Title: Conventional and High-Sensitivity Malaria Rapid Diagnostic Test Performance in Two Transmission Settings: Haiti 2017

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Abstract

Accurate malaria diagnosis is foundational for control and elimination, and Haiti relies on HRP2-based rapid diagnostic tests (RDTs) identifying *Plasmodium falciparum* in clinical and community settings. In 2017, one household and two easy-access group (EAG) surveys tested all participants (N=32,506) by conventional and high-sensitivity RDTs (cRDT/hsRDT). A subset of blood samples (n=1,154) were laboratory tested for HRP2 by bead-based immunoassay and for *P. falciparum* 18S rDNA by PET-PCR. Both RDT types detected low concentrations of HRP2 with sensitivity estimates between 2.6 and 14.6 ng/mL. Compared to the predicate HRP2 laboratory assay,, RDT sensitivity ranged from 86.3% to 96.0% between tests and settings, and specificity from 90.0% to 99.6%. In the household survey, the hsRDT provided a significantly higher number of positive tests, but this represented a very small proportion (<0.2%) of all participants. These data show an hsRDT may have limited utility in a malaria elimination setting like Haiti.

Keywords: *Plasmodium falciparum,* rapid diagnostic test, HRP2, malaria elimination, Haiti

Introduction

Field-deployable diagnostic tests for malaria serve to allow reliable confirmation of infection and appropriate malaria case management [1]. A *Plasmodium falciparum* species-specific antigen, histidine-rich protein 2 (HRP2), is the most common RDT antigenic target in *P. falciparum* endemic settings due to its high expression level and multi-epitope avidity [2, 3], though it is known to linger for weeks to months in human circulation following clearance of parasites [4]. Recent improvements to malaria RDTs include the enhanced sensitivity of antigen detection as well as new antigenic targets more useful for identifying non-falciparum malaria infections [5].

As a nation approaches malaria elimination, a higher proportion of all infections are asymptomatic and do not exhibit treatment-seeking behavior, and monitoring changes in true infection prevalence over time (or changes due to an intervention) becomes particularly difficult [6-9]. The nation of Haiti is currently focused on interruption of local malaria transmission, and identification of a high proportion of all infections is paramount (www.malariazeroalliance.org) [10]. Within this endeavor, the recently developed high-sensitivity RDT (hsRDT, SD Bioline) is being evaluated in direct comparison with a conventional RDT (cRDT) produced by the same manufacturer. Both tests exclusively detect the *P. falciparum* HRP2 antigen, and performance characteristics for these two tests have been considered in other areas of the world from previous studies: Myanmar [11, 12], Uganda [12], and Tanzania [13].

As part of ongoing elimination efforts, three surveys were completed in Haiti in 2017. Two of these occurred in different transmission settings in the country and utilized an easy-access group (EAG) sampling design within schools, health facilities and churches. A third survey was performed in a low-transmission setting using a simple random sample of households from a census-based sampling frame. In total, 32,506 persons who were enrolled from these three surveys received both a cRDT and hsRDT diagnostic test for presence of P. *falciparum* HRP2 antigen. We report here the concordance in test results between these two

RDTs, and evaluation of test performance of a subset of these samples in comparison with a bead-based laboratory assay for the HRP2 antigen and 18S rDNA PET-PCR assay for *P. falciparum* DNA.

Methods

Human subjects

For all surveys, laboratory staff did not have access to personal identifiers. Persons consented for diagnostic tests and blood sample assays for markers of malaria. Activity did not constitute engagement in human subjects research as determined by the CDC Center for Global Health Human Subjects office (#2016-135a), and field activities received approval from Haitian Ministry of Public Health and Population Bioethics Committee (*Comité National de Bioéthique*) and the Institutional Review Boards (IRBs) of Tulane University and the London School of Hygiene & Tropical Medicine.

