL | 10 p/μL | 50 p/μL | 100 p/μL | 200 p/μL | 10 p/μL | 50 p/μL | 100 p/μL | 200 p/μL |
| **A. Overall modelled sensitivities at low density** | | | | | **B. Modelled sensitivity by fever status** | | | | | | | |
| | Overall | | | | **Febrile** | | | | **Afebrile** | | | |
| Microscopy | 7% (4–15) | 45% (34–57) | 69% (54–81) | 86% (72–93) | 11% (2–38) | 71% (48–87) | 90% (67–98) | 97% (79–100) | 6% (2–14) | 34% (22–47) | 56% (38–72) | 76% (55–89) |
| cRDT | 26% (18–36) | 56% (46–66) | 69% (56–80) | 80% (66–89) | 48% (26–71) | 84% (64–94) | 91% (71–98) | 96% (76–99) | 20% (12–30) | 44% (32–56) | 56% (40–71) | 68% (48–83) |
| usRDT | 32% (24–43) | 62% (51–71) | 73% (60–83) | 82% (68–91) | 51% (29–73) | 81% (62–92) | 89% (68–97) | 94% (72–99) | 27% (19–39) | 52% (40–64) | 63% (48–77) | 73% (54–87) |
| LAMP | 55% (45–64) | 74% (64–82) | 80% (69–88) | 86% (73–93) | 58% (35–78) | 87% (68–96) | 93% (73–99) | 96% (77–100) | 53% (42–64) | 68% (56–78) | 73% (58–85) | 78% (60–90) |
| **C. Modelled sensitivity by gravidity** | | | | | | | | | | | | |
| | **Primigravid** | | | | **Secundigravid** | | | | **Multigravid** | | | |
| Microscopy | 10% (3–29) | 47% (30–65) | 69% (45–86) | 85% (56–96) | 7% (2–27) | 49% (27–71) | 74% (47–90) | 90% (63–98) | 7% (2–19) | 39% (22–59) | 62% (37–83) | 81% (51–95) |
| cRDT | 34% (17–57) | 59% (42–74) | 69% (47–84) | 77% (50–92) | 24% (11–44) | 55% (36–73) | 69% (45–86) | 80% (52–93) | 23% (13–37) | 55% (36–72) | 69% (45–86) | 80% (53–94) |
| usRDT | 46% (26–68) | 67% (51–81) | 75% (54–89) | 81% (55–94) | 35% (20–54) | 61% (42–77) | 72% (48–87) | 80% (53–94) | 25% (14–39) | 56% (38–73) | 70% (46–86) | 81% (53–94) |
| LAMP | 56% (33–76) | 77% (60–88) | 83% (62–94) | 88% (63–97) | 67% (48–81) | 81% (62–92) | 86% (63–95) | 89% (63–98) | 47% (33–60) | 65% (48–80) | 72% (50–87) | 79% (51–93) |
| **D. Modelled sensitivity by trimester of pregnancy** | | | | | | | | | | | | |
| | **First** | | | | **Second** | | | | **Third** | | | |
| Microscopy | 7% (2–27) | 39% (18–64) | 61% (30–85) | 80% (40–96) | 5% (2–14) | 48% (32–64) | 76% (56–88) | 91% (75–97) | 20% (6–51) | 45% (23–68) | 57% (27–82) | 69% (29–92) |
| cRDT | 15% (5–37) | 48% (26–70) | 65% (35–87) | 79% (42–95) | 24% (14–36) | 62% (48–75) | 77% (60–88) | 87% (71–95) | 51% (27–75) | 49% (28–71) | 48% (22–76) | 47% (16–81) |
| usRDT | 28% (13–51) | 56% (34–76) | 68% (39–88) | 78% (42–95) | 29% (19–43) | 69% (54–80) | 82% (66–91) | 90% (75–97) | 51% (27–75) | 49% (28–71) | 48% (22–76) | 47% (16–81) |
| LAMP | 61% (39–79) | 86% (59–96) | 91% (60–99) | 95% (60–100) | 53% (41–65) | 76% (63–85) | 83% (68–92) | 88% (72–96) | 52% (27–76) | 56% (33–77) | 58% (29–82) | 60% (23–88) |

Diagnostic test sensitivity at low density derived from logistic models incorporating PET-PCR samples with parasite densities below 500 parasites/μL. (A) Overall modelled sensitivity of diagnostic tests at low density, (B) modelled sensitivity by fever status, (C) by gravidity, and (D) by trimester of pregnancy. Abbreviations: 95% CI, 95% confidence interval; p/μL, parasites per microliter; cRDT, conventional RDT; usRDT, ultra-sensitive RDT; LAMP, loop-mediated isothermal amplification.