Published in final edited form as:

J Infect Dis. 2021 March 29; 223(6): 995–1004. doi:10.1093/infdis/jiaa476.

School-Based Serosurveys to Assess the Validity of Using Routine Health Facility Data to Target Malaria Interventions in the Central Highlands of Madagascar

Laura C. Steinhardt[1], **Elisabeth Ravaoarisoa**[3,4], **Ryan Wiegand**[1], **Aina Harimanana**[5], **Judith Hedje**[1,6], **Annett H. Cotte**[1,2], **Sixte Zigirumugabe**[6,7], **Thomas Kesteman**[3,8], **Tsikiniaina L. Rasoloharimanana**[9], **Emma Rakotomalala**[9], **Anny M. Randriamoramanana**[5], **Jean-Marius Rakotondramanga**[5], **Seheno Razanatsiorimalala**[3], **Odile Mercereau-Puijalon**[10], **Ronald Perraut**[11], **Arsène Ratsimbasoa**[12,13,14], **Jessica Butts**[1,2], **Christophe Rogier**[14,15], **Patrice Piola**[5,16], **Milijaona Randrianarivelojosia**[3], **Inès Vigan-Womas**[9]

[1]Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

[2]U.S. President's Malaria Initiative, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

[3]Malaria Research Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar

[4]Faculty of Sciences, University of Antananarivo, Antananarivo, Madagascar

[5]Epidemiology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar

[6]US President's Malaria Initiative, Antananarivo, Madagascar

[7]United States Agency for International Development, Washington, District of Columbia, USA

[8]Fondation Merieux, Lyon, France

[9]Immunology of Infectious Diseases Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar

[10]Parasites and Insect Vectors Department, Institut Pasteur, Paris, France

[11]Immunology Unit, Institut Pasteur de Dakar, Dakar, Senegal

[12]National Malaria Control Program of Madagascar, Ministry of Public Health, Antananarivo, Madagascar

Correspondence: Laura Steinhardt, PhD, MPH, Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Mailstop A-06, Atlanta, GA 30329 (LSteinhardt@cdc.gov).

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

[13]Faculty of Medicine, University of Antananarivo, Antananarivo, Madagascar

[14]L'Institut Hospitalo-Universitaire en Maladies Infectieuses de Marseille, Marseille, France

[15]Institut Pasteur de Madagascar, Antananarivo, Madagascar

[16]Epidemiology and Public Health Unit, Institut Pasteur du Cambodge, Phnom Penh, Cambodia

Abstract

Background.—In low-malaria–transmission areas of Madagascar, annual parasite incidence (API) from routine data has been used to target indoor residual spraying at subdistrict commune level. To assess validity of this approach, we conducted school-based serological surveys and health facility (HF) data quality assessments in 7 districts to compare API to gold-standard commune-level serological measures.

Methods.—At 2 primary schools in each of 93 communes, 60 students were randomly selected with parents and teachers. Capillary blood was drawn for rapid diagnostic tests (RDTs) and serology. Multiplex bead-based immunoassays to detect antibodies to 5 *Plasmodium falciparum* antigens were conducted, and finite mixture models used to characterize seronegative and seropositive populations. Reversible catalytic models generated commune-level annual seroconversion rates (SCRs). HF register data were abstracted to assess completeness and accuracy.

Results.—RDT positivity from 12 770 samples was 0.5%. Seroprevalence to tested antigens ranged from 17.9% (MSP-1) to 59.7% (PF13). Median commune-level SCR was 0.0108 (range, 0.001–0.075). Compared to SCRs, API identified 71% (95% confidence interval, 51%–87%) of the 30% highest-transmission communes; sensitivity declined at lower levels. Routine data accuracy did not substantially affect API performance.

Conclusions.—API performs reasonably well at identifying higher-transmission communes but sensitivity declined at lower transmission levels.

Keywords

serology; school-based surveys; malaria; Madagascar; stratification

---

Although malaria has declined in Madagascar in the last 2 decades, transmission has increased recently, with increasing focal outbreaks in the last several years [1–5]. Indoor residual spraying (IRS) campaigns with effective insecticides are one of the primary approaches for vector control to reduce malaria burden and prevent outbreaks in epidemic-prone areas. The Central Highlands (CHL) of Madagascar represent an area of unstable malaria transmission >800 meters in altitude that is prone to malaria epidemics. Generalized IRS, that is spraying all houses in all communes (a subdistrict administrative unit) within targeted districts, was carried out using dichlorodiphenyltrichloroethane (DDT) in the CHL from 1993 until 1998; from 1999 to 2007, focalized spraying (only certain communes per district) took place using DDT until 2003 and pyrethroids thereafter.

After the Ministry of Health declared in 2005 a goal of malaria elimination in Madagascar, generalized IRS in the CHL was restarted in 2008 and soon expanded to the Fringe areas

surrounding the CHL, using pyrethroid insecticides (CHL where long-lasting insecticidal nets [LLINs] were not distributed) and carbamates (Fringe areas with LLIN distributions). After 4 years of generalized district-wide spraying, declining resources forced Madagascar to switch to focalized spraying in the CHL/Fringe areas, targeting subdistrict-level communes in 2012. Health facility (HF)-confirmed malaria case data were used to identify and prioritize approximately 30% of communes (within the 33 districts) with the highest malaria incidence in 2011 for focalized spraying.

Using routine malaria case data for stratifying communes by transmission intensity for focalized IRS is potentially problematic because of variable quality and completeness of HF data [6]. In addition, rates of care-seeking in the formal public sector are generally low in Madagascar [7], with only 35.2% of febrile children <5 years old taken to public facilities or community health workers [8]; this rate is 36.0% in the CHL but only 20.1% in the surrounding Fringe areas, where part of the study took place. Accurate stratification of malaria transmission intensity is important for targeting interventions, especially as countries pursue malaria elimination [9]. Better understanding the validity of HF malaria case data for targeting IRS to higher-transmission areas is an important programmatic exercise as countries decide how to deploy more effectively and efficiently limited vector control resources.

Serological markers of malaria exposure can detect malaria hotspots in low-transmission settings [10–12]. Longer-lived antibodies to malaria, such as AMA-1 and MSP-1, can persist over time and represent a more stable measure of malaria transmission compared to parasite prevalence, which can vary substantially between and within transmission seasons [13, 14]. Serology has recently been shown to be a valid tool for measuring variation in local transmission intensity from samples collected in communities [15] and HFs [16]. School-based surveys are significantly less expensive than household surveys [17, 18], and serological measures from school-attending children can be valid for generalizing serological estimates to the surrounding commune [19].

We conducted school-based serological and parasite prevalence surveys to assess the validity of using HF malaria case data to target communes for focalized IRS in Madagascar.

