

Evaluating Field Performance of Highly Sensitive Malaria RDT: Detection of Infection Among Febrile Patients, Asymptomatic Pregnant Women and Household Contacts in Mpigi, Uganda

Daniel Kyabayinze ([✉ kyabayinzed@gmail.com](mailto:kyabayinzed@gmail.com))

World Health Organization Country Office for Uganda <https://orcid.org/0000-0002-0539-1341>

Dan Kajungu

Makerere University Centre for Health and Population Researchs

Proscovia M. Nambuya

Ministry of Health

Christopher Okiira

Republic of Uganda Ministry of Health

Bbaale Ndawula

Ministry of Health

Moses Kawooya

Mpigi Local Government

Isaac Ssewanyana

Republic of Uganda Ministry of Health

Maureen Amutuhaire

Republic of Uganda Ministry of Health

Damian Rutazaana

Republic of Uganda Ministry of Health

Jimmy Opigo

Republic of Uganda Ministry of Health

Susan.N. Nabadda

Republic of Uganda Ministry of Health

Research

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Abstract

Background

World Health Organisation recommends that malaria case management be based on parasite-based diagnosis in all cases, but currently available tools for clinical use have limitations, including the inability to detect low-level infections. Currently, next-generation highly sensitive rapid diagnostic tests (hsRDT) for *Plasmodium falciparum* (Pf) are commercially available, but require field-based validation. This study evaluated the performance of the highly sensitive NxTek™ Eliminate Malaria Pf (NxTek) diagnostic test in health facilities and community settings in Mpigi district, Uganda in comparison to the conventional rapid diagnostic tests (cRDTs).

Methods

Between April and December 2019, symptomatic and asymptomatic participants were randomly enrolled from the Outpatient Department (OPD), Antenatal Care (ANC), and community settings. The participants were tested with both cRDTs and an hsRDT to detect *Plasmodium falciparum* HRP2 antigen and quality assured results with PCR as a reference. Geo-coded real time data were transmitted by health facility and community-based providers using a smartphone with custom designed software.

Results

At the OPD, the parasite positivity rate was 13.3% for HS-RDT, compared to 6.4% with cRDT. The HS-RDT detected twice the number of positives compared to cRDT. At ANC, 11% (384/3,490) of the pregnant women were malaria parasite positive. HS-RDT detected more positive cases (10.4%) than cRDT (7.0%). At the community setting, 79.7% (2,397/3,009) of the under-five age group were positive for malaria parasites. Index clients in households resulted in a detection of 21.1% (1,877) asymptomatic positive especially among school going children. Health workers were able to learn how to effectively perform the HS-RDT, from a one-day training session. The additional support supervision was provided for VHTs to enable all to transmit results through the android phones.

Conclusion

Use of HS-RDTs increased case findings of low parasite density infections compared to cRDTs in study populations and provides the opportunity to eliminate malaria reservoirs through treatments. There were minimal additional training requirements when the HS-RDT kits were introduced. The observed increased positivity rates among school-age children call for integrating targeted interventions into school health programs.

Background

Malaria infections remain common worldwide and cause an estimated 229 million illnesses and 409,000 deaths per year, despite the extensive deployment of diagnostics, improved treatment of symptomatic

disease, increased investments in vector control and research [1]. Accurate diagnosis is a critical step in initiating effective management of malaria as well as breaking the malaria transmission chain[2]. In research settings, the use of molecular diagnostic tests has uncovered a large reservoir of malaria parasites previously not detected by microscopy and RDTs[3, 4], that contribute to malaria transmission[5-7]. Children and pregnant women are some of the most vulnerable population. Worldwide, in 2019, of the estimated 9 million deaths in children younger than 5 years of age, 8% were attributable to malaria. Uganda has the 3rd highest global burden of malaria cases (5% of the cases worldwide) and the 8th highest level of deaths (3% of all malaria death worldwide) [2]. Frequent malaria infections and illness in children also increases the risk of stunting, and makes the child vulnerable to other infections that may cause permanent neurological and cognitive damage [8]. Malaria in pregnancy (MIP) is associated with an increased risk of poor foetal outcomes such as low birth weight (LBW), prenatal, neonatal and infant mortality [9-11]. Infection with *P. falciparum* towards the end of gestation increases the likelihood of placental infection [12]. A review by Desai *et al.* estimated that approximately one in every four pregnant women in malaria-endemic areas have evidence of placental infection at the time of delivery [13]. In high transmission settings including Uganda, the World Health Organisation (WHO) recommends the use of Sulphadoxine-pyrimethamine (SP) for intermittent preventive treatment in pregnancy (IPTp), as an effective method of preventing malaria in pregnancy. Intermittent screening and treatment (IST) of malaria during pregnancy has been proposed as an alternative, or to augment intermittent preventive treatment in pregnancy (IPTp), where IPTp is failing due to drug resistance. IST of malaria during pregnancy has been proposed to better target efficacious antimalarial treatment of asymptomatic women with evidence of malaria infection, and have been found to be beneficial for women to receive targeted therapy [14]. However, the antenatal parasitaemias are frequently very low density, and the most appropriate screening test for IST has not been defined. For vulnerable children, integrated community case management (ICCM) is a key intervention for malaria control with the potential to test, treat, and track cases, and enhance community-based surveillance systems. Village health team (VHT) have been equipped to provide care at the community in Uganda.

