1 **Full Title: Diagnostic performance of loop-mediated isothermal amplification and ultra-sensitive**
2 **rapid diagnostic tests for malaria screening among pregnant women in Kenya**

3 **Short Title:** Malaria diagnostics at first ANC visit

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

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 (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.

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

67 require significant laboratory capacity and resources not readily available in many malaria-endemic
68 settings [11].

69 Two diagnostic tests with reported high sensitivity that can be used at POC in resource-limited settings
70 include loop-mediated isothermal amplification (LAMP), a molecular test with similar sensitivity to PCR
71 [12], and ultra-sensitive malaria RDT (usRDT). usRDTs are reported to be up to ten times more sensitive
72 than cRDTs.[13] We compared the diagnostic performance of usRDT and LAMP against cRDTs and
73 microscopy among pregnant women attending their first ANC visit in a highly endemic setting for
74 malaria.

75 Methods

76 Study design and participants

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

84 Following written informed consent, all pregnant women attending their first ANC visit at one of the nine
85 study facilities between May and September, 2018 were consecutively enrolled, and a finger-prick blood
86 sample of 200 µL was collected in BD Vacutainers® Plastic K2 ethylene diamine tetra-acetic acid
87 (EDTA) tube (Franklin Lakes, NJ, USA). . The only exclusion criterion was inability to provide informed
88 consent. Data on gravidity, trimester of pregnancy, axillary temperature, and history of fever in the last 48
89 hours were prospectively extracted from the Ministry of Health Routine ANC Register and double-

90 entered into a database. Women were classified as febrile if they had a history of fever or an axillary
91 temperature of ≥37.5°C at the clinic.

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

95 **Sample processing and malaria infection detection**

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

106 Blood samples were transported at room temperature to a central laboratory in Siaya County, Kenya
107 within 8 hours of collection. All efforts were made to test samples by microscopy and LAMP on the day
108 of collection, but when not possible, they were stored at room temperature for 7 days or at 2-8° C for 14
109 days before testing as recommended by the manufacturer (LAMP). Thick and thin blood smears were
110 prepared at the laboratory in Siaya, using 9 µL of blood according to WHO research-grade microscopy
111 standards [19]. All smears were independently examined by two microscopists who had passed an
112 external quality assurance program provided by the National Institute of Communicable Diseases, South
113 Africa and certified at the equivalent of WHO competence level 1 or 2 for the accuracy in detection of ,
114 species identification, and parasite counts [20]. Microscopists were blinded to each other's results.

115 Parasite densities were calculated as the arithmetic mean of the two reads. A malaria smear was
116 considered negative if no parasites were found in 200 high-power microscopic fields. A third
117 microscopist, blinded to the results of prior examinations, confirmed discordant results (Supplemental
118 Methods).

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

129 PET-PCR was selected as the reference standard due to its high sensitivity (as sensitive as many
130 quantitative polymerase chain reaction assays), specificity, and ease of use [21]. Readers of cRDT and
131 usRDT results had access to individual-level clinical information, whereas readers of expert microscopy,
132 LAMP and PET-PCR did not. This study was conducted according to STARD Statement for Reporting
133 studies of diagnostic accuracy (Supplemental Table 1).

134 **Sample size**

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

137 **Statistical analyses**

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

150 Results

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

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

161 **Diagnostic accuracy**

162 Of the 482 women, 69 (14.3%), 97 (20.1%), 107 (22.2%), and 173 (35.9%) were positive for malaria by
163 expert microscopy, cRDT, usRDT, and LAMP respectively (Table 2, Figure 2). Relative to PET-PCR,
164 expert microscopy was the least sensitive test (40.1%; 95% CI 32.7-47.9), followed by cRDT (49.4%;
165 41.7-57.1), usRDT (54.7%; 46.9-62.2), and LAMP (68.6%; 61.1-75.5). LAMP was the least specific
166 (82.3%; 95% CI 77.5-86.4) and had the lowest PPV (68.2%; 60.7-75.1). The specificity and PPV of
167 usRDT, cRDT, and microscopy were each above 95% and 85%, respectively. The NPV and diagnostic
168 accuracy were similar for all four tests (Table 2).

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

175 **Diagnostic sensitivity by fever status, gravidity, and trimester of pregnancy**

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

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

187 usRDTs were slightly more sensitive than cRDTs in afebrile women, primigravidae, and first and second
188 trimesters.

189 **Comparison of diagnostic test sensitivity among afebrile women in early pregnancy and by**
190 **gravidity**

191 When afebrile women were further stratified by trimester, only LAMP had a sensitivity greater than 50%
192 in any trimester (Figure 4). Among afebrile women in their first (n=28) and second trimester (n=70),
193 LAMP detected 71.4% (51.3-86.8) and 61.4% (49.0-72.8) of the infections, respectively. usRDT detected
194 46.4% (27.5-66.1) and 41.4% (29.8-53.8) of the infections in afebrile women in their first and second
195 trimester, respectively. usRDT detected >60% more infections than cRDT (sensitivity ratio [SR] 1.63,
196 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
197 >60% (SR=1.61, 0.99-2.66) more infections than cRDT and microscopy, respectively, in the second
198 trimester. The sensitivity of each test among afebrile pregnant women in their third trimester (n=20) was
199 low (Figure 4).

200 When afebrile women were stratified by gravidity, only LAMP and usRDT had a sensitivity >50% among
201 primigravid and secundigravid women. Among afebrile primigravid women (n=32), LAMP detected
202 71.9% (53.3-86.3) and usRDT detected 59.4% (40.6-76.3) of all infections; usRDT detected >25%
203 (SR=1.27, 0.79-2.02) more infections than cRDT and microscopy. The sensitivity of LAMP and usRDT
204 among afebrile secundigravidae (n=37) was similar to afebrile primigravidae, but the difference in
205 sensitivity between usRDT and microscopy increased (SR=1.58, 0.90-2.77). Among afebrile
206 multigravidae, the sensitivity of each test was below 50%.