METHODS

Study Sites

Malaria transmission in the CHL is unstable and episodic. In the Fringe areas, approximately 500 to 1000 meters in altitude, transmission patterns are seasonal, lasting from November to May (rainy season). In 2013, malaria prevalence in children aged 6–59 months was 0.7% and 2.5% by microscopy in the CHL and Fringe areas, respectively [8, 20]. Beginning in 2008, 32 districts in the CHL and Fringe areas, covering 29% of Madagascar's 114 districts, received IRS. The study area included 7 districts, 2 in the CHL (Ambohimahasoa, Ambositra) not covered by LLIN distributions, and 5 in the Fringe areas (Ambatofinandrahana, Anjozorobe, Ankazobe, Betafo, and Mandoto), covered by LLIN distributions, which had all undergone 4 consecutive years of blanket (district-wide) IRS, plus 2 years of focalized spraying, by the time of the survey in May 2014 (Figure 1). These

7 districts comprise 107 communes. Each commune has an average of 14.6 primary schools (average of 154 children per school).

Each commune has at least 1 primary health center, or *centre de santé de base* (CSB), and in most cases 2 lower-level health centers, which should be staffed by a paramedical worker and an assistant, and serve approximately 4000 people. Midlevel health centers should be staffed by a medical doctor, nurse, and midwife, and serve approximately 8000 people. Unfilled positions and frequent absenteeism mean that few are fully staffed. During data collection in May–July 2014, 14 of the 107 communes were not accessible due to insecurity or inaccessibility (eg, heavy rains, lack of roads), leaving 93 communes able to be surveyed.

Data Collection

School-Based Serological Surveys—Within each commune, each public primary school was mapped by Euclidean distance to the midlevel CSB (or lower-level CSB if there was no midlevel CSB in the commune); 1 nearby primary school within 5 km of the midlevel CSB and 1 far primary school (>10 km distance), each with enrolment of at least 50 children, were randomly selected to ensure balance regarding healthcare access. Sampled schools were contacted in advance and parents invited to attend on the day of the survey. Thirty children with parents present (6 children from each of the 5 class levels) and their parents were randomly sampled per school. All teachers present were sampled. If parents brought younger children with them, they were also included if parents consented. A total of 120 children, parents, and teachers were targeted per commune based on simulations that indicated a sample size of 100 observations or greater with a seroconversion rate (SCR) of 0.05 and a seroreversion rate of 0.01 had a small expected bias for estimating the SCR (Wiegand, personal communication).

A brief questionnaire on demographics, residence, bed net use, and recent travel history for the parent and child was administered to parents and teachers. Finger prick blood was collected for malaria rapid diagnostic tests (RDTs) (CareStart Malaria RDT, HRP2/pLDH [Pf/PAN] Combo; Access Bio). Approximately 300–500 μL of capillary blood was collected in microvette tubes (Microvette 500 Z-Gel; Sarstedt) for later serological analysis. The microvettes were centrifuged for 10 minutes at 8000 rpm and stored at −20°C until use. Results of RDTs were disclosed to individuals or their guardian; individuals with a positive RDT were given artesunate-amodiaquine (ASAQ) according to national guidelines. The first treatment dose was administered at the school, and parents were instructed on how to give/take the remaining ASAQ doses at home.

Health Facility Data Quality Assessments—Survey teams visited all open and accessible public lower-level and midlevel CSBs in the 7 districts (estimated total of 179) to conduct rapid data quality assessments. The purpose of this exercise was to explore how varying degrees of data quality affect utility of routine data for estimating malaria transmission intensity. Clinical register data were abstracted for 4 preselected months and assessed for completeness. Register data reporting accuracy was assessed through comparisons with health management information system (HMIS) data (full details in the Supplementary Materials).

Routine Malaria Data—Routine data on HF-based RDT-confirmed malaria cases in 2013 were obtained from the National Malaria Control Program (NMCP) and divided by the estimated commune-level population to calculate the annual parasite incidence (API). For IRS targeting, the NMCP primarily used rank-ordered commune API, selecting communes with APIs in the highest 30% (due to budgetary constraints), although occasionally RDT testpositivity or whether a commune had submitted a malaria epidemic alert in the previous year were considered as well.

Serological Analysis

Three soluble recombinant proteins (PF13, PfMSP1, and PfAMA1) and 2 bovine serum albumin (BSA)-conjugated peptides (PfCSP and PfGLURP) from *Plasmodium falciparum* were included. BSA (GeneCust) was used as carrier control. Full details of the antigen preparation are in the Supplementary Materials. Carboxylated magnetic MagPlex beads (Luminex) were covalently coupled with recombinant proteins, peptide-BSA complexes, or BSA as background control using the xMAP Antibody Coupling Kit (Luminex) following manufacturers' instructions, and using procedures previously described [21–23]. Antigen-coupled beads and plasma were deposited in 96-well plates (additional details in Supplementary Materials) and analyzed using the Luminex-MAGPIX system (Luminex) and xPONENT 4.1 software. IgG levels were expressed as median fluorescence intensity (MFI). A pool of sera from malaria-immune African adults and plasma samples from malaria-naive European individuals were included in each assay as positive and negative controls, respectively.

Data Analyses

Full details on statistical methods can be found in the Supplementary Materials. Briefly, finite mixture models were used to determine which participants were considered negative (unexposed) and positive (exposed) for each antigen. MFI values were $\log_{10}$-transformed due to skewness in all distributions; participants with negative MFI- background values were recoded to 1 so all participants could be included. Using MFI data for all *P. falciparum* antigens, a latent class model was fit to determine an overall *P. falciparum* seropositivity latent variable for each participant. Seropositivity status from the latent class model was then used in reversible catalytic models to calculate SCRs for each commune.

Given the right-skewed distributions of both API and SCR, values of each measure were $\log_{10}$-transformed in analyses. All communes had 0.1 added to incidence values prior to transformation to include communes with zero incidence. Relationships between commune-level SCRs, as a gold standard, and APIs were assessed via regression models. All models attempted were univariable models with $\log_{10}$-transformed API as the outcome variable and $\log_{10}$-transformed SCR as the predictor. Different models were attempted and best-fitting regression models were used (see Supplementary Materials). Final models either included $\log_{10}$-transformed SCR as a linear term or as piecewise linear with 2 intercepts and slopes.

The sensitivity and specificity of API for correctly identifying the 30% of communes with the most intense transmission according to SCR were assessed. This process was then duplicated for other percentages of communes with highest transmission by SCR. Sensitivity

and specificity were separately evaluated for the subsets of districts with lower (n = 3) and higher (n = 4) accuracy and completeness scores. Prevalence estimate confidence intervals across communes used the delta method to account for clustering at the school level or Wilson method [24] when no participants were positive. Analyses were carried out in R and QGIS version 2.18.1 (QGIS Development Team) was used for mapping, and the 5% level of significance was used.

Ethics Approval and Consent to Participate

The study protocol was approved by the National Ethics Committee of the Ministry of Public Health of Madagascar (approval number CNE 011-MSANP/CE, 26 March 2014) and by the US Centers for Disease Control and Prevention Institutional Review Board. At sampled schools, after explaining the study objectives and procedures, individual, informed consent was obtained from caregivers of sampled students, younger children, and from teachers. Assent was obtained from students aged 7–17 years old.

