

1 **Modelling the incremental benefit of introducing malaria screening strategies** 2 **to antenatal care in Africa**

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26

27 **Abstract**

28 ***Plasmodium falciparum* in pregnancy is a major cause of adverse pregnancy outcomes. We**
29 **combine performance estimates of standard rapid diagnostic tests (RDT) from trials of**
30 **intermittent screening and treatment in pregnancy (ISTp) with modelling to assess whether**
31 **screening at antenatal visits improves upon current intermittent preventative therapy with**
32 **sulphadoxine-pyrimethamine (IPTp-SP). We estimate that RDTs in primigravidae at first antenatal**
33 **visit are substantially more sensitive than in non-pregnant adults (OR=17.2, 95% Cr.I. 13.8-21.6),**
34 **and that sensitivity declines in subsequent visits and with gravidity, likely driven by declining**
35 **susceptibility to placental infection. Monthly ISTp with standard RDTs, even with highly effective**
36 **drugs, is not superior to monthly IPTp-SP. However, a hybrid strategy, recently adopted in**
37 **Tanzania, combining testing and treatment at first visit with IPTp-SP may offer benefit, especially**
38 **in areas with high-grade SP resistance. Screening and treatment in the first trimester, when IPTp-**
39 **SP is contraindicated, could substantially improve pregnancy outcomes.**

40 **Introduction**

41 Infection with *P. falciparum* malaria in pregnancy (MiP) is associated with a wide range of adverse
42 pregnancy outcomes including maternal anaemia, low birthweight and neonatal death¹. These
43 adverse effects largely result from sequestration of the parasite within the placenta particularly in

44 women not exposed to *P. falciparum* in any previous pregnancy¹. Despite declines in malaria
45 transmission in many settings², MiP risk remains high³. Approximately a third of all pregnancies
46 (9.5m of 30.6m) occurring in areas of sustained transmission in 2015 were liable to be affected by
47 malaria³. In the absence of pregnancy-specific protection, this could lead to 750,000 malaria-
48 attributable low birthweight deliveries in sub-Saharan Africa each year³. Observed increases in the
49 average density of placental infection in areas where transmission has fallen suggest declining
50 immunity will ensure MiP continues to represent a pressing public health concern even if the
51 current stall in reducing global malaria transmission is overcome⁴⁻⁶.

52 Despite significant improvements in access to antenatal care (ANC) in the past decade, uptake of
53 proven effective tools for MiP prevention has been slow. In areas where intermittent preventative
54 therapy in pregnancy (IPTp) is recommended, 22% of women received the recommended three or
55 more doses of intermittent preventative therapy in pregnancy (IPTp) in 2017⁶. Moreover LLINs use is
56 low in adolescents who are the most at risk of high-density placental infection³. The emergence of
57 parasite resistance to sulphadoxine-pyrimethamine (SP), the only drug currently recommended by
58 the World Health Organisation (WHO) for IPTp, has led to attempts to find alternative strategies.
59 One such alternative, intermittent screening and treatment in pregnancy (ISTp), has been evaluated
60 in a number of countries⁷⁻¹⁰. Whilst, IPTp provides SP to all women at each visit without testing, ISTp
61 involves testing of all pregnant women regardless of the presence of malaria symptoms (screening)
62 with rapid diagnostic tests (RDTs) and treating test-positive women with highly efficacious
63 artemisinin combination therapy (ACT). However, ISTp using the current generation of RDTs has not
64 proven more effective than IPTp-SP¹¹, and is therefore not recommended by WHO¹².

65 WHO has recommended further studies into alternative strategies involving routine screening for
66 MiP¹². These include evaluating whether more sensitive RDTs could make ISTp a viable alternative
67 and whether hybrid strategies, involving adding RDT-based screening to existing IPTp regimens,
68 provide additional benefit¹³. One such hybrid approach is now national policy in Tanzania (where
69 quintuple mutants are ubiquitous and highly resistant sextuple mutants have been identified in
70 some areas): all women are tested for malaria parasites at the first ANC visit (booking) and provided
71 with an ACT if test-positive or, starting in the second trimester, with SP if test-negative. All women
72 then receive IPTp-SP at subsequent scheduled ANC visits¹⁴. However, given the additional costs and
73 complexities of such approaches, it is important to understand where, and the extent to which, they
74 can provide greater protection from malaria during pregnancy than standard IPTp-SP regimens.

75 The negative consequences of first trimester infection^{11,15}, at which time the use of SP is contra-
76 indicated, and reassuring data regarding first trimester safety of ACTs¹⁶ has resulted in increased
77 interest in screen and treat approaches using ACTs for women attending ANC during the first
78 trimester. A recent study from Benin tracking women prior to conception supports previous
79 modelling¹⁷, which suggested that a high proportion of placental infections are likely to be caused by
80 infections acquired prior to conception. Genotyping of infection showed that the densities of
81 infection acquired prior to pregnancy, rather than declining over time, had substantially increased by
82 the time the women attended ANC¹⁸.

83 Trials assessing the impact of MiP interventions are expensive, and time-consuming, requiring up to
84 2 years longitudinal follow-up. Moreover, large sample sizes are required to adequately measure
85 effectiveness and cost-effectiveness as the key drivers of attributable burden are outcomes such as

86 pregnancy loss, low birthweight and neonatal mortality are increasingly rare in the context of clinical
87 trials. Although not a substitute for clinical trials, modelling provides a means to explore the
88 potential of multiple alternative interventions to guide prioritisation of research.

89 A key determinant of the need for alternatives to IPTp-SP, the level of parasite resistance to SP and
90 the associated decline in efficacy of IPT-SP¹⁹, varies across Africa. In much of West Africa, SP provides
91 near perfect curative efficacy and a period of prophylaxis of approximately one month. In East
92 Africa, where there is a very high prevalence of parasites harbouring K540E 'quintuple' SP resistance
93 mutation, SP fails to clear approximately 20% of infections during pregnancy and provides limited
94 prophylaxis²⁰. Though there have been no efficacy studies in areas of high prevalence of the
95 'sextuple' SP resistance mutation (an additional mutation at A581G on top of the quintuple),
96 currently limited to specific foci in East Africa²¹, there are concerns that IPTp-SP effectiveness may
97 be heavily compromised within these settings¹⁹.