Participant Enrollment

Information regarding participant consent for surveys is presented in Supplementary Data. EAG surveys received approval from the Haitian Ministry of Public Health and Population Bioethics Committee (*Comité National de Bioéthique*)(#1516-30), and the Institutional Review Boards (IRBs) of Tulane University (#794709), and the London School of Hygiene & Tropical Medicine (#10393). A single finger-prick was performed on consenting participants to collect capillary blood for cRDT (SD Bioline Malaria Antigen P.f.; 05FK50; Standard Diagnostics), hsRDT (also known as ultrasensitive RDT, Alere Malaria Ag P.f.; 05FK141; Standard Diagnostics), and blood spots on filter paper (Whatman 903, GE Healthcare). Blood was dried on filter paper overnight, and each filter paper stored in individual baggie with desiccant at ambient temperature protected from light. On a weekly basis, samples were shipped to the

Haitian national lab where they were stored at 4°C until laboratory processing. Individuals with a positive cRDT result received free treatment as per the national policy in Haiti.

For the household survey, blood was collected for RDTs and prepared on filter papers in the same manner as the EAG surveys. The household survey was approved by the Haiti Ministry of Public Health and Population National Bioethics Committee (#1516-29 and 1617-31) and the IRBs of the U.S. Centers for Disease Control and Prevention (#6821) and the London School of Hygiene & Tropical Medicine (#10466).

Selection of Samples for HRP2 Detection and DNA analysis

Due to the inherent differences in sampling design, analyses for the two EAG surveys are presented together, and household survey estimates presented separately. All the participants from the EAG and household surveys with a positive cRDT or hsRDT were selected for quantification of HRP2 antigen by bead-based assay and parasite density determined by photo-induced electron transfer polymerase chain reaction (PET-PCR). For the EAG studies, selection of samples from persons not RDT positive were biased towards the venues where infections had been found, with 80% of samples selected from 'high risk' venues (meaning at least 1 RDT positive was identified). Additional samples from persons sampled in the 'low risk' venues (no RDT positives) were randomly selected until a sample size of 300 for Artibonite EAG and 750 for Grand Anse EAG was reached.

Only RDT positive persons were selected from the household survey for further laboratory analysis. Of 161 persons in whom either RDT was positive (and results were available for both RDT types), 153 (95.0%) had a blood sample available for antigen detection and PET-PCR.

Laboratory assays for HRP2 and parasite DNA

Presence and quantification of antigens utilizing bead-based Luminex® based MAGPIX platform (Luminex Corp., Austin, TX)[14], and parasite DNA detected by PET-PCR [15] was performed as described in Supplementary Data.

Statistics

A logistic regression model was fit to the HRP2 concentration versus RDT result dose–response data, and was used to estimate the HRP2 concentrations at which 50, 75, 90, and 95% of the RDTs would be expected to be positive in the study population [16]. Both locally estimated scatterplot smoothing (LOESS) and logistic regression curves with 95% confidence intervals were created by the R software version 3.3.0 using the **stats** package (R Foundation for Statistical Computing). Characteristics of test performance through receiver operating characteristic curve (ROC) analysis was performed through the SAS PROC LOGISTIC command with the ROC statement (SAS v9.4). Youden's J statistic as a measure of informedness was calculated for each RDT for each of the two EAG surveys by the equation J = sensitivity – specificity – 1 [17]. Testing for statistical significance between estimated parasite densities of cRDT versus hsRDT results (in Figure 3, Table 2) was performed by the PROC TTEST procedure in SAS. All Cohen's kappa statistics were generated in SAS. McNemar's Chi-square statistic with one degree of freedom was generated for age categories within the household-based survey, but modified by the Edwards continuity correction due to low numbers of discordant results [18], and test statistic generated by the equation:

$$\chi^2 = \frac{(|b - c| - 1)^2}{b + c}$$

to approximate the exact binomial test with b and c indicating cells with discordant results.

Results

Presentation of results by separate sampling designs

The majority of RDT positives in the two EAG surveys were found in the treatment seeking population, with 74.5% (301/404) of all RDT positive persons enrolled at health facilities. Conversely, all persons enrolled from the household survey were at their residence at time of enrollment, so not seeking standard medical treatment by definition. Due to this inherent difference between the EAG and household survey participant enrollment, and the fact that symptomatic malaria infections will skew blood antigen concentrations higher, results are presented separately for these two survey types.