RESULTS

Survey teams visited 185 schools (2/commune) of an estimated 1372 public primary schools and 141 HFs of an estimated 179 HFs in the 93 accessible communes (Supplementary Figure 1). Altogether, 6447 children and 6448 parents and teachers were surveyed, and 12 770 of the 12 895 (99.0%) surveyed participants had complete serology and demographic data. Most participants were either school aged (5–14 years old, 49.2%) or older than 20 years (48.8%) (Table 1).

Overall RDT positivity was very low at 0.5% and ranged from 0% to 13.3% by commune (Table 1, Supplementary Table 1, and Figure 2). Of the 93 communes surveyed, 68 (73%) had zero positive RDTs (Supplementary Table 1). Seropositivity to the *P. falciparum* antigens ranged from 17.9% of participants seropositive for PfMSP1 to 59.7% seropositive to PF13 (Table 1). Median SCRs by commune for the *P. falciparum* antigens followed the same trend as overall mean seropositivity, with PfMSP1, PfAMA1, PfCSP, PfGLURP, and PF13 having increasing values of median SCR/seropositivity (Table 1). Seropositivity for the *P. falciparum* latent antigen was 24.3% overall, ranging by commune from 2.0% to 67.2% (Table 1 and Supplementary Table 1). The SCR for the *P. falciparum* latent antigen had a median of 0.010 across communes, ranging from 0.001 to 0.075 (Table 1 and Figure 2). Commune-level API for 2013 from HF data ranged from 0.0 to 177.3 positive RDTs per 1000 population (Supplementary Table 1), with a median of 0 positive RDTs per 1000.

Routine Data Quality

Missingness of data in register fields was able to be assessed at 140 HFs in 91 communes, and data quality assessments were able to be conducted at HFs in 89 communes (HFs were inaccessible in 2 communes and HMIS data were not available for accuracy comparisons in an additional 2 communes). Missingness of the 4 register fields assessed was very low (average 1.9% missingness across all communes; range, 0.0%–23.6%) (Supplementary Table 2). It was not possible to gauge completeness of malaria data in terms of entire HFs

not reporting into the HMIS, as only HFs reporting data were included as a denominator and no master list beyond this existed.

Data accuracy, however, was much poorer, with a 34.4% mean absolute value discordance (range by commune, 0.0%–275%) between register tallies and numbers reported in HMIS for indicators examined (number of consultations, number of RDTs done, and number of positive RDTs). Overall district data quality scores combining data missingness and accuracy indicated that 6 of 7 districts had roughly similar quality scores; 1 district (Mandoto) had much worse scores (Supplementary Table 3). Weights for the data quality were then calculated as 1/(1 + mean proportion discordance). The median weight was 0.834 with a range from 0.267 to 1.000.

Relationship Between HF Incidence and Seropositivity Measures

The Akaike information criterion and Bayesian information criterion values indicated that spatial models did not provide a better fit but those accounting for commune-level data quality through an accuracy weight provided a marginally better fit (Supplementary Table 4). The final models selected were linear models for PF13, PfMSP1, PfCSP, and PfGLURP and piecewise linear models for PfAMA1 and the latent antigen (Figure 3). All models incorporated the data quality weights. For the 4 antigens with linear models, there was a significant positive relationship between SCR and API, although with wide variability and confidence intervals. For PfAMA1 and the *P. falciparum* latent antigen, there was a relatively flat relationship at lower levels of SCR (up to 0.0263 and 0.0219 for PfAMA1 and latent antigen, respectively), after which there was a significant positive relationship between SCR and API (Figure 3).

Using commune-level SCRs as a gold standard, we identified the 30% of communes with the highest malaria transmission. Using each commune's API, the sensitivity of detecting the top 30% ranged from 60.7% to 75.0%, and specificity ranged from 83.1% to 89.2%, depending on the antigen used (Figure 4). Sensitivity and specificity of API were 71% (95% confidence interval [CI], 51%–87%) and 88% (95% CI, 77%–95%), respectively, using the *P. falciparum* latent antigen as a gold standard (Figure 4). The performance of API versus gold standard SCRs, as measured by area under the curve (AUC), typically improved when targeting the highest transmission communes (eg, the top 10% or 20%, according to SCR); sensitivity of API and AUC declined, although specificity remained relatively constant, when targeting a broader band of communes (eg, the top 50% or 60%) (Table 2). However, variability in the AUC and sensitivity does not allow us to conclude that 1 quantile has a stronger association with SCR values.

To further explore the impact of data quality on performance of API for targeting high-transmission communes, we calculated sensitivity and specificity of API for identifying the top 30% of communes after stratifying by district quality (4 districts with higher quality versus 3 districts with lower quality), as well as for all districts except Mandoto, the outlying, low-data–quality district. Using API only in districts with higher-quality data did not improve prediction of higher-transmission communes (Supplementary Figure 2).

DISCUSSION

Similar to other settings [25–29], antibody data were much more sensitive and informative than RDT positivity, which was extremely low: only 25 of 93 (27%) communes had any positive RDTs among surveyed participants. Serological data were used in this study as a gold standard for assessing validity of using routine data from the HMIS to target higher-transmission communes for IRS. Previous studies have indicated high correlations between SCRs and clinical malaria incidence in cohort studies [16], as well as between SCRs and the entomological inoculation rate [14, 30], which has traditionally been the gold standard measure of malaria transmission.

The overall SCR for the study area for the latent antigen of 0.010 translates into roughly 1 seroconversion per 100 population per year. This is low and expected, given the low overall API from HF data in the study area of 3.5 reported cases per 1000 population. However, serological data revealed wide heterogeneity among the communes, where SCRs ranged from 0.001 to 0.075. Our inclusion of several antigens, which might represent different exposure histories, has been recommended for maximizing the utility of serology for malaria [30], and latent class analysis has been proposed as an approach to combine results from multiple tests when no gold standard exists [31, 32]. The latent class analysis analyzed the quantitative MFI values from the 5 antigens instead of using the binary classifications from each antigen. Therefore, this approach avoided the loss of information created from a binary classification for each antigen.

In our study area within the low-transmission CHL and Fringes areas, we found that API is relatively good at identifying higher-transmission communes, or hotspots, but sensitivity of this measure degrades at lower transmission levels, that is when trying to detect a larger percentage of communes as ranked by SCR. It should be noted that these findings are in the context of relatively low levels of public sector HF utilization in Madagascar (only 35.8% of children aged 6–59 months with fever were taken to the public sector for care according to a 2016 survey [20]) and poor data quality in the HMIS [6]. Interestingly, data quality did not appear to modify substantially the relationship between API and SCRs. Few studies have looked at the relationship between API from routine data and seropositivity. One study in a very low-transmission area of South Africa found no significant linear relationship between seroprevalence and historical ward-level malaria incidence [33].