98 In this analysis, we combine data on the sensitivity of standard RDTs during pregnancy collected
99 during ISTp trials with equivalent data from a review of RDT sensitivity outside of pregnancy²². We
100 then use modelling to estimate the impact of pregnancy on the detectability of infection using RDTs,
101 incorporating the role of pregnancy-specific immunity in controlling parasite densities in the
102 placental and peripheral blood. Finally, we incorporate these estimates within a model of the
103 relationship between malaria transmission and effectiveness of IPTp-SP, incorporating the effects of
104 SP resistance, to assess the potential for different strategies involving antenatal screening, either
105 with current or more sensitive RDTs, to improve protection for pregnant women from MiP.

106 **Results**

107 **Impact of pregnancy upon detectability of infection by RDT**

108 There have been four large-scale trials comparing ISTp with IPTp-SP, three of them had matched RDT
109 (First Response Malaria pLDH/HRP2 Combo Test, Premier Medical Corporation, India) and PCR
110 samples collected from 1,559 women based in 6 countries (Burkina Faso, The Gambia, Ghana, and
111 Mali in West Africa and Kenya and Malawi in East Africa)⁸⁻¹⁰. West African studies recruited only
112 women in their first and second pregnancy, whereas the studies in East Africa recruited women of all
113 gravidities. In all studies women were enrolled in their first visit after 16 weeks gestation provided
114 this visit was before 28 weeks, 30 weeks and 32 weeks in Malawi, Kenya and West Africa
115 respectively. In three countries, infection by conventional RDT and PCR was measured throughout
116 pregnancy: Ghana, Kenya and Malawi (in Burkina Faso, The Gambia and Malawi PCR was only
117 measured at enrolment). Both prevalence and detectability (measured by RDT sensitivity relative to
118 PCR) were consistently higher at enrolment than at subsequent ANC visits, particularly in
119 primigravidae (Figure 1).

120 In all six countries, RDT sensitivity at enrolment, defined throughout this paper as the level of
121 detection relative to PCR, showed a declining trend with gravidity (Figure 2a). Overall sensitivity in
122 primigravidae was very high, with 88.9% [640/720, 86.4-91.1% 95% C.I.] of PCR positive infections
123 detected by RDT, but showed substantial heterogeneity between sites ranging between 65.8%
124 [27/41, 49.4-79.9% 95% C.I.] sensitivity in Bassé, The Gambia, the setting with lowest transmission
125 (PCR prevalence in primigravidae: 13.4% [41/316, 9.8-17.8% 95% C.I.]), to close to that of PCR in
126 Navrongo, Ghana (95.0% [192/202, 91.1%-97.6% 95% C.I.]), where PCR prevalence was 65.8%

127 [202/307,60.2-71.1% 95% C.I.]. We also compared the sensitivity observed within primigravidae at
128 enrolment against the RDT sensitivity in other non-pregnant populations from data obtained from a
129 recent review²². Incorporating the relationship between transmission and RDT sensitivity from this
130 analysis (Figure 2), we estimated that the odds of detecting a PCR positive infection with an RDT
131 were substantially higher at enrolment in primigravidae than in asymptomatic non-pregnant
132 individuals over 15 years old (Odds Ratio (OR) 17.2 [13.8-21.6 95% Cr.I.]) or asymptomatic children
133 under 5 years of age (OR 3.8 [2.9-4.9 95% Cr.I.]).

134 To obtain estimates of how acquisition of pregnancy-specific immunity influences detectability of
135 infection with RDTs, we then used a previous model of the relationship between prevalence in the
136 general population and cumulative exposure to MiP to account for likely patterns of prior exposure
137 to infection during pregnancy by gravidity¹⁷. Our estimates suggest that the odds ratio for the
138 pregnancy-related increase of detectability relative to non-pregnant adults, falls from 17.2 [13.8-
139 21.6 95% Cr.I.] in primigravidae to 4.05 [3.14-5.16 95% Cr.I.] when a woman has experienced
140 infection in one previous pregnancy and to 1.67 [1.22-2.34 95% Cr.I.] if she has experienced infection
141 in two previous pregnancies. By the fourth infected pregnancy, our estimates of sensitivity in
142 pregnancy are no longer distinguishable from those outside of pregnancy [Figure 2b].

143 We used multivariable logistic regression to find the best-fitting predictors of RDT sensitivity at
144 subsequent ANC visits, using random effects intercepts to account for unknown or unmeasured
145 factors between sites. This suggested gravidity remains a significant factor at later visits (OR 0.87
146 [0.78-0.96 95% C.I.] per additional previous pregnancy, $p=0.005$), as does the presence of infection
147 at the preceding visit (OR 0.70 [0.53-0.92 95% C.I.]). Other potential variables explored that were not
148 kept within the best fitting model as measured by Akaike Information Criterion (AIC), after
149 accounting for gravidity and PCR status, included a measure of parasite density of this previous
150 infection (patent (RDT positive) or sub-patent), the number of times a woman had been previously
151 tested, or the number of previous ANC visits she had attended. Despite the exploration of these
152 factors, there remained substantial unexplained between-site variation within this best-fitting model
153 ($p<0.0001$).

154 In addition to these factors, RDT sensitivity in pregnancy is also likely to depend upon maternal age.
155 This factor was not included in our analysis as sufficient granularity was not available for the
156 relationship with RDT sensitivity outside of pregnancy²². Given the correlation between age and
157 gravidity, it is likely that some age-dependent effects have been attributed to pregnancy-specific
158 immunity. However, our results, which found that RDT sensitivity in primigravidae is substantially
159 higher than would be expected in children (Figure 2a), suggest the major determinants of the
160 observed patterns are pregnancy- rather than age-specific.

161 **Dynamics of infection throughout pregnancy with ISTp**

162 We updated a previously model of the relationship between malaria transmission and exposure to
163 malaria infection throughout pregnancy to incorporate the factors described above¹⁷. We then
164 assessed the extent to which this model replicated patterns of PCR prevalence throughout
165 pregnancy within the three trials which conducted PCR at each visit. This was done by restricting our
166 analysis to women who received the modal number of screens in each trial (3 screens prior to
167 delivery in Kenya and Ghana, and 4 screens in Malawi). We also assumed that these dynamics could
168 be approximated by simulations with an initial screen occurring at the median gestational age at

169 which women could be enrolled into each trial (24 weeks in Kenya, 20 weeks in Ghana and 20 weeks
170 in Malawi for women receiving 4 screens), with subsequent visits spaced regularly until delivery at
171 40 weeks gestation.