Recent world malaria reports showed that the global malaria burden remained unchanged in the last four years, undermining the progress previously achieved between 2000 and 2016[15].

Inclusion of more accurate diagnostics for detection of malaria infections supported by efficient capture and use of data is a necessary improvement required for malaria elimination generally. RDTs have been widely deployed to achieve universal parasite-based diagnosis and as a surveillance tool. RDTs are visual lateral flow tests that detect parasite-specific antigens in blood—Antigen detection thresholds vary widely among RDTs, and the distribution of target antigens in peripheral blood circulation is expected to differ. Currently, the best *P. falciparum* malaria RDTs have a limit of detection (LOD) in the range of 600-1,000pg/mL HRP2 [16, 17]. The limitation of the cRDTs is that it may not detect infections of parasite density less than 100 parasites per microliter (ml) or one parasite per 200 White blood cells in comparison to expert microscopy.

The Alere™ malaria HS-RDT (now NxTek™ Eliminate Malaria Pf) was developed for field use and was evaluated for the detection of low-density infections. Compared with the commercially available HRP2-based conventional cRDTs) the HS-RDT uses the same immunochromatographic cassette platform, has the same whole blood volume requirements (5ul), and takes 5 minutes longer to obtain results, making this device a promising and highly sensitive field tool.

Inability of current diagnostic tools to detect asymptomatic low-density infections will hinder the elimination efforts. Detection and treatment of low-density infections will reduce transmission, delay emergency care and spread of drug resistance. Therefore, the potential value of RDTs in this population can best be established through comparative evaluation using highly-sensitive RDTs.

An observational comparative study was conducted to evaluate the utility of highly sensitive NxTek™ Eliminate Malaria RDT (NxTek™) HS-RDTs in detecting low parasite infection in health facilities and for active case detection (ACD) at the community level in Mpigi district, Uganda.

Methodology

This report conforms to 'Strengthening the Reporting of Observational Studies in Epidemiology' (STROBE) guidelines for reporting results of observational cohort studies[18] and Standards for Reporting of Diagnostic Accuracy (STARD) guidelines[19].

Study team training and data collection

All health workers from the selected facilities and ICCM providers based in the community were invited to a district-based training on the use of RDTs for malaria in a back-to-back fashion that ensured no interruption of the service delivery in the health care facilities. The training curriculum was based on the WHO guidelines for use of RDTs [20]. In addition, the training introduced testing using HS-RDTs, reporting of test results using the android phone as a data logger, and how to collect blood on filter paper for future PCR evaluation. Health workers were instructed to adhere to the test results as the study standard, or treat according to the national guidelines on IMCI. At the community level, when a child presented to the VHT, a malaria RDT was performed and treatment was offered based on test results. They were supervised by trained coordinators linked to public health facilities.

Study site and participants

The study took place in all the sub-counties of Mpigi district, Uganda, an area where malaria transmission has been reported as low and perennial with two peaks in April and November[21], with a parasite prevalence of <5% and entomological inoculation rate of less than 150 bites/person/year in 2006 [22]. *P. falciparum* is the dominant malaria species accounting for 99% of infections[21]. Between April and December 2019, a prospective observational evaluation was conducted within the health centers and surrounding communities in Mpigi, with a catchment population of approximately 250,508. At a community level, the community-based VHT service providers test, treat and manage common childhood

illnesses such as malaria, pneumonia and diarrhoea. When a child presents to the VHT, a malaria RDT is performed and treatment is offered based on test results. They are supervised by trained coordinators linked to public health facilities. For the study, children with positive cRDTs test results were considered index cases and were enrolled in the study for active case detection (ACD).

Study design and testing procedures

The study enrolled participants into three cohorts: (1) out-patient cohort (OPD), (2) antenatal cohort (ANC), and (3) community cohort (ICCM). Only participants who provided consent, were of the required age category and without severe disease that required emergency attentions were enrolled in the study.

OPD Cohort: Participants were screened and enrolled at both OPD clinics within the catchment facilities of the 28 health facilities. All children 6 months and older, and adults presenting at the OPD with fever or history of febrile illness in the previous 24 hours, were enrolled. Participants agreed to be tested/investigated multiple times according to the study protocol and testing algorithm used for the day.