This study had several limitations. We were only able to sample 2 schools (from an average of 14) per commune, thus our use of SCR as the gold standard for transmission intensity in this study relies on several assumptions, including that schoolchildren and their parents accurately represent commune transmission and that the 2 sampled schools are representative of the commune. Although appropriate for a low-transmission setting, the use of finite mixture models to produce seropositivity cutoffs assumes only 2 underlying components when in reality there could be more than that [34]. Further, our sample included only a handful of children younger than 5 years, who might be most informative for assessing recent transmission. However, lack of data from the youngest age group should in theory have affected only the confidence intervals on the SCRs but not the SCRs themselves [35]. Another potential limitation is that routine data for only the previous year

(2013) were used to calculate the API for comparison with the SCR, which might reflect more cumulative exposure than simply the previous year; however, comprehensive routine malaria data were not available before this and some studies have shown that several of the antibodies assessed have very short half-lives in children [36, 37]. Finally, community-level data were not captured during this time period in the routine data system. However, this should not compromise the utility of APIs as a relative measure of transmission intensity if the proportion of cases seen in the community does not vary substantially by commune.

Despite its limitations, this study provides important information on the validity of routine data to characterize relative malaria transmission intensity at subdistrict levels. Reassuringly, despite imperfect routine data, API performed reasonably well for identifying the highest-transmission communes. In many cases, routine data are the most readily-available—and sometimes the *only* available—information that program managers have for stratification efforts. Malaria program managers in Madagascar are increasingly using routine data to assess trends and predict and prevent outbreaks at the facility and commune level, as cases have increased nationwide beginning in 2017. In response to findings from this study, managers have also worked to improve the quality of routine data especially in outbreak-prone, low-transmission areas.

CONCLUSIONS

This study used serological data from multiple *P. falciparum* antibodies to estimate commune-level malaria transmission for identifying the highest-transmission communes, and for evaluating the validity of using routine data to target IRS. In low-transmission settings of Madagascar, API had a sensitivity of slightly above 70% compared to gold standard commune SCRs for identifying the 30% of communes with highest transmission. API performed better at differentiating communes on the higher end of transmission, but its performance declined when trying to target a greater percentage of communes. Factoring in data quality did not appear to change substantially the relationship between API and SCR. Although school-based surveys have the advantage of being relatively rapid and less costly than household surveys (total cost for this survey was approximately US $300 000), program managers must weigh their costs against using existing routine data or other less costly measures such as climate and vegetation data, which are increasingly being used to predict malaria transmission.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments.

We express our gratitude to the population of the districts and communes investigated and especially to the children, parents, guardians, and teachers who participated to the study. We also thank those who facilitated the survey, that is heads of communes and *fokontany*, local administration authorities, and health authorities from Ministry of Health and National Malaria Control Program. We also thank the survey teams.

Financial support.

This work was supported by the US President's Malaria Initiative program (grant number AID-687-G-13-00003 Surveillance and Data for Management Project).

References

1. World Health Organization (WHO). World malaria report 2017. Geneva, Switzerland: WHO, 2017.
2. World Health Organization (WHO). World malaria report 2018. Geneva, Switzerland: WHO, 2018.
3. Howes RE, Mioramalala SA, Ramiranirina B, et al. Contemporary epidemiological overview of malaria in Madagascar: operational utility of reported routine case data for malaria control planning. Malar J 2016; 15:502. [PubMed: 27756389]
4. Ihantamalala FA, Rakotoarimanana FMJ, Ramiadantsoa T, et al. Spatial and temporal dynamics of malaria in Madagascar. Malar J 2018; 17:58. [PubMed: 29391023]
5. Kang SY, Battle KE, Gibson HS, et al. . Spatio-temporal mapping of Madagascar's malaria indicator survey results to assess Plasmodium falciparum endemicity trends between 2011 and 2016. BMC Med 2018; 16:71. [PubMed: 29788968]
6. Ly M, N'Gbichi J-M, Lippeveld T, Ye Y. Rapport d'évaluation de la performance du système d'information sanitaire de routine (SISR) et de la surveillance intégrée de la maladie et la riposte (SIMR). Chapel Hill, NC: MEASURE Evaluation, 2016.
7. Mattern C, Pourette D, Raboanary E, et al. "Tazomoka is not a problem". Local perspectives on Malaria, fever case management and bed net use in Madagascar. PLoS One 2016; 11:e0151068.
8. Institut National de la Statistique (INSTS), Programme National de Lutte Contre le Paludisme (PNLP), Institut Pasteur de Madagascar IPM), ICF International. Madagascar. Enquete sur les indicateurs du paludisme 2013. Antananarivo, Madagascar and Calverton, MD: INSTAT, PNLP, IPM, ICF International, 2013.
9. World Health Organization (WHO). A framework for malaria elimination. Geneva, Switzerland: WHO, 2017.
10. Bousema T, Drakeley C, Gesase S, et al. Identification of hot spots of malaria transmission for targeted malaria control. J Infect Dis 2010; 201:1764–74. [PubMed: 20415536]
11. Bousema T, Griffin JT, Sauerwein RW, et al. Hitting hotspots: spatial targeting of malaria for control and elimination. PLoS Med 2012; 9:e1001165.
12. Lynch CA, Cook J, Nanyunja S, et al. Application of serological tools and spatial analysis to investigate malaria transmission dynamics in highland areas of Southwest Uganda. Am J Trop Med Hyg 2016; 94:1251–8. [PubMed: 27022156]
13. Corran P, Coleman P, Riley E, Drakeley C. Serology: a robust indicator of malaria transmission intensity? Trends Parasitol 2007; 23:575–82. [PubMed: 17988945]
14. Drakeley CJ, Corran PH, Coleman PG, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. Proc Natl Acad Sci U S A 2005; 102:5108–13. [PubMed: 15792998]
15. Satoguina J, Walther B, Drakeley C, et al. Comparison of surveillance methods applied to a situation of low malaria prevalence at rural sites in The Gambia and Guinea Bissau. Malar J 2009; 8:274. [PubMed: 19954532]
16. Stewart L, Gosling R, Griffin J, et al. Rapid assessment of malaria transmission using age-specific sero-conversion rates. PLoS One 2009; 4:e6083.
17. Gitonga CW, Karanja PN, Kihara J, et al. Implementing school malaria surveys in Kenya: towards a national surveillance system. Malar J 2010; 9:306. [PubMed: 21034492]
18. Brooker S, Kolaczinski JH, Gitonga CW, Noor AM, Snow RW. The use of schools for malaria surveillance and programme evaluation in Africa. Malar J 2009; 8:231. [PubMed: 19840372]
19. Stevenson JC, Stresman GH, Gitonga CW, et al. Reliability of school surveys in estimating geographic variation in malaria transmission in the western Kenyan highlands. PLoS One 2013; 8:e77641.
20. Institut National de la Statistique (INSTST), Programme National de Lutte Contre le Paludisme (PNLP), Institut Pasteur de Madagascar (IPM), ICF Internatinal. Enquete sur les indicateurs du

paludisme a Madagascar 2016. Antananarivo, Madagascar and Rockville, MD: INSTAT, PNLP, IPM, ICF International, 2017.