RDT concordance and test results relative to blood HRP2 concentration

Five additional HRP2-positive persons were found by the hsRDT for the Artibonite EAG, and fourteen additional positives in the Grand Anse EAG survey (Figure 1A, and 1B respectively). Additional hsRDT positives represented a 10.2% increase in RDT+ numbers in Artibonite and 3.9% increase in Grand Anse, with only the augmented numbers in the Grand Anse EAG significantly different. For both surveys, agreement was strong between the reported results for the two RDTs, with a Cohen's kappa coefficient of 0.946 for the Artibonite EAG and 0.978 for the Grand Anse EAG. Of 1,001 blood samples selected for further laboratory analyses (RDT concordance for these in Supplementary Figure 2), both parametric and non-parametric regression models provided a similar dose-response relationship with high convergence for both survey sites and RDTs (Figure 1C and D). One visible deviation among the LOESS and logistic curves occurred due to a single blood sample found to have high levels of HRP2, but that participant's RDT results were called negative (Figure 1C). Estimates are provided for each RDT and EAG study site for test sensitivity at 50%, 75%, 90%, and 95% confidence of a positive test result at a given concentration of HRP2 antigen in the person blood sample (Table 1). At 50% confidence, all estimates of HRP2 concentration for the cRDT and hsRDT from both

EAG survey locations were similar and had overlapping confidence intervals, but estimates began to diverge by survey location as level of confidence increased. At the 95% confidence level, estimates for HRP2 concentration were significantly different for the cRDT between the Artibonite and Grand Anse EAGs (2.84 versus 14.61 ng/mL respectively). No significant differences were observed between the cRDT and hsRDT estimates within the same survey area for any of the confidence levels.

Histograms showing the entire range of HRP2 concentrations for the samples designated for laboratory analysis are shown in Supplementary Figure 3, and many more persons selected from Grand Anse were positive for HRP2 (n=322, 45.9%) than from Artibonite (n=50, 16.7%). As the difference in dose-response regression estimates for HRP2 concentration at the 95% confidence level was minor between the two RDT types, few additional positives would be predicted to be discovered by the high-sensitivity test (as reflected by the actual counts in Figure 1A, B).

In direct comparison with the bead-based HRP2 laboratory assay, both RDT types performed well at both survey sites with receiver operating curve (ROC) area under the curve (AUC) values 0.93 or greater when modeling for RDT result based on HRP2 antigen positivity (Figure 2). If considering the laboratory assay to be the gold standard for HRP2 detection, the cRDT had sensitivities of 86.3% and 95.0% at the Artibonite and Grand Anse sites, respectively, and specificities of 99.6% and 92.9%. The hsRDT had slightly higher sensitivities of 88.2% and 96.0% at the Artibonite and Grand Anse sites, but slightly lower specificities of 98.0% and 90.0%.

RDT result relative to estimated parasite density at time of sampling

Though the only target for these RDTs was the HRP2 antigen, comparison to *P. falciparum* parasite densities provides valuable information regarding the utility of the tests to identify active infections. Most individuals (97.5%) who tested RDT negative in the EAG surveys

did not have detectable *P. falciparum* parasite DNA, and persons with positive RDT results had evidence of *P. falciparum* DNA >80% of the time for both surveys and both tests (Table 2). No statistically significant differences were observed in comparing the mean estimated parasite densities among all four RDT negative categories ([two RDT categories] x [two survey sites]), or among all four RDT positive categories.

Dose-response modelling of cRDT and hsRDT result as a function of estimated parasite density is shown in Supplementary Figure 4. Since so few RDT negative persons were found to harbor *P. falciparum* DNA, the logistic sigmoidal curve did not reach a lower asymptote, and estimates for lower levels of RDT performance could not be derived. For both RDT tests at both survey sites, reliability of a positive RDT test in identifying active *P. falciparum* infections was shown to decrease only at the lowest estimated parasite densities (under 100 p/uL).