172 We calibrated the model to match the observed PCR prevalence in primigravidae at enrolment in
173 each trial, whereupon the model captured (Figure 3) many of the observed dynamics of infection
174 throughout the trials, namely:

175 i) decreases in PCR prevalence at enrolment by gravidity: driven in the model by reduced prevalence
176 at conception due to the acquisition of non-pregnancy specific immunity between pregnancies and
177 improved clearance of parasitaemia during pregnancy related to pregnancy-specific immunity;

178 ii) a large decline in prevalence in primigravidae between first and second screens: explained in the
179 model by the higher proportion of women testing positive by RDTs at first screen and associated
180 treatment and post-treatment prophylactic effect;

181 iii) diminishing impact upon prevalence with increasing gravidity: driven by reduced detectability of
182 infection by gravidity due to pregnancy-specific immunity acquired in previous pregnancies;

183 iv) similar prevalence between tests from the second screen until delivery across all trials and
184 gravidity categories: explained by the shorter window of exposure in which women can acquire new
185 detectable infection (~20 weeks duration of gestation prior to first screen plus any residual infection
186 acquired pre-conception versus ~4-8 gestation between screens) and the correspondingly smaller
187 proportion of women benefiting from any post-treatment prophylactic effect from second screen
188 onwards.

189 **Alternatives to IPTp-SP involving screening with standard RDTs**

190 To capture differential effectiveness of IPTp-SP as a function of the accumulation of parasite
191 resistance mutations we defined three resistance scenarios (summarised in Figure 4) as 'low
192 prevalence quintuple' and 'high prevalence quintuple' SP resistance that map to those commonly
193 observed in West and East Africa, along with a hypothetical scenario in which SP retains no
194 antiparasitic activity, referred to as 'high sextuple' SP resistance areas. Within each scenario we
195 compared six MiP prevention strategies: no intervention; IPTp-SP; ISTp; a hybrid approach (Hybrid-
196 SSTp) wherein women are screened at first visit during the second trimester, provided an ACT if test-
197 positive and SP if test-negative, then provided with IPTp-SP at subsequent visits; a second hybrid
198 approach (Hybrid-ISTp) where women are tested at each ANC visit, provided an ACT whenever they
199 test positive and SP otherwise (after the start of 2nd trimester); and IPTp with an ACT. For each
200 scenario involving ACTs we considered two possible drug combinations: artemether-lumefantrine
201 (AL), which provides prophylaxis for around 10 days, and dihydroartemisinin-piperaquine (DP) which
202 we assume provides prophylaxis of similar longevity to SP in the absence of resistance (see Methods
203 and Discussion).

204 We compared scenarios in terms of two measures of exposure we consider likely to correspond to
205 distinct sets of pathologies¹:

- 206 • The proportion of women left with uncleared infection post-enrolment (Figure 5), to
207 capture impact upon pathologies associated with chronic placental infection such as

208 intrauterine growth restriction and LBW. To incorporate the interaction between infection
209 detectability using RDT and immunity, these estimates are then weighted by estimates of
210 the number of LBW these infections would cause if left untreated (Figure 5c).

- 211 • The risk of new infection later in pregnancy (Figure 6), associated with a range of negative
212 outcomes including preterm delivery, neonatal mortality and stillbirth^{23,24}.

213 Our results suggest that in low quintuple resistance areas, as in West Africa, where SP retains high
214 efficacy and longevity, improving upon IPTp-SP strategies when delivered correctly, is likely to be
215 challenging (Figure 4, a,d). The choice of ACT generally had limited impact upon prevalence when
216 only provided to test-positive women (i.e. ISTp or hybrid strategies). However, hybrid strategies using
217 AL resulted in higher infection prevalence than IPTp-SP between first and second visits, driven by the
218 longer period of prophylaxis provided by SP²⁰ than AL²⁵ in areas of low quintuple resistance. This
219 highlights the need to prioritise longer-lasting ACTs when screening for infection at scheduled ANC
220 visits in order to ensure women are provided with at least equivalent protection to IPTp-SP.

221 The ISTp trial in Malawi and Kenya were conducted in areas of high quintuple mutation SP
222 resistance. Overall RDT sensitivity across all visits was 47% in these trials, meanwhile the risk that
223 presumptive SP fails to clear existing infections is approximately 20% in these settings. Thus, it is
224 unsurprising that our model incorporating these data suggests that IPTp-SP is more effective than
225 ISTp in terms of cumulative exposure to infection during pregnancy measured by PCR (the 47%
226 average risk of an untreated infection due to a false-negative RDT outweighs the approximately 20%
227 risk of treatment failure with presumptive SP). However, accounting for higher sensitivity of RDTs
228 earlier in pregnancy and in women with lower immunity, our results suggests that both IPTp-SP and
229 ISTp have a large impact upon prevalence when compared to the counterfactual of no intervention
230 (Figure 4, b,e). Moreover, our results suggest screening with RDTs early in the second trimester is
231 effective at detecting the majority of early infections that would cause chronic intrauterine growth
232 restriction leading to low birthweight if not treated (Figure 5).

233 In Figure 4, b,c,e,f we show the effectiveness of IPTp with AL and DP. Neither drug is currently
234 recommended for this purpose. Both are predicted to show incremental effectiveness in preventing
235 infection over SP in areas where resistance has reached high levels of quintuple mutation or above.
236 However, the incremental impact of DP, the focus of several ongoing studies, is substantially higher
237 than the shorter-lasting AL. However, until a suitable, more effective, alternative drug to SP for IPTp
238 has been recommended, our results suggest a hybrid strategy could be more effective than IPTp-SP
239 alone in areas of high quintuple resistance or above. It ensures that RDT-positive infections, which
240 are the higher-density, potentially more severe, infections are treated with a highly effective ACT,
241 for which the curative efficacy is higher than for SP. Meanwhile, in contrast to ISTp strategies,
242 women testing negative (both truly and falsely) are still receive the same level of protection
243 standard IPTp-SP (Figure 5). Our model suggests that hybrid approaches at all scheduled IPTp visits,
244 instead of just at the first visit, provides marginal incremental impact over the single screen-and-
245 treat hybrid strategy (Figures 4 and 6), whilst requiring substantially more resources due to repeated
246 screening.