21. Kerkhof K, Canier L, Kim S, et al. Implementation and application of a multiplex assay to detect malaria-specific antibodies: a promising tool for assessing malaria transmission in Southeast Asian pre-elimination areas. Malar J 2015; 14:338. [PubMed: 26337785]
22. Perraut R, Richard V, Varela ML, et al. Comparative analysis of IgG responses to Plasmodium falciparum MSP1p19 and PF13-DBL1α1 using ELISA and a magnetic bead-based duplex assay (MAGPIX®-Luminex) in a Senegalese mesoendemic community. Malar J 2014; 13:410. [PubMed: 25326042]
23. Perraut R, Varela M, Mbengue B, Guillotte M, Mercereau-Puijalon O, Vigan-Womas I. Standardization of a multiplex magnetic bead-based for simultaneous detection of IgG to Plasmodium antigens. J Immunol Tech Infect Dis 2015; 4:1–8.
24. Wilson EB. Probable inference, the law of succession, and statistical inference. J Am Stat Assoc 1927; 22:209–12.
25. Kattenberg JH, Erhart A, Truong MH, et al. Characterization of Plasmodium falciparum and Plasmodium vivax recent exposure in an area of significantly decreased transmission intensity in central Vietnam. Malar J 2018; 17:180. [PubMed: 29703200]
26. Birhanu Z, Yihdego YY, Yewhalaw D. Quantifying malaria endemicity in Ethiopia through combined application of classical methods and enzyme-linked immunosorbent assay: an initial step for countries with low transmission initiating elimination programme. Malar J 2018; 17:152. [PubMed: 29618357]
27. Nyunt MH, Soe TN, Shein T, et al. Estimation on local transmission of malaria by serological approach under low transmission setting in Myanmar. Malar J 2018; 17:6. [PubMed: 29304809]
28. Sáenz FE, Arévalo-Cortés A, Valenzuela G, et al. Malaria epidemiology in low-endemicity areas of the northern coast of Ecuador: high prevalence of asymptomatic infections. Malar J 2017; 16:300. [PubMed: 28747199]
29. Stevenson JC, Stresman GH, Baidjoe A, et al. Use of different transmission metrics to describe malaria epidemiology in the highlands of western Kenya. Malar J 2015; 14:418. [PubMed: 26502920]
30. Greenhouse B, Smith DL, Rodríguez-Barraquer I, Mueller I, Drakeley CJ. Taking sharper pictures of malaria with CAMERAs: combined antibodies to measure exposure recency assays. Am J Trop Med Hyg 2018; 99:1120–7. [PubMed: 30298804]
31. van Smeden M, Naaktgeboren CA, Reitsma JB, Moons KG, de Groot JA. Latent class models in diagnostic studies when there is no reference standard–a systematic review. Am J Epidemiol 2014; 179:423–31. [PubMed: 24272278]
32. Wiegand RE, Cooley G, Goodhew B, et al. Latent class modeling to compare testing platforms for detection of antibodies against the Chlamydia trachomatis antigen Pgp3. Sci Rep 2018; 8:4232. [PubMed: 29523810]
33. Biggs J, Raman J, Cook J, et al. Serology reveals heterogeneity of Plasmodium falciparum transmission in northeastern South Africa: implications for malaria elimination. Malar J 2017; 16:48. [PubMed: 28126001]
34. Rogier E, Wiegand R, Moss D, et al. Multiple comparisons analysis of serological data from an area of low Plasmodium falciparum transmission. Malar J 2015; 14:436. [PubMed: 26537125]
35. Sepúlveda N, Drakeley C. Sample size determination for estimating antibody seroconversion rate under stable malaria transmission intensity. Malar J 2015; 14:141. [PubMed: 25889960]
36. Akpogheneta OJ, Duah NO, Tetteh KK, et al. Duration of naturally acquired antibody responses to blood-stage Plasmodium falciparum is age dependent and antigen specific. Infect Immun 2008; 76:1748–55. [PubMed: 18212081]
37. Kinyanjui SM, Conway DJ, Lanar DE, Marsh K. IgG antibody responses to Plasmodium falciparum merozoite antigens in Kenyan children have a short half-life. Malar J 2007; 6:82. [PubMed: 17598897]