207 When evaluating primigravid women in their first trimester of pregnancy, LAMP identified 83.3% (51.6-
208 97.9), and usRDT identified 66.7% (34.9-90.1), while cRDT and microscopy identified just 50.0% (21.1-
209 78.9 for both) of the malaria infections (Supplemental Figure 2). Among secundigravid women in their
210 second trimester of pregnancy, LAMP identified 80.0% (63.1-91.6) of the infections while usRDT,
211 cRDT, and microscopy identified 53.3% (26.6-78.7), 40.0% (16.3-67.7), and 40.0% (16.3-67.7),

212 respectively. Sample sizes for this group were very small and results should be interpreted with caution as
213 indicated by the wide confidence limits around sensitivity estimates. The sensitivity of each test was
214 slightly lower, but the observations remained similar among primi- and secundigravid women in their
215 second trimesters of pregnancy.

216 Discussion

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

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

230 While the overall added value of usRDT over cRDT was marginal, analyses of subgroups with lower
231 GMPD, corroborated the model findings, suggesting usRDTs may have more utility over cRDTs in these
232 sub-populations. For example, among afebrile women (GMPD 29 parasites/µL), usRDTs detected about
233 21% more infections than cRDTs (43.2 vs 35.6%, SR 1.21) and LAMP 74% more. Our findings are
234 consistent with four similar screening studies in afebrile pregnant women [24-27], and suggest that

235 LAMP and usRDT are likely to detect more infections than cRDTs and microscopy when screening
236 afebrile pregnant women attending their first ANC.

237 A recent model estimated that a diagnostic test with 75% sensitivity would substantially reduce placental
238 infections and low birthweight when used as a screening test for malaria in the first trimester [9]. Only
239 LAMP approached this threshold with a 68.6% sensitivity overall, 75.0% in the first trimester, and 71.4%
240 among afebrile women in their first trimester. By contrast, usRDT detected 54.7% overall, 52.5% in the
241 first trimester and 46.4% among afebrile women in the first trimester.

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

253 Among febrile pregnant women, we found that LAMP (83.3%), usRDT (79.6%), and cRDT (79.6%)
254 performed similarly to one another, which is consistent with three previous studies comparing the
255 sensitivity of LAMP (100%) [24], usRDT [25] (range 95.2-100%) or both [27] to cRDTs (range: 80.0-
256 95.2%) or microscopy (range: 95.2-100%). In a fourth study, conducted in a high transmission setting in
257 Benin, the sensitivity of usRDT and cRDT among febrile women was 66.7% and 50.0%, respectively,
258 relative to quantitative PCR [26, 28]. In this latter study, the GMPD in this population was not presented,
259 but may have been lower, as 85% of the women had received at least one dose of IPTp, which is known

260 to suppress parasite densities [29]. Together, these findings suggest that cRDTs may be sufficient for
261 screening pregnant women attending their first ANC visit who are febrile [27, 28].

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

278 In conclusion, LAMP was the most sensitive point-of-care diagnostic test and approached the 75%
279 diagnostic sensitivity estimated to substantially reduce adverse pregnancy outcomes when used in
280 screening and treatment strategies in the first trimester. However, most pregnant women in endemic
281 countries seek ANC care in rural facilities. LAMP may not be a viable solution in these settings due to the
282 training requirements, cost, and need for basic infrastructure, including electricity. However, usRDTs
283 detected 1.21 fold more infections in afebrile women and 63% more in afebrile women in the first
284 trimester; the sub-group most likely to benefit from screen-and-treat strategies at the first antenatal clinic

285 visit. Although it may be tempting to conclude that in rural settings without basic infrastructure, usRDTs
286 should be the preferred choice for screening pregnant women, a thorough assessment of their cost, storage
287 and shelf-life will need to be conducted. Second-generation usRDTs are being developed, which may
288 address some of the limitations of first-generation usRDTs, such as the storage temperature and shelf-life,
289 and may have further increased sensitivity. Studies with the second generation of usRDTs are urgently
290 needed when they become commercially available.

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Footnote Page:

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

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343 # References

344 1. Rogerson SJ, Desai M, Mayor A, Sicuri E, Taylor SM, van Eijk AM. Burden, pathology, and costs of
345 malaria in pregnancy: new developments for an old problem. Lancet Infect Dis **2018**; 18:e107-e18.
346 2. Desai M, ter Kuile FO, Nosten F, et al. Epidemiology and burden of malaria in pregnancy. Lancet Infect
347 Dis **2007**; 7:93-104.
348 3. World Health Organization. A Strategic Framework for Malaria in Prevention and Control During
349 Pregnancy in the African Region, **2004. AFR/MAL/04/01.**
350 4. Desai M, Hill J, Fernandes S, et al. Prevention of malaria in pregnancy. Lancet Infect Dis **2018**; 18:e119-
351 e32.
352 5. Desai M, Gutman J, Taylor SM, et al. Impact of Sulfadoxine-Pyrimethamine Resistance on
353 Effectiveness of Intermittent Preventive Therapy for Malaria in Pregnancy at Clearing Infections and
354 Preventing Low Birth Weight. Clin Infect Dis **2016**; 62:323-33.
355 6. Elphinstone RE, Weckman AM, McDonald CR, et al. Early malaria infection, dysregulation of
356 angiogenesis, metabolism and inflammation across pregnancy, and risk of preterm birth in Malawi: A
357 cohort study. PLoS Med **2019**; 16:e1002914.
358 7. Moeller SL, Nyengaard JR, Larsen LG, et al. Malaria in Early Pregnancy and the Development of the
359 Placental Vasculature. J Infect Dis **2019**; 220:1425-34.
360 8. Kitojo C, Chacky F, Kigadye ES, et al. Evaluation of a single screen and treat strategy to detect
361 asymptomatic malaria among pregnant women from selected health facilities in Lindi region, Tanzania.
362 Malar J **2020**; 19:438.
363 9. Walker PGT, Cairns M, Slater H, et al. Modelling the incremental benefit of introducing malaria
364 screening strategies to antenatal care in Africa. Nat Commun **2020**; 11:3799.
365 10. Dellicour S, Sevene E, McGready R, et al. First-trimester artemisinin derivatives and quinine
366 treatments and the risk of adverse pregnancy outcomes in Africa and Asia: A meta-analysis of
367 observational studies. PLoS Med **2017**; 14:e1002290.
368 11. Lucchi NW, Gaye M, Diallo MA, et al. Evaluation of the Illumigene Malaria LAMP: A Robust Molecular
369 Diagnostic Tool for Malaria Parasites. Sci Rep **2016**; 6:36808.
370 12. Lucchi NW, Ndiaye D, Britton S, Udhayakumar V. Expanding the malaria molecular diagnostic
371 options: opportunities and challenges for loop-mediated isothermal amplification tests for malaria
372 control and elimination. Expert Rev Mol Diagn **2018**; 18:195-203.
373 13. Das S, Jang IK, Barney B, et al. Performance of a High-Sensitivity Rapid Diagnostic Test for
374 Plasmodium falciparum Malaria in Asymptomatic Individuals from Uganda and Myanmar and Naive
375 Human Challenge Infections. Am J Trop Med Hyg **2017**; 97:1540-50.
376 14. Odero NA, Samuels AM, Odongo W, et al. Community-based intermittent mass testing and
377 treatment for malaria in an area of high transmission intensity, western Kenya: development of study
378 site infrastructure and lessons learned. Malar J **2019**; 18:255.
379 15. Samuels AM, Odero NA, Odongo W, et al. Impact of Community-Based Mass Testing and Treatment
380 on Malaria Infection Prevalence in a High-Transmission Area of Western Kenya: A Cluster Randomized
381 Controlled Trial. Clin Infect Dis **2021**; 72:1927-35.
382 16. Huijben S, Macete E, Mombo-Ngoma G, et al. Counter-Selection of Antimalarial Resistance
383 Polymorphisms by Intermittent Preventive Treatment in Pregnancy. J Infect Dis **2020**; 221:293-303.
384 17. National Malaria Control Programme (NMCP), Kenya National Bureau of Statistics (KNBS), and ICF
385 International. Kenya Malaria Indicator Survey 2015. Nairobi, Kenya, and Rockville, Maryland, USA:
386 NMCP, KNBS, and ICF International, **2016**.