247 Our simulations also suggest that in areas with high quintuple mutant resistance, IPTp using a long-
248 lasting drug such as DP would be considerably more effective than IPTp-SP or any alternative
249 strategies involving screen-and-treat strategies in terms of their impact upon newly occurring

250 infections from the second trimester onwards (i.e. following the timing when the first dose of IPTp
251 would be scheduled to occur) (Figure 6).

252 Given the limited data on the incremental sensitivity for infection in pregnancy of new highly
253 sensitive RDTs (hs-RDTs) ²⁶ we do not model the impact of specific hs-RDTs. Instead, we explored the
254 extent to which more highly sensitive tests than standard RDTs in general could potentially improve
255 the incremental impact of the strategies considered above.

256 For ISTp strategies, the added benefits of more sensitive RDTs may be small in high transmission
257 areas if the bulk of adverse outcomes results from patent infections. Moreover, sub-patent
258 infections missed by standard RDTs are more concentrated later in pregnancy when evidence for
259 increased risk of adverse pregnancy outcome is not consistent (see discussion). Even in areas of high
260 quintuple resistance, SP is likely to retain relatively high efficacy in clearing low-density infections
261 missed by standard RDTs, resulting in >90% of infections being effectively cleared with a hybrid
262 strategy using existing RDTs (Figure 5). As a result, we estimate the incremental effectiveness of
263 more sensitive diagnostics within hybrid strategies would be limited.

264 **Potential value of screening in the first trimester**

265 Although a large proportion of infections are likely to have been sub-patent at the beginning of
266 pregnancy, by the time primigravidae receive the first dose of IPTp-SP, the density of infection has
267 increased to the extent that very few remain below the limit of detection of standard RDTs (Figure
268 7a)¹⁸. Clearing these infections during any first trimester ANC visit irrespective of the immediate
269 density of the infection, is likely to have a large impact on the overall exposure to placental infection
270 (Figure 7c). Moreover, (Figure 7b), such testing is predicted to have a large proportional impact on
271 remaining exposure to placental infection in the presence of IPTp-SP (Figure 7c) which leaves the
272 first trimester entirely unprotected.

273 Our model suggests that a substantial number of infections acquired before or during the first
274 trimester would lead to adverse outcomes if left untreated⁵. It also suggests that the impact of first
275 trimester testing will depend strongly on gravidity, transmission, and the sensitivity of the test. The
276 latter is likely to depend strongly upon poorly understood temporal dynamics of parasite replication
277 in early pregnancy (Figure 7a). Moreover, it is difficult to assess the extent to which future IPTp-SP
278 will modify the impact of these early infections upon birth outcome.

279 **Discussion**

280 By reanalysing malaria testing data from trials of ISTp we were able to generate the first quantitative
281 estimates of the impact of pregnancy upon the detectability of infection using RDT. These
282 relationships provide more nuanced understanding as to the failure of ISTp to show incremental
283 effectiveness compared to IPTp-SP in trials Our estimates suggest that infections missed by standard
284 RDTs lead to a greater proportion of inadequately treated infected women than providing SP
285 presumptively (i.e. the negative effects of misdiagnosed infections outweigh those of treatment and
286 prophylaxis failures). However, in these settings our simulations suggest that both IPTp-SP and ISTp,
287 whilst failing to provide optimal protection, effectively prevent the majority of infections when
288 compared to women without any intervention.

289 This finding, that ISTp has substantial intrinsic impact relative to no intervention despite not being
290 superior to IPTp, is supported by a recent meta-analysis of four trials comparing these two
291 strategies: when pooled these studies show that babies born in the IPTp-SP arms had a 25g higher
292 mean birthweight than in the ISTp arm (95% CI 7–44, $p=0.0088$, I^2 0%, 8659 pregnancies)¹¹. In
293 absolute terms, this difference is small compared to the 79g (95% CI 13-145) seen with IPTp-SP when
294 compared to placebo or passive case detection.. Consequently, ISTp , has potential advantages over
295 current practice in some countries that do not deploy IPTp due to concerns about SP efficacy, or,
296 if an adequate replacement drug for SP within IPTp regimens cannot be identified, if SP became
297 completely ineffective due to further development of resistance in the future (Figure 4). This might
298 be the case if the ‘sextuple’ A581G resistance mutation, established in specific foci in East Africa,
299 becomes more prevalent and widespread.

300 There is not sufficient data to specifically include recently developed highly sensitive tests for HRP2
301 within our analysis. However, our results suggest more highly sensitive diagnostics in general could
302 improve ISTp strategies, and if sufficiently sensitive, could provide incremental effectiveness over
303 IPTp-SP in terms of parasitological outcomes such as infection prevalence by PCR at delivery in areas
304 of high quintuple mutation SP resistance. However, the clinical implications of any increased
305 effectiveness to detect low-density infections, which are more common in multigravidae and later in
306 pregnancy remain to be determined. The association between low-density infection in the second
307 trimester onwards and pregnancy outcome is not consistent^{27–31}. However, as transmission falls, the
308 density of peripheral and placental infection at delivery in multigravidae increases⁴, presumably
309 reflecting a lower level of exposure to malaria during previous pregnancies. Of all trial sites, RDT
310 sensitivity at enrolment in primigravidae was lowest In The Gambia, the trial site with the lowest
311 transmission.. This may reflect lower density of infection prior to placental development, as the
312 sensitivity of infection by RDT outside of pregnancy falls as transmission declines^{22,32}. In these areas,
313 more sensitive RDTs could substantially improve the ability of ISTp to detect and treat what would
314 otherwise be long-lasting infections in women lacking pregnancy-specific antimalarial immunity.
315 More data are required from studies measuring RDT sensitivity in pregnancy in areas of low
316 transmission in order to assess this hypothesis.

317 In areas with high prevalence of quintuple SP resistance, hybrid strategies show promise as a
318 solution to offset the respective weaknesses of IPTp-SP and ISTp. A potential advantage of hybrid
319 strategies over IPTp is that they prioritise the use of highly effective ACTs to those with the higher
320 density infections early in pregnancy most likely to cause harm. Retaining IPTp-SP for women who
321 test negative still receive the current standard of care and ensures that women with low-density
322 sub-patent infections are not left untreated.. Standard RDTs perform well at the first antenatal visit
323 in the second trimester, when prevalence and parasite densities are highest, largely offsetting the
324 need for more sensitive diagnostics, at least whilst SP retains the majority of its curative efficacy
325 (which is the case even in high quintuple resistant areas^{3,20}). However, given the complexity of multi-
326 day ACT dosing regimens, the theoretical advantages (in terms of efficacy with 100% adherence) and
327 real-life advantages(,accounting for adherence, need to be carefully considered to ensure that
328 switching strategies does not lead to lower protection relative to IPTp-SP in practice.