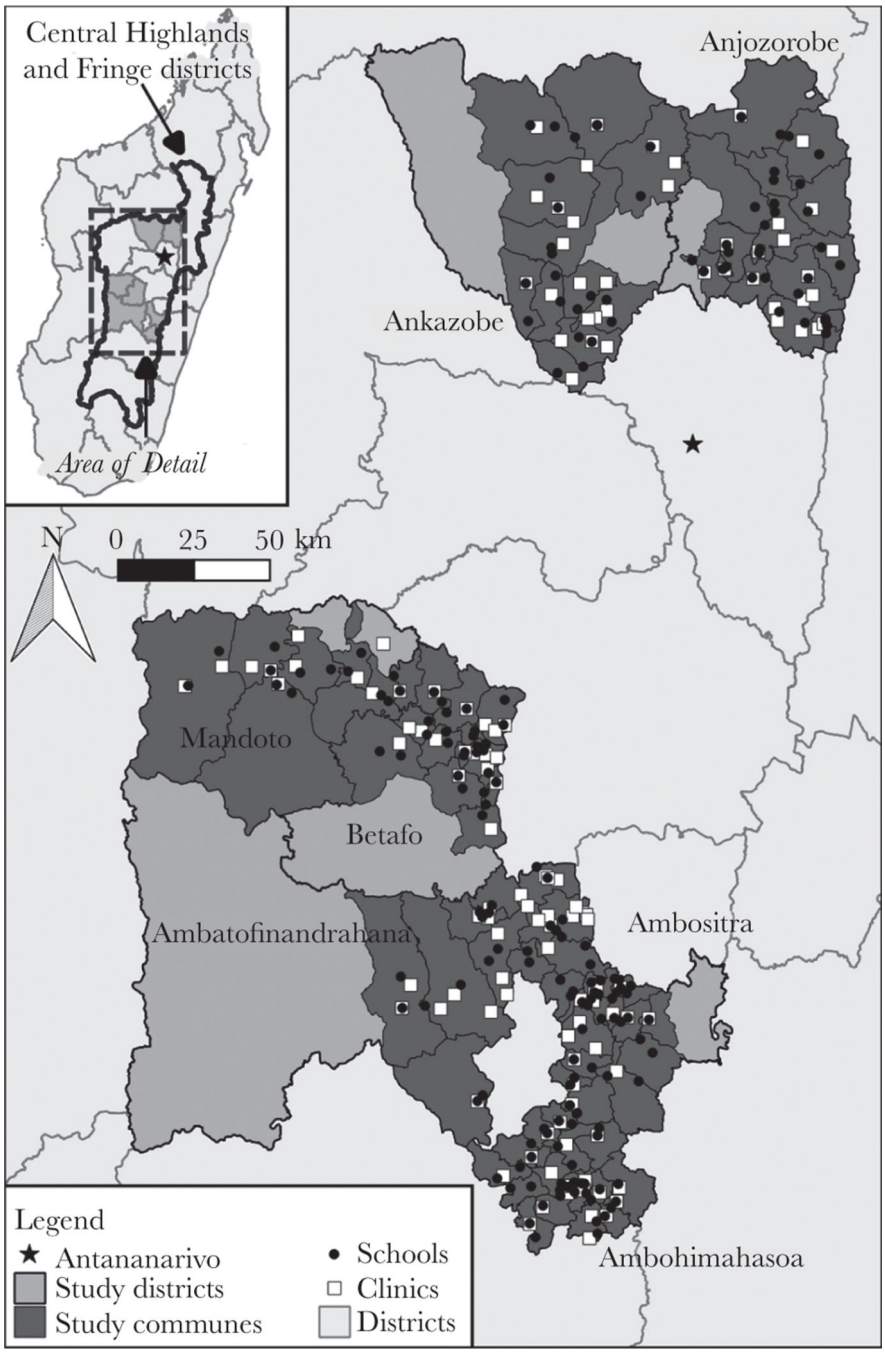


Figure 1.

Map of sampled districts, schools, and health facilities. Source: Database of Global Administrative Areas (GADM) and QGIS.

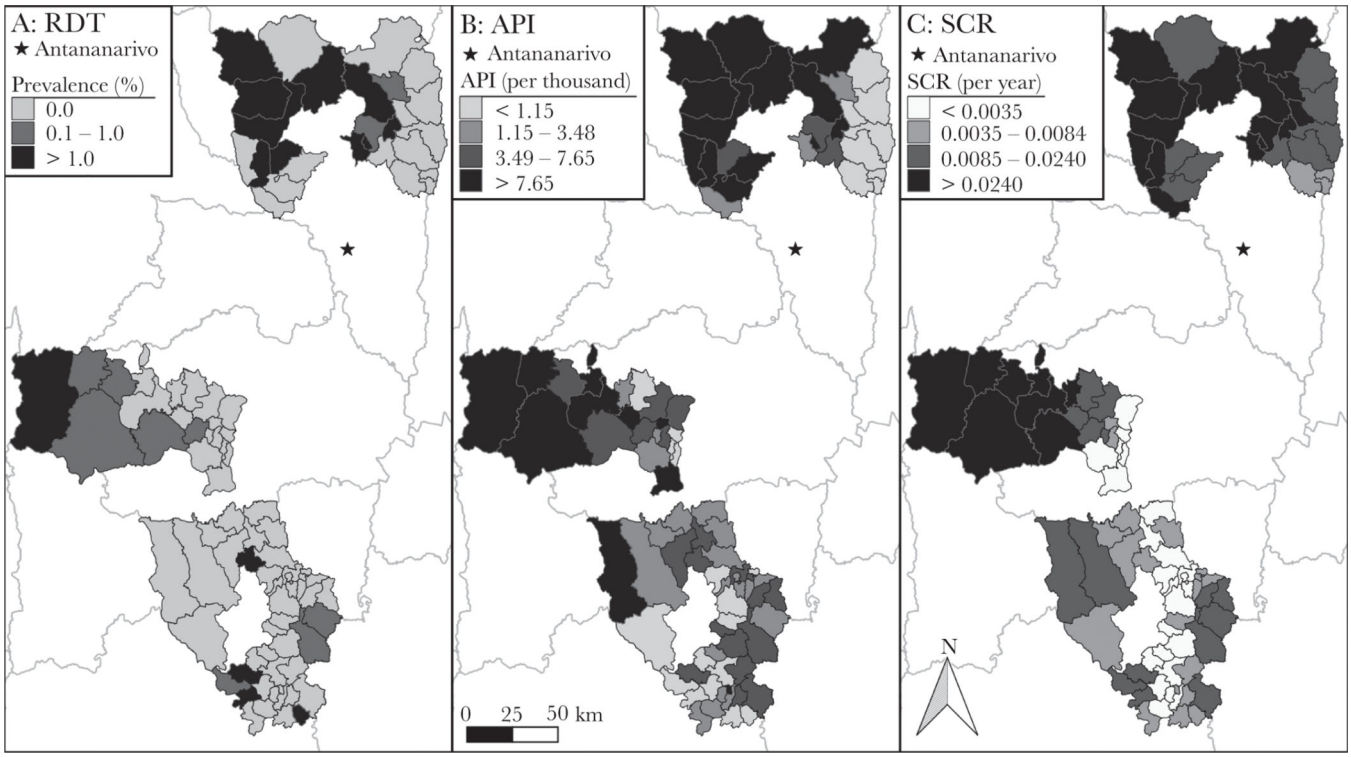
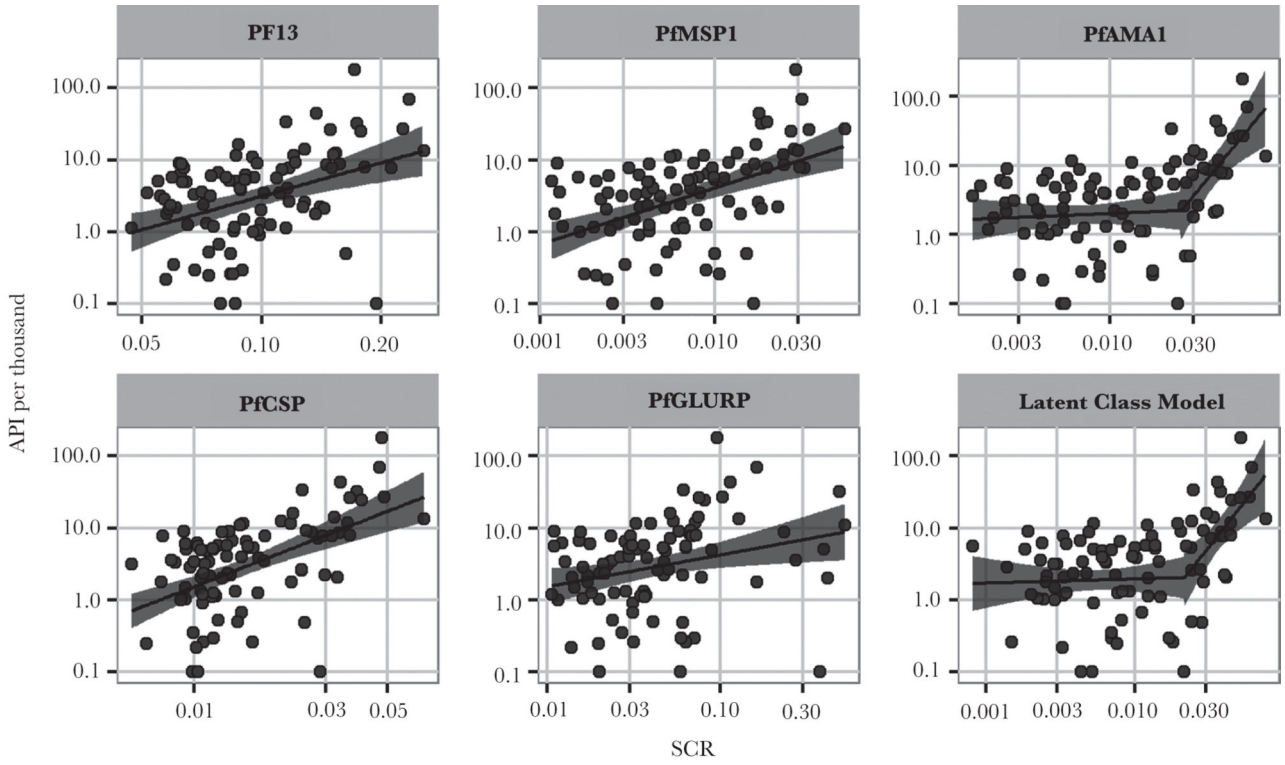