Household survey cRDT and hsRDT concordance and differences in HRP2 antigen and parasite densities

In the Artibonite household survey, 21,517 persons were administered both types of RDTs, and agreement between the cRDT and hsRDT was strong for all age categories: 0–5, 6–15, and >15 years (Figure 4A). Persons tested cRDT negative but hsRDT positive in all three age categories, with increasing test statistic of discordant results as age increased. Among the three age categories, only slight variation was observed in the distribution of HRP2 concentrations among the RDT positive persons (Supplementary Figure 5), and positive correlation was observed for antigen concentration and parasite density (Supplementary Figure 6). One person tested positive by cRDT, but negative by hsRDT, but a blood sample was not available for investigation of HRP2 antigen or parasite presence. Overall, the hsRDT identified 36 more HRP2 positive persons (28.8% more positives than the cRDT alone), but this only represented 0.17% of the total study population. Highly significant differences were seen in HRP2 concentrations between the blood samples of those who tested positive by both RDTs

(median = 23,577 pg/mL) or the hsRDT only (median = 1,168 pg/mL) (Figure 4B). Additionally, highly significant differences were observed in PET-PCR estimated parasite density in persons testing positive by both RDTs (median = 8.64 p/uL; range: 0.0-2045.8 p/uL) or the hsRDT alone (median = 0.90 p/uL; range: 0.0-11.4 p/uL).

cRDT to hsRDT comparison summary across all three surveys

In total, 32,506 total persons had both a cRDT and an hsRDT performed. Of these, 565 (1.74%) tested positive by any RDT. Of these 565 positives, 509 (90.1% of all positives, 1.57% of total population) were positive by both RDTs, 55 (9.73% of all positives, 0.17% of total population) were positive by only the hsRDT, and 1 person was positive by only the cRDT.

Discussion

A nation faces many challenges as it progresses from very low malaria to no malaria, and traditional field diagnostic tests of microscopy and cRDTs were not designed for detecting low-density infections [7, 19]. Newer field-deployable diagnostics have undergone testing in field settings with promising results in their ability to detect low-density *P. falciparum* infections by molecular [20-23] and antigen detection [11, 12] methods. Though these high-sensitivity field diagnostic tests consistently detect more infections than their conventional counterparts of microscopy or cRDT, the significance of detected very low-density infections is still being investigated with respect to potential transmissibility and overall population-based estimates [24, 25].

Transmission of *P. falciparum* in Haiti is seasonal and heterogeneous [26-28] with some regions of the country estimated to have parasite prevalence exceeding 5%, whereas other regions have a complete absence of reported malaria [10, 29]. This current study included two sites in Haiti representing two different transmission strata: Grand Anse as the area of the nation currently reporting the most clinical cases, and Artibonite as a setting of lower *P.*

falciparum prevalence [10] (and internal data from national malaria program). A previous Artibonite prevalence estimate of *P. falciparum* infection was approximately 3% with most of these individuals being asymptomatic carriers at the time of the survey [28]. For this current study, EAG test positivity rates for any RDT in Artibonite and Grand Anse confirmed the disparity in number of *P. falciparum* infections with 0.83% (49/5876) of participants in the Artibonite EAG and 7.1% (355/5014) of participants in the Grand Anse EAG surveys testing RDT positive. If assessing the added number of positives provided by the hsRDT in these two surveys, 0.08% more of the sampled population in Artibonite (5 persons) and 0.28% more in Grand Anse (14 persons) had levels of HRP2 antigen low enough to be missed by the cRDT, but detectable by the hsRDT. This finding has been observed previously, with a low overall percentage of the study population found to harbor HRP2 antigen levels in the "opportunistic" zone of detection between the sensitivities of the two tests [12, 25]. However, studies in some higher-transmission settings have found considerably greater percentages of the population that tested positive with an hsRDT alone [11, 12]. Even within a country of heterogeneous transmission patterns, the hsRDT may have value for some areas in providing more accurate (and substantial) prevalence estimates, yet not be significantly advantageous over a cRDT in other settings. Importantly, most (75%) of the RDT positive persons from the Haiti EAG surveys came from treatment-seeking individuals enrolled in health facilities, and higher parasite and antigen densities would negate any benefit of detecting low levels of the HRP2 antigen [13].