329 Hybrid strategies may only represent an interim solution if SP resistance continues to increase and
330 SP effectiveness progressively declines¹⁹. Adding screening at first IPT-SP visit only alleviates some of
331 the risk associated with these infections, and more effective chemoprevention with longer-lasting

332 drugs such as DP is likely required to provide larger incremental benefits to pregnancy
333 outcome^{9,11,33,34}. Two confirmatory trials of IPTp-DP are currently ongoing in Kenya, Malawi and
334 Tanzania (clinicaltrials.gov NCT03208179 and NCT03009526). Estimates of SP prophylactic longevity
335 outside of pregnancy suggest equivalent prophylactic longevity to DP (approximately one month)³⁵
336 in areas where the quintuple SP resistance mutation is largely However, there remains a dearth of
337 data allowing the direct comparison of the effectiveness of the two drugs in pregnancy in such
338 settings, limiting our ability to provide guidance on the relative merits of IPTp when SP resistance is
339 low. Such data could also help to provide insight into the extent to which SP has impact upon non-
340 malarial causes of adverse pregnancy outcome which we do not capture in our analysis.

341 Our analysis highlights that a high proportion of pregnant women are already infected prior to the
342 second trimester, the earliest stage at which doses of IPTp-SP can be initiated.. A large proportion of
343 these infections are likely to have been acquired early in pregnancy or prior to conception, as
344 evidenced by genotyping pre-conception infecting parasites in Benin¹⁸ and the high prevalence of
345 infection in women first attending ANC outside of the transmission season in seasonal settings³⁶.
346 Our results support the findings from Benin in suggesting that in primigravidae, low-density
347 infections at conception persist, multiply and sequester within the placenta at crucial stages of
348 development¹⁸. Thus, adding screening for malaria in the first trimester could have important
349 benefits. This relies upon women being aware of their pregnancy and ANC provision and attendance
350 during this period, though, first trimester ANC is a strong focus of updated 2016 WHO ANC
351 guidelines³⁷ which now recommends a first ANC visit prior to 12 weeks gestation, and the drive to
352 improve ANC as part of the wider Sustainable Development Goals³⁸.

353 Estimating the impact of treating first trimester infections upon birth outcome, and the extent to
354 which this depends upon subsequent IPTp uptake, is challenging as most studies measuring
355 associations between early infection and birth outcome do so in the context that these infections
356 are effectively treated upon detection. Some adverse pregnancy outcomes associated with first
357 trimester infection, such as disruption of the development of aspects of placental vasculature, may
358 be irreversible³⁹, whereas for others, e.g. intrauterine growth restriction, IPTp-SP may allow recovery
359 and catch-up growth later in pregnancy⁴⁰. In the absence of randomised controlled trials of the
360 impact of first-trimester screening, the findings that parasite densities are likely to be on the rise
361 early in pregnancy, and the increasing data suggesting a negative impact of these infections upon
362 placental and fetal development, even in the presence of IPTp-SP^{15,39}, suggest there is no threshold
363 level of parasitaemia under which women can be safely exposed during the first trimester. Providing
364 presumptive antimalarial treatment or prophylaxis at this stage of pregnancy is challenging as ACTs
365 are only recommended for case-management in the first trimester. However, the ability to identify
366 women carrying infections at this stage by testing with a highly sensitive diagnostic test, and thus
367 treat infections before they have the chance to multiply and sequester within the placenta, has the
368 potential to provide substantial and lasting benefits to maternal, fetal, neonatal, and infant health..
369 The only published study assessing the performance of existing next-generation highly-sensitive
370 RDTs during pregnancy detected a statistically insignificant higher number of PCR positive infections
371 than conventional RDTs⁴¹. However, this study was conducted in an area of low transmission, with
372 testing conducted throughout gestation and at delivery. Interpreting these results in terms of the
373 value of such tests for first trimester screening in areas of higher transmission is challenging.

374 The extent to which hybrid strategies would affect uptake of ANC-based interventions aimed
 375 towards preventing MiP (IPTp and ITNs) is unknown and will be a large determinant of the impact of
 376 such a shift in policy. Since adopting a single screen and treat hybrid approach as policy, the uptake
 377 of routine testing as an ANC-based intervention in Tanzania has been rapidly increasing, from 36.7%
 378 in 2014 to 88.8% in 2017¹⁴. This uptake is particularly impressive in the history of scale-up of IPTp,
 379 both in Tanzania, where IPTp-SP became policy in 2001 but where only 56% of pregnant women
 380 received two or more doses of SP in 2017, and more generally across Africa⁶. Understanding
 381 whether this rate of uptake would be mirrored in other countries, and whether it leads to a higher
 382 proportion of ANC attendees receiving any malaria-specific intervention, will be key to
 383 understanding the overall role testing may have in improving the limited protection from malaria
 384 currently provided to pregnant women.

385 This study has several limitations. The epidemiology of MiP is complex, particularly placental
 386 infection, which can only be reliably measured at delivery. As a result, and given the challenges
 387 associated with quantifying the attributable burden of multifactorial negative pregnancy outcomes
 388 such as LBW, preterm delivery, and fetal loss, we were not able to include direct estimates of the
 389 impact of these interventions upon many of the negative effects of malaria in pregnancy. Although
 390 we can estimate the impact of different strategies on the incidence of new infection, we could not
 391 quantify these effects on the burden of clinical malaria, neither could we quantify impact on
 392 prematurity and stillbirth, which are likely to depend upon timing during gestation and transmission
 393 intensity²³. Our analysis does not include any consideration of optimal strategies to protect HIV-
 394 infected pregnant women who currently receive daily cotrimoxazole, which provides sub-optimal
 395 protection from malaria⁴². Finally, we do not capture the potential value data from ANC-based
 396 screening to improve malaria surveillance⁴³.