387 18. Young N, Achieng F, Desai M, et al. Integrated point-of-care testing (POCT) for HIV, syphilis, malaria
388 and anaemia at antenatal facilities in western Kenya: a qualitative study exploring end-users'
389 perspectives of appropriateness, acceptability and feasibility. BMC Health Serv Res **2019**; 19:74.
390 19. World Health Organization. Microscopy for the detection, identification and quantification of malaria
391 parasites on stained thick and thin blood films in research settings. Geneva: World Health Organization,
392 **2015**.
393 20. Swysen C, Vekemans J, Bruls M, et al. Development of standardized laboratory methods and quality
394 processes for a phase III study of the RTS, S/AS01 candidate malaria vaccine. Malar J **2011**; 10:223.
395 21. Lucchi NW, Narayanan J, Karell MA, et al. Molecular diagnosis of malaria by photo-induced electron
396 transfer fluorogenic primers: PET-PCR. PLoS One **2013**; 8:e56677.
397 22. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J
398 Epidemiol **2004**; 159:702-6.
399 23. Danwang C, Kirakoya-Samadoulougou F, Samadoulougou S. Assessing field performance of
400 ultrasensitive rapid diagnostic tests for malaria: a systematic review and meta-analysis. Malar J **2021**;
401 20:245.
402 24. Vasquez AM, Zuluaga L, Tobon A, et al. Diagnostic accuracy of loop-mediated isothermal
403 amplification (LAMP) for screening malaria in peripheral and placental blood samples from pregnant
404 women in Colombia. Malar J **2018**; 17:262.
405 25. Vasquez AM, Medina AC, Tobon-Castano A, et al. Performance of a highly sensitive rapid diagnostic
406 test (HS-RDT) for detecting malaria in peripheral and placental blood samples from pregnant women in
407 Colombia. PLoS One **2018**; 13:e0201769.
408 26. Briand V, Cottrell G, Tuike Ndam N, et al. Prevalence and clinical impact of malaria infections
409 detected with a highly sensitive HRP2 rapid diagnostic test in Beninese pregnant women. Malar J **2020**;
410 19:188.
411 27. Vasquez AM, Velez G, Medina A, et al. Evaluation of highly sensitive diagnostic tools for the
412 detection of P. falciparum in pregnant women attending antenatal care visits in Colombia. BMC
413 Pregnancy Childbirth **2020**; 20:440.
414 28. Briand V, Cottrell G, Tuikue Ndam N, et al. Correction to: Prevalence and clinical impact of malaria
415 infections detected with a highly sensitive HRP2 rapid diagnostic test in Beninese pregnant women.
416 Malar J **2020**; 19:328.
417 29. Kyabayinze DJ, Zongo I, Cunningham J, et al. HRP2 and pLDH-Based Rapid Diagnostic Tests, Expert
418 Microscopy, and PCR for Detection of Malaria Infection during Pregnancy and at Delivery in Areas of
419 Varied Transmission: A Prospective Cohort Study in Burkina Faso and Uganda. PLoS One **2016**;
420 11:e0156954.
421 30. Idris ZM, Chan CW, Kongere J, et al. High and Heterogeneous Prevalence of Asymptomatic and Sub-
422 microscopic Malaria Infections on Islands in Lake Victoria, Kenya. Sci Rep **2016**; 6:36958.

423

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)	**<200**	**200 to <2000**	**2000 to <20,000**
	43 (33–58)	(n=135; 78.5%)	(n=29; 16.9%)	(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)										
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)
<u>Gestational Age</u>										
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

	Sensitivity (95% CI)											
Diagnostic Test	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.