Figure 2.

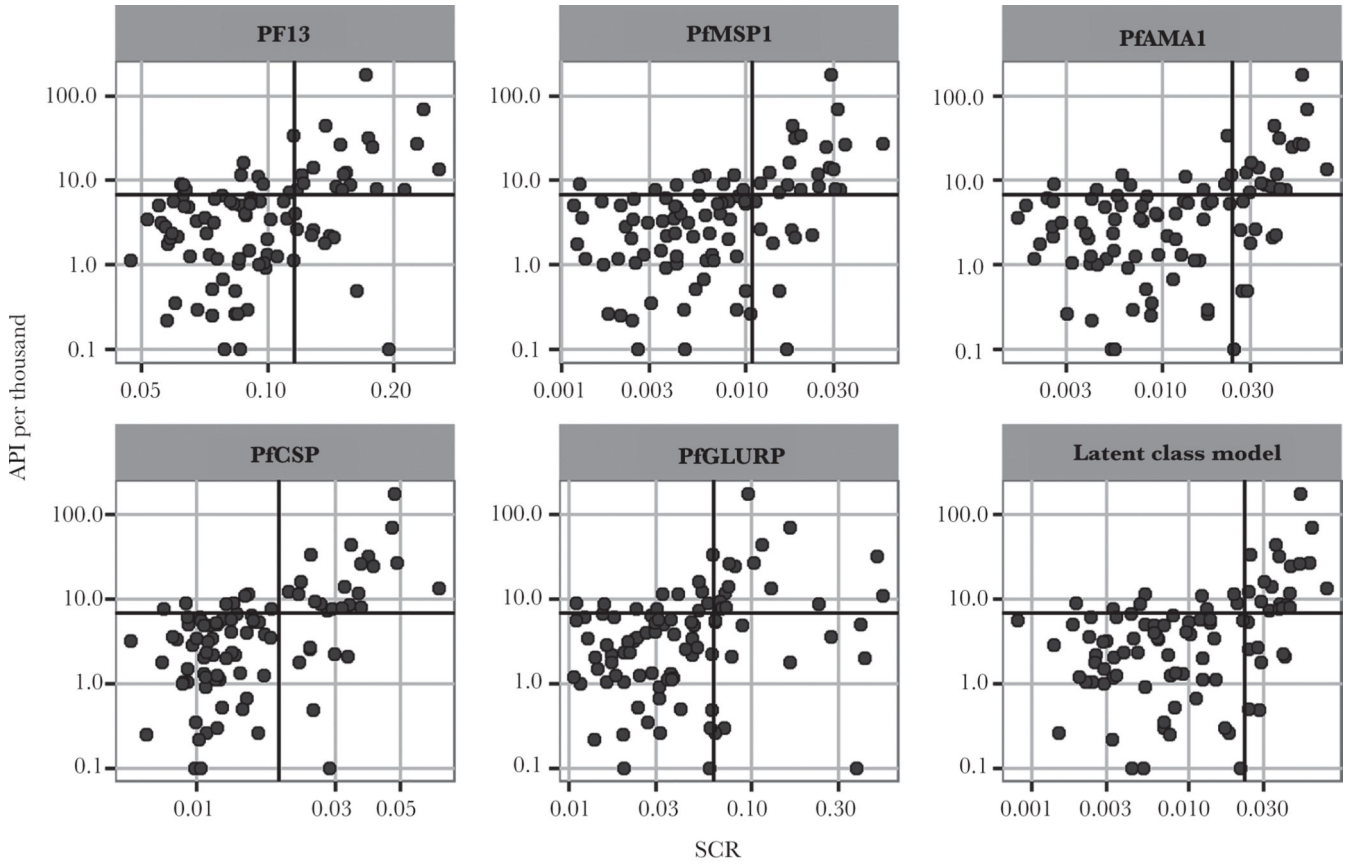
Maps of commune-level RDT prevalence (*A*), 2013 health facility API (*B*), and SCR for the Pf latent antigen (*C*). Abbreviations: API, annual parasite incidence; RDT, rapid diagnostic test; SCR, seroconversion rate. Source: Database of Global Administrative Areas (GADM) and QGIS.



Marker	Term	Intercept (95% CI)	Slope (95% CI)	Difference (95% CI)	Changepoint (95% CI)
PF13		2.05 (1.26–2.83)	1.55 (0.79–2.31)		
PfMSP1		2.18 (1.51–2.85)	0.78 (0.48–1.08)		
PfAMA1	Segment 1	0.52	0.11 (–0.33–0.54)	2.98 (1.15–4.80)	–1.59 (–1.76 to –1.43)
PfAMA1	Segment 2	5.26	3.08 (1.31–4.85)		
PfCSP		3.18 (2.25–4.11)	1.49 (0.99–2.00)		
PfGLURP		1.07 (0.58–1.55)	0.44 (0.10–0.78)		
Latent class model	Segment 1	0.41	0.06 (–0.37–0.49)	2.56 (1.03–4.10)	–1.67 (–1.86 to –1.48)
Latent class model	Segment 2	4.68	2.62 (1.15–4.09)		

Figure 3.

Best-fitting regression models (above) and coefficients (below) for SCRs of different antibodies versus API. Abbreviations: API, annual parasite incidence; CI, confidence interval; SCR, seroconversion rate.



Marker	Sensitivity (95% CI)	Specificity (95% CI)	SCR*	API*
PF13	68 (48–84)	86 (75–93)	0.116	6.77
PfMSP1	71 (51–87)	88 (77–95)	0.011	6.77
PfAMA1	68 (48–84)	86 (75–93)	0.024	6.77
PfCSP	75 (55–89)	89 (79–96)	0.019	6.77
PfGLURP	61 (41– 78)	83 (72–91)	0.062	6.77
Latent class model	71 (51–87)	88 (77–95)	0.023	6.77

Figure 4.