397 In conclusion, our modelling suggests that screening and treatment with the current generation of
 398 RDTs would not provide incremental effectiveness relative to WHO's existing IPTp-SP strategy, even
 399 in areas with high quintuple mutation SP resistance. However, screen-and-treat strategies may have
 400 incremental benefit if the effectiveness of IPTp-SP is reduced further by resistance, especially in
 401 areas with high prevalence of sextuple SP mutants. Our model suggests that hybrid strategies
 402 integrating g screening at the first antenatal visit into existing IPTp-SP regimens are potentially
 403 beneficial in areas with high prevalence quintuple mutation SP resistance. Moreover, screening
 404 women routinely for malaria in the first trimester and providing effective treatment could provide
 405 substantial benefit, particularly if suitable highly sensitive diagnostics for first trimester infection can
 406 be identified.

407 **Methods**

408 **Estimating the effects of pregnancy on RDT performance within ANC**

409 We related the observed sensitivity of RDTs at enrolment in the ISTp trials to RDT sensitivity in the
 410 general population and the acquisition of pregnancy-specific immunity due to prior exposure to MiP
 411 according to the following function (see supplementary information for full details of models and
 412 model fitting):

$$413 \quad \text{Odds}(S_{ij}^W) = \text{Odds}(S^A(x_j)) \left(1 + \frac{\beta}{(1 + y_{ij}/\delta)^v} \right). \quad (1)$$

414

415 Here $S^A(x_j)$ – describes the probability, p , that a PCR positive infection in the general population is
416 detected by RDT, following a function of overall PCR prevalence within the setting, x_j . Odds are
417 related to probability p by the general relationship $\text{Odds}(p) = \frac{p}{1-p}$.

418 The sensitivity S_{ij}^W represents the probability that a PCR-positive infection of a newly enrolled
419 pregnant woman i within site j is detected by RDT. The odds of detection in primigravidae are
420 boosted by a constant β relative to the equivalent odds for the probability of detecting infection by
421 RDT outside of pregnancy. This pregnancy-related boost in detectability, relative to adults in the
422 general population, then decreases with increasing number of pregnancies in which a woman has
423 previously been exposed to malaria, denoted y_{ij} . This follows a Hill function with offset parameter δ
424 and power parameter ν .

425 Neither the sensitivity of RDTs outside of pregnancy nor the exposure history in previous
426 pregnancies were available in the data. Instead, we relied on the following fitted relationship
427 between RDT sensitivity and PCR prevalence obtained by *Wu et al*²²:

428
$$S^A(x_j) = \left[x_j \left(1 + \exp \left(-(\mu_A * \text{Odds}(x_j) + \sigma_A) \right) \right) \right]^{-1}, \quad (2)$$

429

430 where $\mu_A = 1.30$ and $\sigma_A = -1.38$ are the best fitting parameters obtained by fitting this model to
431 matched cross-sectional RDT and PCR samples in people aged over 15 (parameters obtained from
432 fitting to matched RDT and PCR data from children under 5, young adults aged 5-15 and all-age
433 surveys were also included within separate model fits for comparison)²². Working within a Bayesian
434 framework we were then able to simultaneously fit this model and a previously developed model of
435 the relationship between malaria transmission and exposure to MiP¹⁷ (see supplementary materials
436 for full details) to the gravidity-specific patterns of RDT and PCR detection across each setting,
437 accounting for uncertainty in y_{ij} , the number of previous pregnancies during which each women
438 would have been exposed to malaria. This provided inference on the parameters β , δ and ν
439 determining the impact of pregnancy upon detectability of infection using RDT. This model was
440 fitted alongside models where the sensitivity of RDTs at enrolment were independent of either
441 transmission intensity or gravidity, or independent of both, and compared using the Deviance
442 Information Criterion (DIC) (see supplementary information for full details of model fitting).

443 The probability of detecting infection at later ISTp visits was modelled as a separate logistic
444 multivariable regression accounting for random effects between study sites. Gravidity, infection
445 status of the previous test and overall throughout pregnancy, and the number of previous visits or
446 tests were all included as potential predictors of RDT sensitivity. Model selection was carried out
447 using AIC, and parameters of the best-fitting regression were included in the dynamical model (see
448 supplementary information for a detailed description of this analysis).

449 **Modelling the impact of interventions upon parasite prevalence**

450 We extended our existing model, linking transmission in the general population to the risk and
451 burden of MiP and effectiveness of IPTp³ to incorporate our estimates of RDT sensitivity by gravidity
452 and throughout pregnancy (see supplementary information for full details). The overall fit of this
453 model was assessed by visually comparing PCR prevalence throughout pregnancy in the ISTp arm in
454 each trial site, with data restricted to women with the modal number of visits in each site, with 95%
455 uncertainty intervals of trajectories of PCR prevalence throughout pregnancy generated by the
456 model using 1000 draws from the joint posterior distribution of β , δ and ν and from the parameters
457 within the final regression model of RDT sensitivity after enrolment, with the model calibrated by
458 varying EIR to match a draw from the 95% confidence interval PCR prevalence at enrolment in
459 primigravidae in each site (see Figure 3).

460 For all protocols involving the use of SP we considered three separate scenarios with respect to the
461 resistance of *Pf* to the drug on the basis of the prevalence of the quintuple K540E resistance
462 mutation and *in vivo* efficacy data: ‘Low quintuple resistance’ (K540E mutation prevalence < 15%),
463 women are protected with almost no treatment failures over the period of a month (here we
464 assume a Weibull-distributed period of protection which SP prevents over 50% of infections until
465 mean period of prophylaxis of 28 days, reflecting the observation that reinfections following
466 treatment in these areas appear to begin occurring around this duration post-treatment), ‘high
467 quintuple resistance’ (K540E mutation prevalence > 85%) where the risk of infection recrudescence
468 has been estimated to be 21.6% but re-infections appear to occur readily around a week after
469 treatment, and an ‘intermediate quintuple resistance’ category (15% < K540E prevalence < 85%)
470 where treatment fails to clear infection around 10% of the time and prophylaxis appears to last
471 around two weeks²⁰. There remains no efficacy data on the effects of the A581G sextuple mutation
472 in pregnant women, but there is evidence to suggest that IPTp-SP efficacy may be severely
473 compromised in settings with prevalence greater than 37%¹⁹. As a result, we also carried out
474 simulations under the scenario that SP is provided but has no impact. In the absence of data, we
475 assume that treatment failure occurs randomly with respect to gravidity, gestational time, or
476 whether an infection is detectable by RDT. The ACTs AL and DP were also assumed to have near
477 perfect efficacy in clearing ongoing parasitaemia. AL was assumed to have a mean prophylactic half-
478 life of 14, matching that estimated outside of pregnancy by *Okell et al* (13.8 days [range 10.2–22.8
479 days])²⁵. In the same analysis *Okell et al* estimated a prophylactic half-life of DP outside of pregnancy
480 of 29.4 days [range 16.4–48.8 days]²⁵, similar to our assumed duration of effectiveness of SP in areas
481 of low quintuple resistance. As a result, in the absence of specific data comparing duration of
482 effectiveness of SP and DP in pregnancy in areas of low quintuple resistance, we assumed the same
483 prophylactic profile for both drugs and avoid drawing conclusions as to the relative merits of the two
484 combinations in such settings.