Figure Legends

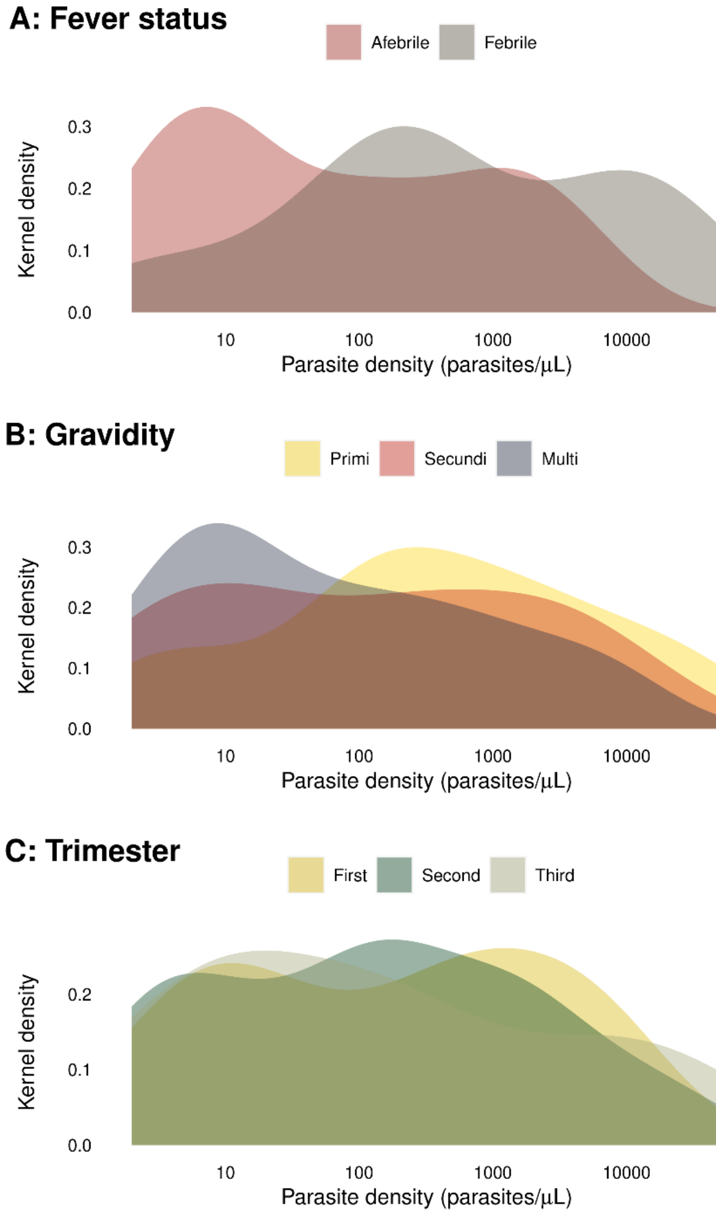

Figure 1. Distribution of PET-PCR positive samples by parasite density stratified by fever status, gravidity and trimester of pregnancy

Legend: 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.

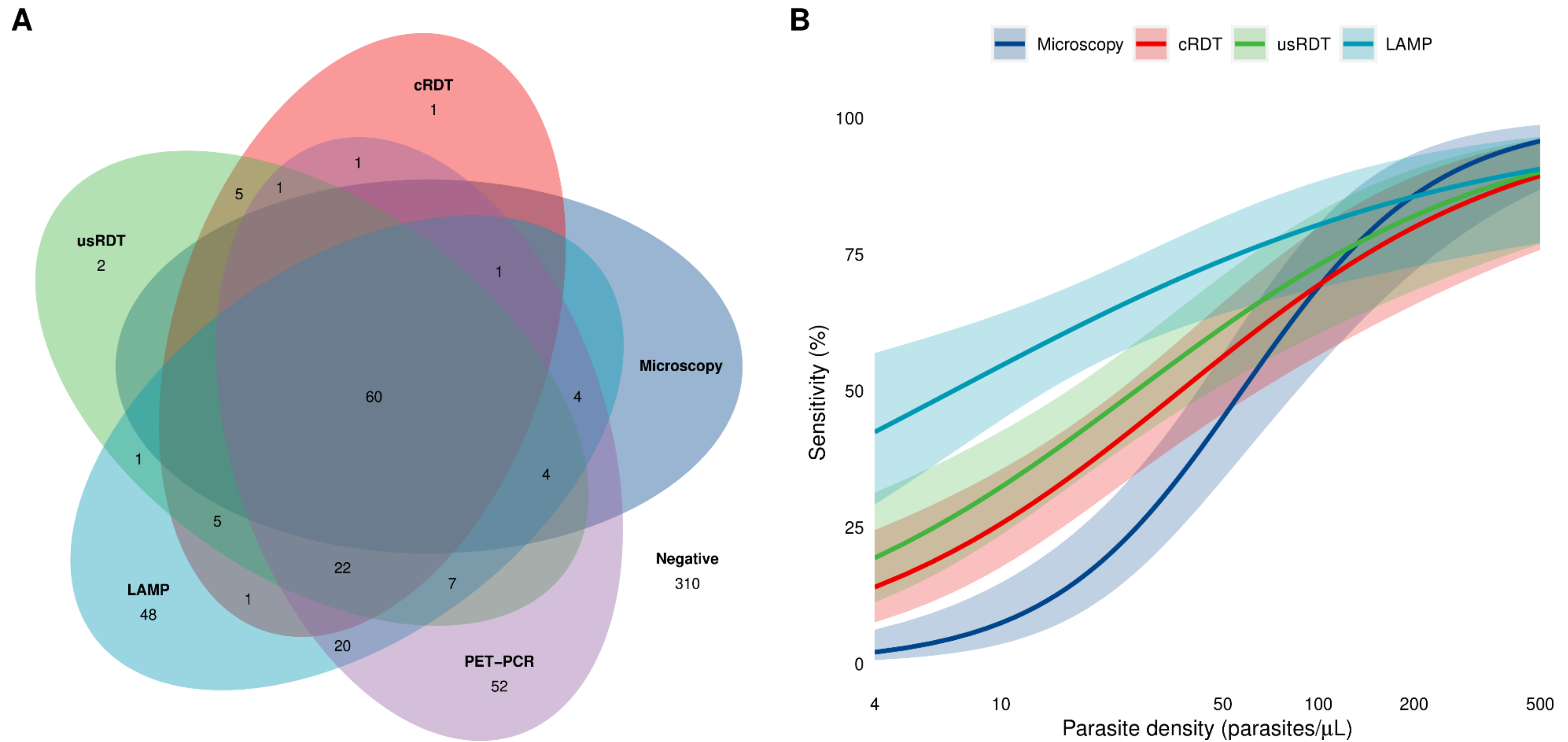

Figure 2. Distribution of positive samples by diagnostic test and modelled sensitivity to PET-PCR at densities below 500 parasites/µL

Legend: (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.