A key factor of RDT performance is overall HRP2 carriage in the population, which itself is determined by the level of *P. falciparum* infection [30], recent infection (lingering HRP2 in host blood [4, 31]), and potential accumulation of HRP2 from tandem and/or frequent infections. In the lower-transmission Artibonite setting, there was only a small number of individuals with HRP2 levels near RDT detection limits. However, in Grand Anse, where many more infections were found (and higher likelihood of *P. falciparum* exposure in the preceding few months), a much higher percentage of persons had intermediate and low levels of HRP2 antigen – giving

the hsRDT an opportunity to be the sole detector of these low HRP2 concentrations. Antigen persistence potentially explains our finding in the higher-transmission Grand Anse setting: 18.4% of hsRDT positive persons were not found to have P. falciparum DNA. However, some of these "false positive" tests may have actually been very low density infections undetectable by the PET-PCR assay since this nucleic acid assay is less analytically sensitive than the hsRDT [15, 30]. Additionally, sampling from this one point in time does not take into account fluctuations of parasites through replication cycles or parasite load from the recent past. Though being a “false positive” in the sense of no active parasitemia, detection of the HRP2 antigen following a *P. falciparum* infection could still be useful indicator for a malaria program as a clear proxy of current or recent *P. falciparum* exposure at individual and population levels [14].

Among both tests and both EAG sites, overall sensitivity and specificity estimates were high (86.3% or greater) when defining the laboratory HRP2 detection assay test as the gold standard. Though cRDT and hsRDT performance within a single study site was basically equivalent, consistent differences were seen between surveys with both types of RDTs providing a lower sensitivity in Artibonite, but higher specificity. The Youden’s J statistic (as a measure of a probability of making an informed decision with 1.0 being perfect decision making [17, 32]) was strong for all scenarios with values of 0.86 or higher. In Grand Anse, multiple samples were positive by the HRP2 lab assay only, but an even higher number of persons were RDT+ only for both RDT types. The RDT+s only would lower specific estimates, but this is potentially an artificial depression due to inherent differences in the RDT and laboratory tests. The field RDT uses fresh, undiluted blood immediately drawn from a participant, whereas the sample type for the laboratory test dried blood on filter paper. To rehydrate blood and remove from filter paper, an intrinsic dilution of the blood sample is needed [14]. Additionally, antigen degradation is possible from drying of blood, storage conditions, or length of storage. Even with the increased detection limit of the laboratory test, some very low HRP2 concentrations have the potential to be detected by field tests but missed in the laboratory.

Active *P. falciparum* infections detected from the household survey in Artibonite were typically found to be low density, with 80.3% under 100 p/µL and 54.9% under 10.0 p/µL. Of 21,591 persons in the household survey, 0.72% of 0–5 year olds, 1.1% of 6–15 year olds, and 0.59% of >15 year olds tested positive by any RDT. Concordance between the cRDT and hsRDT was very good, alluding to the high sensitivity and specificity in detecting HRP2 antigen in participants' blood. Though the hsRDT detected statistically-higher number of positives in all age categories, this was 0.2% or less from any category, and 0.17% overall regardless of age. In endemic settings, younger persons are at greater risk for high-density *P. falciparum* infections, whereas older individuals more able to suppress parasite replication [33, 34], but our study found little difference in the distribution of HRP2 concentrations among age categories. Comparison of persons testing positive to both RDTs versus the hsRDT alone found significantly lower antigen (and parasite) levels in persons detected by the hsRDT alone, but there was simply just a low number of Artibonite residents with these low antigen levels at the time of sampling.

In the malaria elimination setting of Haiti, both conventional and high-sensitivity HRP2-based RDTs performed well, and high concordance between the two tests was observed in two different transmission zones. In comparison with the cRDT, statistically higher numbers of positive tests were observed with the hsRDT, supporting the increased HRP2 detection capacity of this test, but this was a very small percentage of the overall population sampled. These findings are in line with other studies in malaria elimination settings that have noted more positives when employing the hsRDT, but that these slightly higher estimates do not change the overall prevalence for relevant programmatic purposes. Though likely of limited utility in the Haitian setting, further evaluation of novel antigen-based tests is warranted to investigate utility in different transmission settings, *P. falciparum* genotypes, and human populations. As innovative diagnostics are introduced, increased performance of a novel test in a population will also need to be weighed against the increased costs for that test.