485 Estimates of the extent to which screening infection in the first trimester will prevent low-
486 birthweight are based upon a previous analysis looking at different models of the relationship
487 between exposure to malaria and malaria-attributable LBWs, the best fitting of which involved a
488 relationship depending upon the level of chronic placental infection during pregnancy which was
489 modified by exposure to infection during previous pregnancy⁵. We make the conservative
490 assumption that, prior to the beginning of the second trimester, variation in detectability of infection
491 using RDT will be random with respect to gravidity. When estimating the proportion of LBW-causing
492 infections detected by standard RDTs at first visit in the second trimester (e.g. Figure 5), we
493 incorporate the dependence between both LBW risk and RDT sensitivity and pregnancy-specific

494 immunity (see Supplementary information for full details and parameter values of our model of
495 malaria-attributable LBW). However, we again make a likely conservative assumption with respect to
496 any advantage of RDT-based screening that, for a given level of pregnancy-specific immunity, there is
497 no difference between RDT detectable and undetectable infection in terms of attributable LBW risk.
498 As highlighted in the results, we are not able to estimate the potential impact that clearing infection
499 later in pregnancy through IPTp-SP may have upon this risk.

500 WHO recommends IPTp is given at 13 weeks gestation then subsequently every 4 weeks³⁷, however,
501 to avoid presenting an over-optimised picture of interventions in pregnancy, we here model IPTp
502 (and corresponding ISTp or hybrid) schedules of 3 or 4 contacts rather than monthly, which are more
503 reflective of the number of ANC contacts that women generally have across Africa³.

504 **Author Contributions**

505 PGTW, MC, ACHG and FTK conceived and designed the study. PGTW, MC, JG, HS conducted analyses
506 and prepared figures. MC, JG, KK, JEW, SOC, CK, ST, SRM, JH, VM, LKP, KB, SK, HT, MM, MD and FtK
507 contributed data or aided with the interpretation of data. All authors contributed to drafting and
508 revising the manuscript.

509 **Competing Interests**

510 The authors declare no competing interests.

511 **Disclaimer**

512 The findings and conclusions in this report are those of the author(s) and do not necessarily
513 represent the official position of the Centers for Disease Control and Prevention.

514 **Data availability statement**

515 There are three separate primary data sources used in this analysis:

516 1) Matched RDT and PCR samples from a trial of Intermittent Screening and Treatment in pregnancy
517 based in four countries in West Africa (see reference 8 in the main manuscript for full details)

518 2) Matched RDT and PCR samples from a trial of Intermittent Screening and Treatment in pregnancy
519 in Western Kenya (see reference 9 in the main manuscript for full details)

520 3) Matched RDT and PCR samples from a trial of Intermittent Screening and Treatment in pregnancy
521 in Malawi taken from reference (see reference 10 in the main manuscript for full details)

522 4) A review of matched RDT and PCR prevalence in non-pregnant adults (see reference 22 in the
523 main manuscript for full details)

524 Figures 1-3 show data from sources 1-3. Supplementary Figure 1 and Supplementary Tables 2-4
525 show output from fitting to these data. Figure 2c show data from source 4.

526 Source 1 is available subject to agreement with the original authors from the LSHTM Data Compass
527 <https://datacompass.lshtm.ac.uk/4/>

528 Sources 2 and 3 are available for access with the WorldWide Antimalarial Resistance Network
529 (WWARN) at www.WWARN.org. Requests for access will be reviewed by a Data Access Committee
530 to ensure that use of data protects patient privacy according to the terms of consent and ethics
531 approval.

532 Source 4 is freely available to download from a supplementary data file from

533 <https://www.nature.com/articles/nature16039>

534

535 **Code availability statement**

536 Source code of the mathematical model developed and used within this analysis, along with a
537 compiled version and compilation and running instructions are available open access at the
538 following repository: www.github.com/patrickgtwalker/malaria_in_pregnancy_istp_model_open

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661

662 **Figure 1 | Prevalence by and RDT and PCR during ISTp.** Figure shows prevalence of RDT positive infection, confirmed by
663 PCR, (height of darker bars) and the additional prevalence of RDT negative, PCR positive infection prevalence (height of
664 lighter bars) at each ANC visit at which RDT testing was carried out during ISTp from enrolment to delivery by trial site.

665 **Figure 2 | Comparing RDT performance at enrolment in ISTp trials to outside of pregnancy.** Figure shows (a) RDT
666 sensitivity relative to PCR by gravidity across each of the six study countries (ordered by PCR prevalence in primigravidae),
667 red dots and error bars show mean and 95% C.I. for RDT sensitivity within the trials. For comparison, the estimated
668 sensitivity in non-pregnant individuals (male or female) aged >15 years old (green dashed line) and for children under 5
669 years old (blue dashed line) based upon the relationship between transmission and RDT sensitivity from *Wu et al.*²² are also
670 shown. The red line and orange polygon show the mean and 95% Cr.I.s for the best fitting model incorporating a declining
671 boost in detectability of infection with RDT dependent upon the level of exposure in previous pregnancy, (b) shows the
672 fitted relationship (see Methods) from this model (blue line shows median and polygon 95% Cr.I.) of the odds ratio of
673 detection at enrolment and non-pregnant individuals (male or female) aged >15 years old and (c) Yellow dots and line
674 show the data and the fitted relationship between PCR and RDT prevalence in non-pregnant individuals (male or female)
675 aged >15 years old from *Wu et al.*²². Remaining colours show the estimated relationship between gravidity-specific PCR
676 prevalence and RDT prevalence from this model (lines) compared to the data (dots with horizontal and vertical bars
677 showing 95% C.I. for PCR prevalence and RDT prevalence respectively).