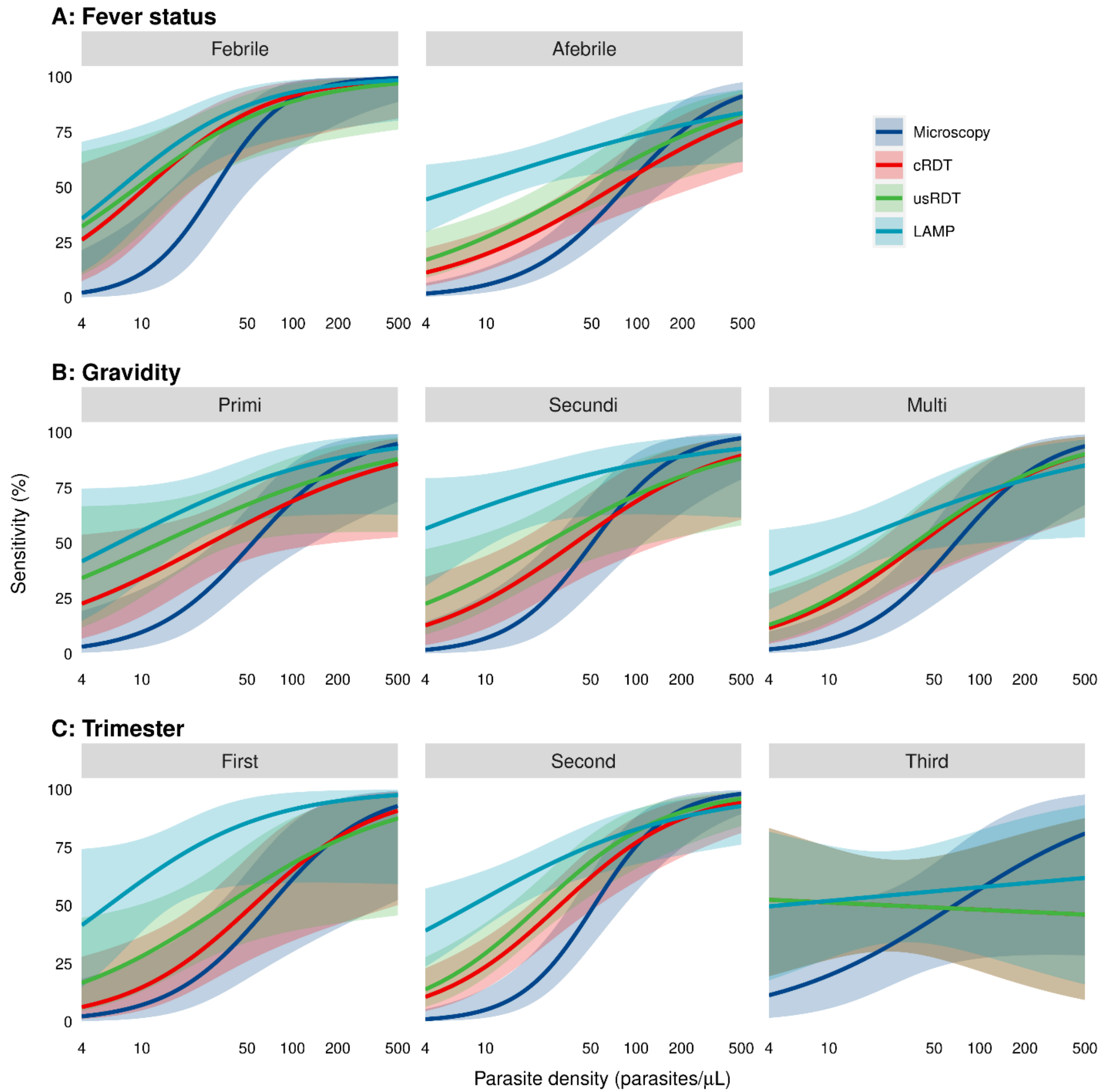

Figure 3. Curves of modelled test sensitivity at low parasite density with PET-PCR as the reference

Legend: 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

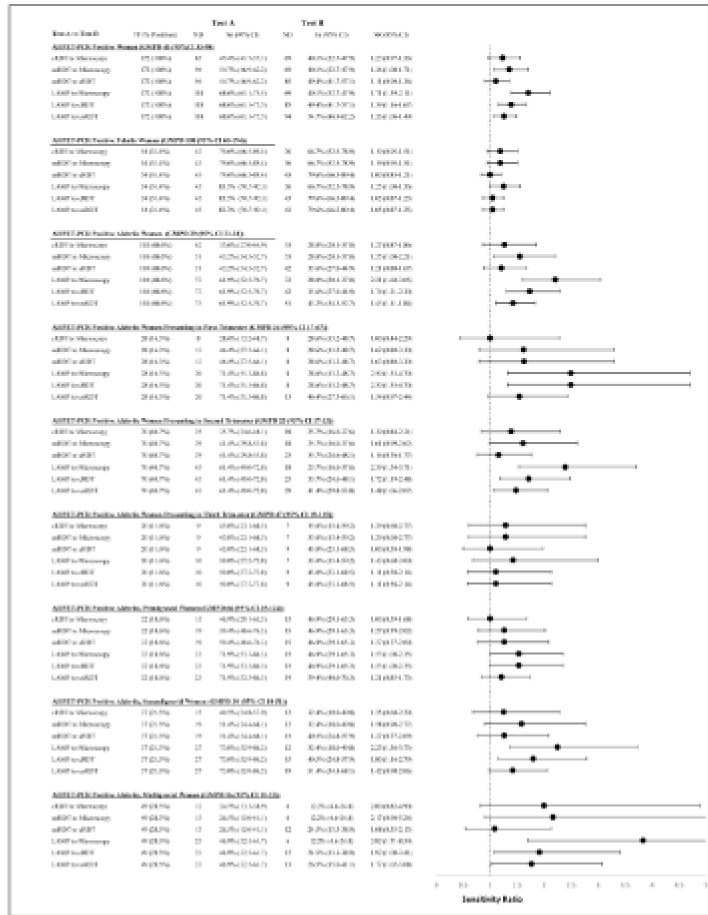

Figure 4. Relative test diagnostic sensitivity to PET-PCR by febrile status and among afebrile women by trimester of pregnancy and gravidity

Legend: 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.