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Conflicts of interest

All authors report no conflicts of interest

Disclaimer

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Figure Legends

Figure 1. Concordance between test results for the two types of RDTs in Artibonite and Grand Anse EAG surveys and dose-response relationship between HRP2 antigen concentration and RDT result. Panels are shown for the Artibonite and Grand Anse study sites for the conventional and high-sensitivity RDTs (cRDT/hsRDT). Two-by-two tables for the two RDTs comparing test concordance for all persons enrolled in the Artibonite (A) and Grand Anse (B) surveys with Cohen's kappa agreement. *, McNemar's test to indicate statistical significance of discordant test results between the two RDTs was modified to adjust for low numbers of discordant results as described in Methods. Logistic and LOESS regression of probability of RDT positivity by antigen concentration in study participants for Artibonite (C) and Grand Anse (D) surveys. Outputs for the logistic regression shown in Table 1.

Figure 2. Performance of the two RDT tests in comparison with the laboratory HRP2 bead assay. Two-by-two tables, ROC curves, and performance measures shown for the two EAG study sites for the two types of RDTs employed. PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve.

Figure 3. Estimated *P. falciparum* parasite densities by RDT result. Panels are shown for the Artibonite and Grand Anse EAG study sites and categories indicate negativity or positivity to the conventional RDT (cRDT) or high-sensitivity RDT (hsRDT). Horizontal bars show median parasite density as estimated by PET-PCR for the respective category.

Figure 4. Concordance of conventional RDT and high-sensitivity RDT test for large household-based survey in Artibonite. (A) Two-by-two tables are separated by age

categories: 0-5, 6-15, and >15 years of age, with estimates for Cohen's kappa statistic for agreement between the two types of RDTs. *, McNemar's test to indicate statistical significance of discordant test results between the two RDTs was modified to adjust for low numbers of discordant results as described in Methods. Differences in HRP2 antigen levels (B) and PET-PCR estimated parasite density (C) for blood samples from persons testing positive for both RDTs, or hsRDT only. Antigen levels are log-transformed and normal and kernel distributions overlaid. Boxes are 25% and 75% percentile with horizontal line displaying median. Whiskers display minimum and maximum values within 1.5 interquartile range and circles are outliers beyond this range.

Tables

Table 1. Modelled Concentrations of HRP2 Detection by Probability of a Positive Test for the Conventional and High-Sensitivity RDTs at the Two EAG Study Sites

	HRP2 concentration (ng/mL) (95% CI)			
Sensitivity	Artibonite cRDT	Artibonite hsRDT	Grand Anse cRDT	Grand Anse hsRDT
50%	0.318 (0.18-0.66)	0.213 (0.12-0.46)	0.458 (0.32-0.66)	0.326 (0.23-0.47)
75%	0.719 (0.33-1.5)	0.542 (0.24-1.2)	1.659 (1.0-2.6)	1.216 (0.77-1.9)
90%	1.623 (0.52-3.6)	1.379 (0.42-3.3)	6.012 (3.2-11)	4.538 (2.4-8.1)
95%	2.849 (0.65-6.4)	2.607 (0.54-6.4)	14.61 (6.5-27)	11.19 (4.8-21)

HRP2: histidine-rich protein 2
CI: confidence interval
cRDT: conventional rapid diagnostic test
hsRDT: high-sensitivity rapid diagnostic test

Table 2. PCR Positivity and Estimated Parasite Density by RDT Result

RDT Result	Pf DNA positive (%)	Estimated parasite density of DNA positives (p/μL)
Artibonite cRDT		
Positive	88.4	511.4
Negative	4.5	6.7
Artibonite hsRDT		
Positive	85.4	463.2
Negative	3.3	10.3
Grand Anse cRDT		
Positive	84.0	1009.9
Negative	6.6	15.7
Grand Anse hsRDT		
Positive	81.6	998.1
Negative	5.6	15.6

Pf: *Plasmodium falciparum*
DNA: deoxyribonucleic acid
cRDT: conventional rapid diagnostic test
hsRDT: high-sensitivity rapid diagnostic test