678 **Figure 3 | Comparison of simulated and observed dynamics of infection throughout trials of ISTp.** Figure shows observed
679 PCR prevalence throughout successive screens during pregnancy (dots with error bars representing 95% C.I.s). Pink areas
680 show the 95% cr.I. for PCR prevalence throughout pregnancy in the absence of intervention. Red datapoints indicate
681 observed prevalence at enrolment in primigravidae to which the model is calibrated for each trial, the remaining
682 datapoints, marked in blue, represents dynamics the model aims to replicate, with sharp drops in prevalence
683 corresponding to ISTp rounds. Blue areas show the 95% cr.I.s generated by the posterior distribution of the fitted model in
684 each scenario (see supplementary information for full details) with blue lines representing the posterior median PCR
685 prevalence. Note for the trial in Ghana only primi- and secundigravidae were recruited but the simulated output is still
686 shown for completeness.

687 **Figure 4 | Simulated Incremental benefit of alternative strategies to IPTp-SP by level of SP resistance.** Simulations are for
688 high transmission settings (EIR=100), top row shows peripheral PCR prevalence in primigravidae alone, bottom row
689 averaged across all pregnant women. Left column, (a) and (d), represent areas with low quintuple SP mutation, centre, (b)
690 and (e), with high quintuple mutation and right, (c) and (f), represents a scenario with sextuple resistance where SP is
691 assumed to no longer provide any protection. Simulations show the following strategies: no intervention (black lines), IPTp-
692 SP (yellow lines), ISTp –DP (orange lines), Hybrid-SSTp (light purple lines), Hybrid-ISTp (dark purple lines) and IPTp-DP (blue
693 lines). In general, for scenarios involving ACTs, simulations with DP are shown. In select situations simulations with shorter-
694 acting AL are shown with dashed line. NB: In settings with low quintuple SP mutations, SP and DP are assumed to have
695 equivalent efficacy so IPTp and hybrid strategies involving these drugs are indistinguishable when SP has no impact, ISTp
696 and hybrid strategies using the same treatment drug are indistinguishable.

697 **Figure 5 | The relative effectiveness of IPTp, ISTp and hybrid strategies in clearing early infection.** (a) shows the
698 percentage of primigravidae with ongoing parasitaemia following their first visit in 2nd trimester if they: receive no
699 intervention (yellow dots), are screened with a standard RDT and treated with an ACT if positive (green dots), receive SP
700 presumptively (light blue dots), are given an ACT if RDT+ and SP otherwise (hybrid strategy – dark blue dots). Error bars
701 show 95% intervals based upon our uncertainty analysis for comparing the relative impact of intervention strategies (see

702 methods), (b) shows the equivalent figure but in women of gravidities 3 and above. (c) shows the percentage of these early
703 infections that would subsequently lead to LBW that are effectively treated based upon our modelled relationship between
704 the detectability and severity of infection (NB: given these are ongoing infections this does not imply that treating these
705 infections would necessarily avert all risk of LBW attributable to these infections- see *Methods* for full details).

706 **Figure 6 | Impact of different strategies upon infection later in pregnancy in areas of high quintuple SP resistance.** Figure
707 shows the impact of different simulated strategies upon the incidence of new (defined as either symptomatic or
708 asymptomatic blood-stage) infection following a first ANC visit in the second trimester at 20 weeks gestation in areas of
709 low, moderate and high transmission (EIRs of 1,10 and 100). (a) shows the percentage of women who will experience any
710 new infection in the second or third trimester (T2/3), (b) shows the average number of new infections occurring
711 throughout T2/3. Each strategy is assumed to involve three scheduled ANC visits occurring at 20, 27 and 34 weeks, except
712 for “Monthly DP” (darkest blue) which involves five visits spaced 30 days apart from 20 weeks onwards. A perfect test
713 refers to a hypothetical diagnostic with perfect sensitivity and specificity for peripheral or placental infection. Error bars
714 show 95% intervals based upon our uncertainty analysis for comparing the relative impact of intervention strategies (see
715 methods)

716 **Figure 7 | Potential impact of routinely testing for malaria during the first trimester. (a)** an illustration of the
717 hypothesised mechanism by which the performance of standard RDTs are modified by gravidity. Women often experience
718 chronic, asymptomatic parasitaemia outside of pregnancy which, as parasites are progressively cleared by the immune
719 system, would eventually fall below the limit of detection of standard RDTs and be cleared if she had not conceived (grey
720 line). If an asymptotically infected woman becomes pregnant, and as her placenta develops so that maternal blood
721 flows into the intervillous space (towards the end of the first trimester), the parasite undergoes antigenic switching,
722 allowing it to bind to placental Chondroitin Sulphate A (CSA) receptors¹, multiplying to higher densities in women who have
723 never experienced placental infection, leading to more severe and, due to higher concentrations of HRP2, more detectable
724 infections (purple line). In subsequent pregnancies women mount a specific, acquired immune response, leading to better
725 controlled, lower density and less detectable infection (turquoise and blue lines). (b) shows a simulated example of the
726 impact of testing and clearing infections at a first ANC visit at 10 weeks upon overall exposure to placental infection in
727 primigravidae in a setting of EIR=10 (ongoing peripheral infections are assumed to begin sequestering from the end of the
728 first trimester onwards), simulations reflect our uncertainty in the sensitivity of the RDT at this time point, ranging from
729 26.8% (RDT sensitivity for asymptomatic infection in adults outside of pregnancy in such a setting based upon the
730 relationship estimated in *Wu et al.*²²) to 90% (the approximate sensitivity of standard RDTs at first visit in areas of high
731 transmission in primigravidae in ISTp trials). (c) shows the proportional impact this screening would have upon the mean
732 duration of placental infection either in the presence or absence of IPTp-SP (assuming low SP resistance) and by
733 transmission intensity. (d) the impact upon the risk of LBW according to our model relationship between the duration and
734 stage of placental infection and LBW⁵, two thirds of these bars are coloured transparently emphasising our uncertainty in
735 impact of IPTp-SP in terms of promoting catch-up growth⁴⁰.

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