Supplemental Content

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Samuels, AM, et al: Diagnostic performance of loop-mediated isothermal amplification (LAMP) and ultra-sensitive rapid diagnostic tests (usRDTs) for malaria screening in pregnant women attending their first antenatal care clinic visit in western Kenya

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Supplemental methods

MICROSCOPY

Blood smear reads were considered discordant if they differed qualitatively by the presence of parasites or species identification. Additionally, reads were considered discordant if they differed quantitively by the following parameters:

- For high and medium parasitemia results (parasite density ≥400 parasites/µL): if the higher count divided by the lower count is ≥2
- For low parasitemia results (parasite density ≤400 parasites/µL): if the higher count divided by the lower count is ≥10
- If one parasitemia result is ≥400 parasites/µL and the other is ≤400 parasites/µL: if the higher count divided by the lower count is ≥10

A third microscopist, blinded to the results of prior examinations, confirmed discordant results. The final results used the results from the third reader combined with those the results of the microscopist most similar to the third reader.

LAMP

50 µL microlitres of whole blood sample were added to a collection tube containing illumigene® buffer and thoroughly mixed by inverting the tube five times. After incubation for 2 minutes at room temperature, 50 µL of the lysate was added to a sample device (SMP PREP IV) containing 900 µL of reaction buffer. After inverting five times, 5-10 drops of the lysate/reaction buffer mixture were gently squeezed into a clean tube. Fifty microlitres of the prepared eluate were added to both the test and control chambers of the illumigene® Malaria Test Device consisting of a TEST tube containing primers targeting the genus Plasmodium and a CONTROL tube with primers targeting the housekeeping human gene, NADH dehydrogenase subunit 1. Amplification and detection of malaria parasites were done by inserting the sample and control tubes in the Illumipro-10™ Incubator/Reader, which detects the change in turbidity associated with the production of magnesium pyrophosphate. A qualitative test result (positive, negative or invalid) is printed out after the run. The limit of detection (LoD) using the WHO standard has been determined to be equivalent to 2 parasites/µl [1].

PET-PCR

Genus-specific photo-induced electron transfer (PET) PCR was used as described previously,[1] with some modifications. Briefly, the PET-PCR assay was performed in triplicate using a 20µl reaction mix containing 2x TaqMan Environmental Master Mix 2.0 (Applied BioSystems), forward (GGCCTAACATGGCTATGACG) and FAM-labeled reverse (aggcgcatagcgcctggCTGCCTTCCTTAGATGTGGTAGCT) *Plasmodium*-specific primers and 5µl of DNA template. All runs included *a P. falciparum* positive lab control (3D7 strain) and PCR water as a no-template control. The cycling parameters used included an initial hot-start at 95°C for 15 minutes, followed by 45 cycles of denaturation at 95°C for 20 seconds, annealing at 63°C for 40 seconds and an extension at 72°C for 10 seconds. Samples with a cycle threshold (Ct) value of <40 Ct were considered positive; otherwise, all Ct values above 40 Ct were considered negative.

The mean cycle threshold (Ct) value from the PET-PCR was used to prepare a standard curve which to obtain parasite densities of the field isolates. Briefly, parasite density was calculated using a standard curve obtained from seven parasite isolates with known parasite density. A 5-fold serial dilution was prepared for each parasite isolate starting from a parasite density of 2000 to 0.64 parasites/µl. The dilutions were evaluated in quadruplicates by PET-PCR as described above.

The reported LOD for detecting *P. falciparum* infections is 3.2 parasites/µL.

Supplemental references

1. Lucchi NW, Narayanan J, Karell MA, et al. Molecular diagnosis of malaria by photo-induced electron transfer fluorogenic primers: PET-PCR. *PLoS One* 2013; **8**(2): e56677.