Figure 1

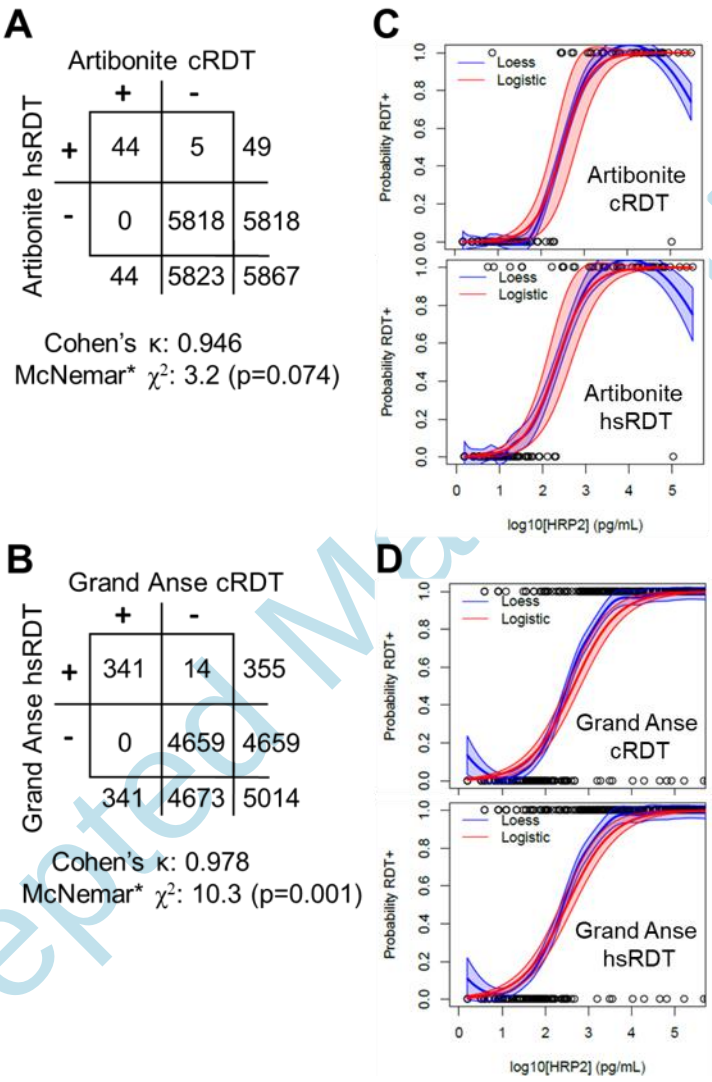

Figure 2