Sensitivity and specificity of API versus gold standard SCR for detecting 30% highest transmission communes. Horizontal and vertical lines represent API and SCR, respectively, for the top 30% communes. * SCR and API values represent cutoff for top 30% communes. Abbreviations: API, annual parasite incidence; CI, confidence interval; SCR, seroconversion rate.

Table 1.

Characteristics of Sampled Individuals and Communes

Characteristic	Sample	Positive RDT		Serological Outcomes						API
				PfMSPI	PfAMAI	PfCSP	PfGLURP	PF13	Pf Latent Antigen[a]	
Individual data										
	n (%)	n	% (95% CI)	Seropositivity, % (95% CI)						
Age, y										
2–4	24 (0.2)	0	0.0 (0.0–17.2)	4.2 (0.1–22.7)	0.0 (0.0–17.2)	12.5 (2.0–35.6)	29.2 (13.6–49.4)	25.0 (10.3–45.7)	0.0 (0.0–17.2)	...
5–9	3071 (24.1)	22	0.7 (0.3–1.3)	6.9 (5.6–8.41	4.0 (3.0–5.3)	13.2 (11.9–14.5)	34.6 (30.6–38.7)	47.0 (45.0–49.1)	3.3 (2.4–4.51	...
10–14	3204 (25.1)	32	1.0 (0.6–1.6)	8.8 (7.3–10.4)	10.0 (7.6–12.7)	18.0 (16.4–19.7)	40.0 (35.8–44.3)	55.3 (53.0–57.5)	7.6 (5.6–9.91	...
15–19	241 (1.9)	1	0.4 (0.0–2.3)	10.0 (6.2–15.0)	12.9 (7.8–19.6)	29.0 (23.4–35.2)	42.3 (34.3–50.7)	56.0 (49.2–62.7)	12.4 (7.3–19.4)	...
20 +	6230 (48.8)	13	0.2 (0.1–0.4)	28.4 (25.2–31.8)	46.3 (41.9–50.8)	44.7 (41.6–47.8)	72.0 (68.5–75.4)	68.5 (65.8–71.0)	43.8 (39.3–48.5)	...
Female	8071 (63.2)	33	0.4 (0.2–0.7)	17.3 (15.2–19.7)	26.9 (23.9–30.1)	30.6 (28.5–32.6)	55.3 (51.9–58.7)	59.3 (57.3–61.3)	24.8 (21.8–28.0)	...
Total	12 770 (100.0)	68	(0.5)	17.9	26.3	30.1	54.3	59.7	24.3	...
Commune data										
	n	Median (min, max)		Seroconversion Rate, Median (min, max)						
	93	0.00 (0.00–13.3)		0.006 (0.001–0.056)	0.011 (0.002–0.078)	0.013 0.006–0.068)	0.037 (0.011–0.524)	0.090 (0.047–0.256)	0.01 (0.001–0.075)	*0.0 (0.0–177.3)*

API derived from health management information system data; all other measures derived from survey data.

Abbreviations: API, annual parasite incidence, Pf, *Plasmodium falciparum;* RDT, rapid diagnostic test

[a]Pf latent antigen created from latent class analysis of all *Plasmodium falciparum* antigens.

Table 2.

Sensitivity, Specificity, and AUC of API Versus SCRs of Various *Plasmodium falciparum* Antigens, at Different Quantiles of the SCR

Percentage of Highest Communes[a]	SCR Threshold[b]	API Threshold[c]	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
PF13					
50	0.0903	5.15	72 (62–83)	61 (46–74)	79 (66–89)
40	0.099	6.83	73 (62–84)	57 (41–73)	88 (79–95)
30	0.1157	7.6	77 (65–89)	68 (50–86)	89 (82–95)
20	0.1371	7.6	79 (65–94)	79 (58–95)	85 (77–92)
10	0.162	7.6	76 (52–99)	80 (50–100)	78 (70–87)
PfMSP1					
50	0.0064	5.02	73 (63–84)	65 (52–78)	81 (68–91)
40	0.0083	6.18	75 (63–86)	62 (46–78)	88 (79–95)
30	0.0109	6.83	81 (69–92)	71 (54–86)	88 (80–95)
20	0.0171	7.6	89 (79–98)	84 (68–100)	86 (78–93)
10	0.0249	7.64	93 (87–99)	100 (100–100)	83 (75–90)
PfAMA1					
50	0.0114	5.02	74 (64–84)	65 (52–78)	81 (70–91)
40	0.0167	6.83	76 (65–87)	59 (43–76)	89 (80–96)
30	0.0239	6.83	76 (64–88)	68 (50–86)	86 (77–94)
20	0.0313	7.6	87 (77–96)	84 (68–100)	86 (78–93)
10	0.0427	7.6	93 (87–99)	100 (100–100)	81 (72–89)
PfCSP					
50	0.0133	6.18	77 (68–87)	59 (43–74)	94 (85–100)
40	0.0151	6.18	78 (68–88)	62 (46–78)	88 (79–95)
30	0.0191	6.83	82 (70–93)	75 (57–89)	89 (82–95)
20	0.0262	6.83	83 (71–96)	84 (68–100)	84 (76–92)
10	0.0338	11.52	97 (94–100)	90 (70–100)	95 (90–99)
PfGLURP					
50	0.0371	6.83	71 (60–82)	50 (35–65)	89 (79–98)
40	0.0505	6.83	73 (61–85)	59 (43–76)	89 (80–96)
30	0.0621	7.6	74 (62–87)	61 (43–79)	86 (77–94)
20	0.0723	7.74	76 (62–90)	63 (42–84)	85 (77–93)
10	0.1254	8.51	63 (42–84)	50 (20–80)	81 (72–88)
Pf latent antigen					
50	0.0097	5.02	73 (63–84)	65 (50–78)	81 (68–91)
40	0.0136	6.83	74 (62–85)	59 (43–76)	89 (80–96)
30	0.0227	6.83	81 (70–92)	71 (54–89)	88 (78–95)
20	0.0297	6.83	89 (81–98)	89 (74–100)	85 (77–93)
10	0.0405	7.74	88 (76–100)	90 (70–100)	83 (75–90)

Abbreviations: AUC, area under the curve; API, annual parasite incidence; Pf, *Plasmodium falciparum;* SCR, seroconversion rate.

[a]Percentage of highest communes refers to the highest X% of communes by transmission level assessed by SCR (eg, the highest 20% of communes). For each antigen, 46 were above the 50% SCR threshold, 37 above the 40% SCR threshold, 28 above the 30% SCR threshold, 19 above the 20% SCR threshold, and 10 above the 10% threshold, although it should be noted that these communes differ by antigen, which gives different results.

[b]SCR threshold is the value that splits the communes such that the number of communes to be identified matches the percentage in the first column.

[c]API threshold was determined by Youden index (see Supplementary Material).