Supplemental figures

FIGURE S1: PARTICIPANT FLOW DIAGRAM OVERVIEW

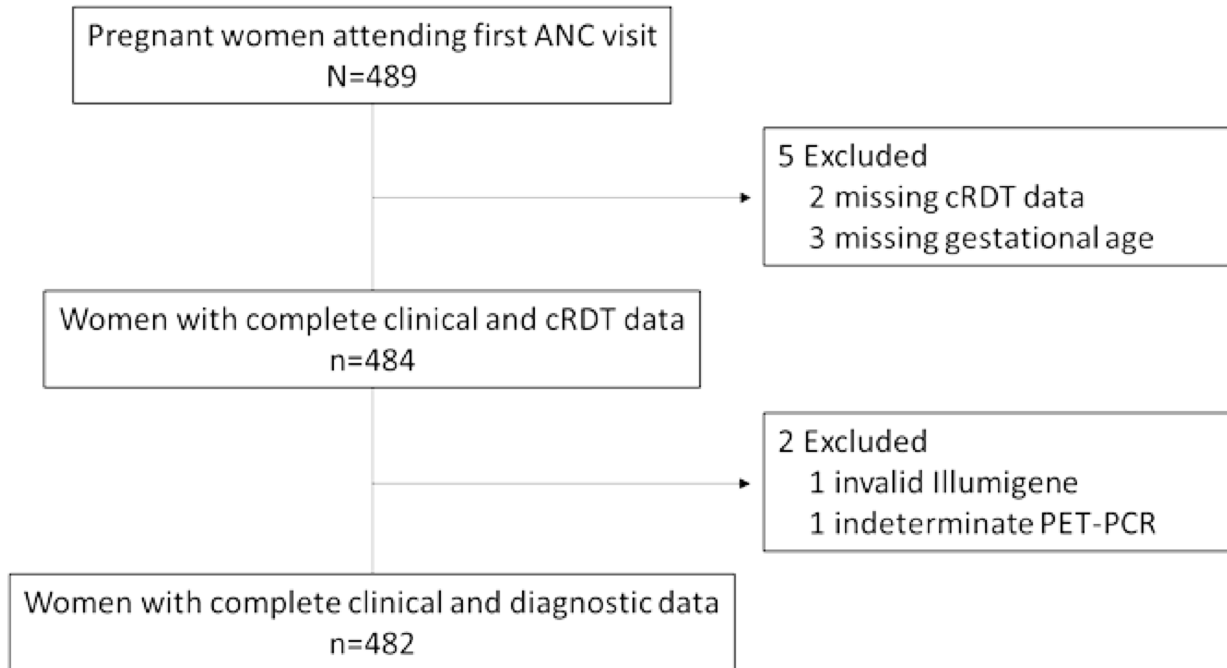
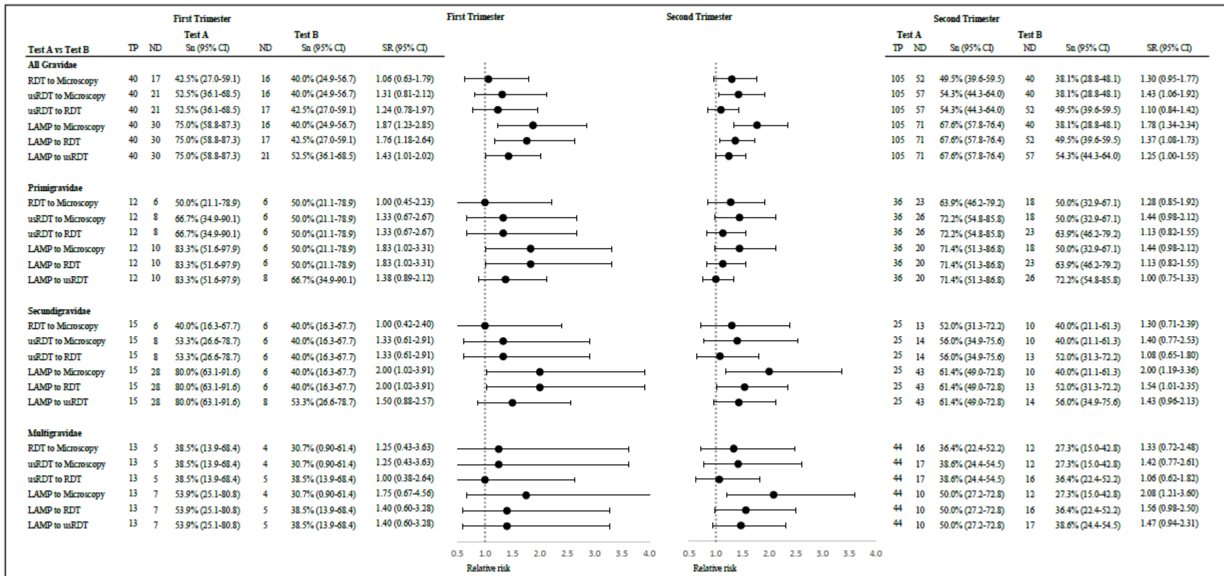


FIGURE S2: RELATIVE DIAGNOSTIC SENSITIVITY BY FIRST OR SECOND TRIMESTER AND GRAVIDITY



First Trimester

Test A vs Test B	Test A TP	Test A ND	Test A Sn (95% CI)	Test B ND	Test B Sn (95% CI)	SR (95% CI)
All Gravidae						
RDT to Microscopy	40	17	42.5% (27.0-59.1)	16	40.0% (24.9-56.7)	1.06 (0.63-1.79)
usRDT to Microscopy	40	21	52.5% (36.1-68.5)	16	40.0% (24.9-56.7)	1.31 (0.81-2.12)
usRDT to RDT	40	21	52.5% (36.1-68.5)	17	42.5% (27.0-59.1)	1.24 (0.78-1.97)
LAMP to Microscopy	40	30	75.0% (58.8-87.3)	16	40.0% (24.9-56.7)	1.87 (1.23-2.85)
LAMP to RDT	40	30	75.0% (58.8-87.3)	17	42.5% (27.0-59.1)	1.76 (1.18-2.64)
LAMP to usRDT	40	30	75.0% (58.8-87.3)	21	52.5% (36.1-68.5)	1.43 (1.01-2.02)
Primigravidae						
RDT to Microscopy	12	6	50.0% (21.1-78.9)	6	50.0% (21.1-78.9)	1.00 (0.45-2.23)
usRDT to Microscopy	12	8	66.7% (34.9-90.1)	6	50.0% (21.1-78.9)	1.33 (0.67-2.67)
usRDT to RDT	12	8	66.7% (34.9-90.1)	6	50.0% (21.1-78.9)	1.33 (0.67-2.67)
LAMP to Microscopy	12	10	83.3% (51.6-97.9)	6	50.0% (21.1-78.9)	1.83 (1.02-3.31)
LAMP to RDT	12	10	83.3% (51.6-97.9)	6	50.0% (21.1-78.9)	1.83 (1.02-3.31)
LAMP to usRDT	12	10	83.3% (51.6-97.9)	8	66.7% (34.9-90.1)	1.38 (0.89-2.12)
Secundigravidae						
RDT to Microscopy	15	6	40.0% (16.3-67.7)	6	40.0% (16.3-67.7)	1.00 (0.42-2.40)
usRDT to Microscopy	15	8	53.3% (26.6-78.7)	6	40.0% (16.3-67.7)	1.33 (0.61-2.91)
usRDT to RDT	15	8	53.3% (26.6-78.7)	6	40.0% (16.3-67.7)	1.33 (0.61-2.91)
LAMP to Microscopy	15	28	80.0% (63.1-91.6)	6	40.0% (16.3-67.7)	2.00 (1.02-3.91)
LAMP to RDT	15	28	80.0% (63.1-91.6)	6	40.0% (16.3-67.7)	2.00 (1.02-3.91)
LAMP to usRDT	15	28	80.0% (63.1-91.6)	8	53.3% (26.6-78.7)	1.50 (0.88-2.57)
Multigravidae						
RDT to Microscopy	13	5	38.5% (13.9-68.4)	4	30.7% (0.90-61.4)	1.25 (0.43-3.63)
usRDT to Microscopy	13	5	38.5% (13.9-68.4)	4	30.7% (0.90-61.4)	1.25 (0.43-3.63)
usRDT to RDT	13	5	38.5% (13.9-68.4)	5	38.5% (13.9-68.4)	1.00 (0.38-2.64)
LAMP to Microscopy	13	7	53.9% (25.1-80.8)	4	30.7% (0.90-61.4)	1.75 (0.67-4.56)
LAMP to RDT	13	7	53.9% (25.1-80.8)	5	38.5% (13.9-68.4)	1.40 (0.60-3.28)
LAMP to usRDT	13	7	53.9% (25.1-80.8)	5	38.5% (13.9-68.4)	1.40 (0.60-3.28)