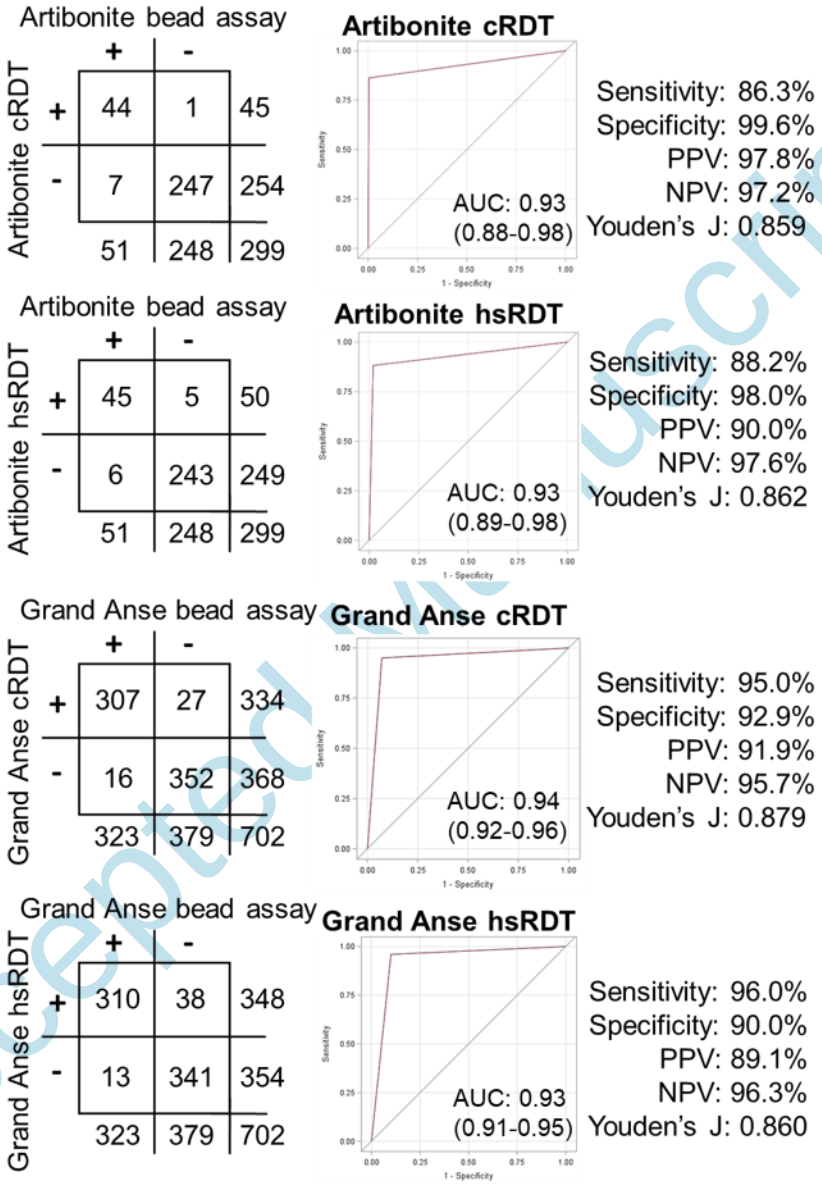

Figure 3

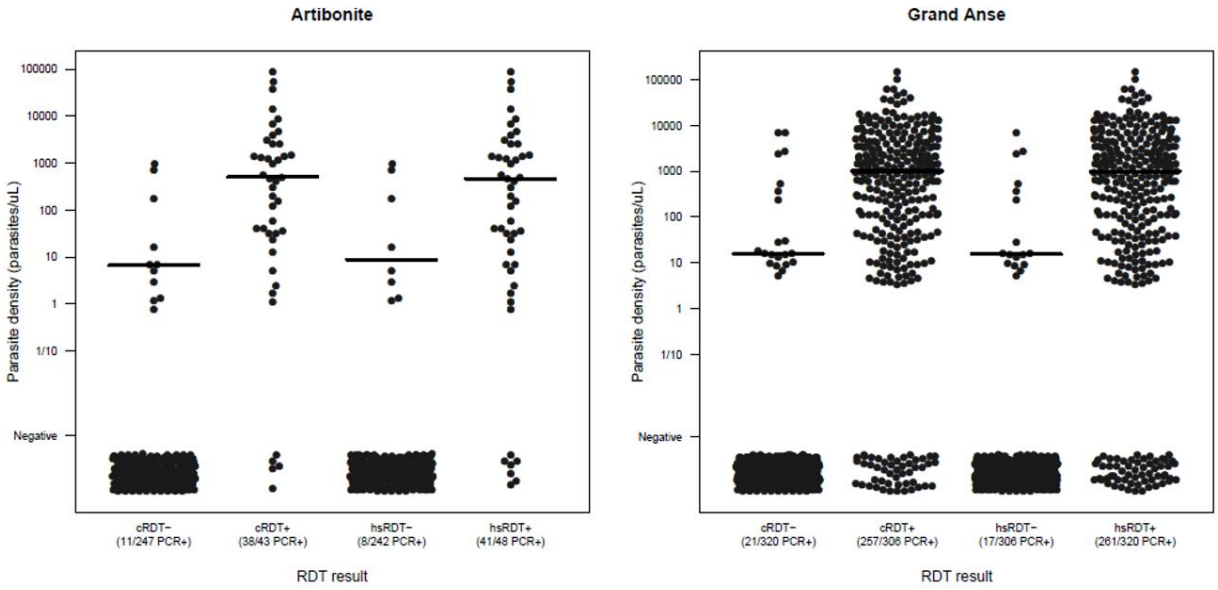

Figure 4