Second Trimester

Test A vs Test B	Test A TP	Test A ND	Test A Sn (95% CI)	Test B ND	Test B Sn (95% CI)	SR (95% CI)
All Gravidae						
RDT to Microscopy	105	52	49.5% (39.6-59.5)	40	38.1% (28.8-48.1)	1.30 (0.95-1.77)
usRDT to Microscopy	105	57	54.3% (44.3-64.0)	40	38.1% (28.8-48.1)	1.43 (1.06-1.92)
usRDT to RDT	105	57	54.3% (44.3-64.0)	52	49.5% (39.6-59.5)	1.10 (0.84-1.42)
LAMP to Microscopy	105	71	67.6% (57.8-76.4)	40	38.1% (28.8-48.1)	1.78 (1.34-2.34)
LAMP to RDT	105	71	67.6% (57.8-76.4)	52	49.5% (39.6-59.5)	1.37 (1.08-1.73)
LAMP to usRDT	105	71	67.6% (57.8-76.4)	57	54.3% (44.3-64.0)	1.25 (1.00-1.55)
Primigravidae						
RDT to Microscopy	36	23	63.9% (46.2-79.2)	18	50.0% (32.9-67.1)	1.28 (0.85-1.92)
usRDT to Microscopy	36	26	72.2% (54.8-85.8)	18	50.0% (32.9-67.1)	1.44 (0.98-2.12)
usRDT to RDT	36	26	72.2% (54.8-85.8)	23	63.9% (46.2-79.2)	1.13 (0.82-1.55)
LAMP to Microscopy	36	20	71.4% (51.3-86.8)	18	50.0% (32.9-67.1)	1.44 (0.98-2.12)
LAMP to RDT	36	20	71.4% (51.3-86.8)	23	63.9% (46.2-79.2)	1.13 (0.82-1.55)
LAMP to usRDT	36	20	71.4% (51.3-86.8)	26	72.2% (54.8-85.8)	1.00 (0.75-1.33)
Secundigravidae						
RDT to Microscopy	25	13	52.0% (31.3-72.2)	10	40.0% (21.1-61.3)	1.30 (0.71-2.39)
usRDT to Microscopy	25	14	56.0% (34.9-75.6)	10	40.0% (21.1-61.3)	1.40 (0.77-2.53)
usRDT to RDT	25	14	56.0% (34.9-75.6)	13	52.0% (31.3-72.2)	1.08 (0.65-1.80)
LAMP to Microscopy	25	43	61.4% (49.0-72.8)	10	40.0% (21.1-61.3)	2.00 (1.19-3.36)
LAMP to RDT	25	43	61.4% (49.0-72.8)	13	52.0% (31.3-72.2)	1.54 (1.01-2.35)
LAMP to usRDT	25	43	61.4% (49.0-72.8)	14	56.0% (34.9-75.6)	1.43 (0.96-2.13)
Multigravidae						
RDT to Microscopy	44	16	36.4% (22.4-52.2)	12	27.3% (15.0-42.8)	1.33 (0.72-2.48)
usRDT to Microscopy	44	17	38.6% (24.4-54.5)	12	27.3% (15.0-42.8)	1.42 (0.77-2.61)
usRDT to RDT	44	17	38.6% (24.4-54.5)	16	36.4% (22.4-52.2)	1.06 (0.62-1.82)
LAMP to Microscopy	44	10	50.0% (27.2-72.8)	12	27.3% (15.0-42.8)	2.08 (1.21-3.60)
LAMP to RDT	44	10	50.0% (27.2-72.8)	16	36.4% (22.4-52.2)	1.56 (0.98-2.50)
LAMP to usRDT	44	10	50.0% (27.2-72.8)	17	38.6% (24.4-54.5)	1.47 (0.94-2.31)

Sensitivity ratios were modelled using Poisson regression. Sensitivities of tests were calculated using PET-PCR results as the gold standard. SRs greater than 1 indicate that test A is more sensitive than test

B for the given criteria. Abbreviations: TP, true positives within sub-group by PET-PCR; ND, number of true positives detected by the given test; Sn (95% CI), sensitivity (95% confidence interval); SR, sensitivity ratio; NE, non-estimable due to sample size limitation.

Supplemental tables

SUPPLMENTAL TABLE 1: STARD CHECKLIST

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4
	4	Study objectives and hypotheses	4
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	4
Participants	**6**	Eligibility criteria	4
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	4
	8	Where and when potentially eligible participants were identified (setting, location and dates)	4
	9	Whether participants formed a consecutive, random or convenience series	4
Test methods	**10a**	Index test, in sufficient detail to allow replication	5
	10b	Reference standard, in sufficient detail to allow replication	5, Supplement Methods
	11	Rationale for choosing the reference standard (if alternatives exist)	5
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	5
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	4, Supplemental Methods
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	5
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	5
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	6
	15	How indeterminate index test or reference standard results were handled	6
	16	How missing data on the index test and reference standard were handled	6
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	6
	18	Intended sample size and how it was determined	5
RESULTS			
Participants	**19**	Flow of participants, using a diagram	5
	20	Baseline demographic and clinical characteristics of participants	6
	21a	Distribution of severity of disease in those with the target condition	6
	21b	Distribution of alternative diagnoses in those without the target condition	N/A
	22	Time interval and any clinical interventions between index test and reference standard	5
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	6

	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	6
	25	Any adverse events from performing the index test or the reference standard	N/A
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	8
	27	Implications for practice, including the intended use and clinical role of the index test	8
OTHER INFORMATION			
	28	Registration number and name of registry	N/A
	29	Where the full study protocol can be accessed	Submitted upon request
	30	Sources of funding and other support; role of funders	6, 10

1. Lucchi NW, Narayanan J, Karell MA, et al. Molecular diagnosis of malaria by photo-induced electron transfer fluorogenic primers: PET-PCR. PLoS One **2013**; 8:e56677.