ANC cohort: At ANC clinics, pregnant women seeking care and eligible for IPTs in the clinic were medically evaluated and enrolled in the study by the study team. All ANC participants were given a long-lasting insecticidal bed net and additional testing according to the national procedures.

Community Cohort: At the community level, the VHT enrolled febrile children under five years of age and provided a diagnostic test and subsequent case management based on results. Children positive for malaria infection confirmed with cRDTs were defined as index cases and selected for subsequent testing of the entire household, described here as active case detection (ACD).

Clinical care and diagnostic testing

Participants presented to the clinic for routine care (monthly for ANC cohort) or with fever at OPD and Community VHT's homes. Patients with severe disease and requiring inpatient management or referral were excluded from the study. At each visit, clinical interviews were performed, and blood by finger prick and dried blood spots (DBS) were collected, regardless of symptoms. Samples were selected for PCR if both HS-RDTs and cRDTs had been performed regardless of the test results (negative and positive included). The DNA extracted from DBS was tested for the presence of *P.falciparum* parasitemia using qPCR at Central Public Health Laboratories (CPHL). Fever was described as a temperature of $\geq 38.0^{\circ}\text{C}$. Routine clinical and antenatal care was provided by health workers at the health center, including clinical officers, midwives, nurses, and other similar cadres of health care staff. Patients with reported or documented fever and any positive RDT result were treated with age-specific standard dosing of artemether-lumefantrine both at ANC and OPD[23]. Participants with asymptomatic parasitaemia were provided anti-malarial therapy at ANC, but had their IPTp delayed for two weeks in accordance with local standard-of-care.

Rapid Diagnostic testing

The Malaria Antigen Pf tests are a one step, rapid, qualitative tests for the detection of HRP-2 specific to *P. falciparum* in human blood samples. The NxTek™ Highly-Sensitive Rapid Diagnostic Test (HS-RDT) complete kits used in the study included the following batch numbers and respective expiration dates: (1) 05LDE001A, 20.02.2020; (2) 05LDE003A, 31.03.2020; and (3) 05LDE004A, 01.04.2020, and was compared to CareStart™ Malaria HRP2 (Access Bio, Inc. (Monmouth Jct, New Jersey, USA). All kits were supplied according to the national system supply chain mechanisms in place without monitoring of temperature and humidity in the storage at testing sites. The malaria RDT testing followed the manufacturer's instructions for each specific malaria RDT brand. The results were recorded by the health worker or CHW at 15 (cRDTs) or 20 (hsRDTs) minutes. Result reading and interpretation were according to the manufacturer's instructions and product package inserts for each malaria RDT brand. If a control line did not appear or only the test result appeared, the result was deemed to be invalid and was repeated.

Testing and Laboratory methods

Participants who met the eligibility criteria were enrolled in the study to undergo testing. A two-phased approach, serial and concurrent, was undertaken to conduct testing of the participants using the RDTs. With serial testing, the patients were first tested with cRDTs at OPD, and only participants who tested negative were offered an additional HS-RDTs test. With the concurrent testing approach, the participants were tested with cRDT and HS-RDT at the same time, which gave two results for each patient (Figure 1).

The DBS were prepared by spotting a drop of blood onto filter paper, air drying it completely, and storing it at room temperature in an individually sealed plastic bag with silica gel (desiccant). *P. falciparum* DNA was extracted and analysed using standardised methods as previously described [24]. PCR technologists were blinded to the RDT results. As a reference standard, PCR testing targeted a minimum of 500 samples from OPD and ANC cohorts used to determine the diagnostic performance (Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV)) of the both cRDTs and HS-RDTs

Data recording and transmission

Clinical notes including demographic information, presenting malaria symptoms (febrile illness, fever, joint pains, headache, nausea, vomiting), cRDT and HS-RDT results, and treatment administered were captured in paper format and stored in a controlled location. The results of both cRDTs and HS-RDTs were recorded and transmitted to a central server using the Data Logger SMAPP for further processing and analysis. Real time data were transmitted and enabled the generation of geospatial maps to track malaria prevalence across villages within all sub-counties of the Mpigi district.

Data analysis

Data were analysed using STATA (version 13; STATA Corp., College Station, TX, USA). The proportion of participants with no detectable infection by both cRDTs, HS-RDTs and PCR were made for different cohorts and age strata. The analysis data set included a hard paper questionnaire, double entered into

the EPIDATA database and digital data was captured by the Data loggers displayed on dashboards. The analysis compared test results between HS-RDTs and cRDTs to estimate the magnitude of undetected low parasite densities, with qPCR as quality control. Qualitative methods were used to understand suitability of the tools. Kappa statistics with 95% CI were used to assess the agreement between the cRDT and the HS-RDT. Using qPCR as the reference standard, clinical performance of the cRDT and HS-RDTs were calculated (sensitivity, specificity, PPV and NPV) for the presence of *P. falciparum*. Focus Group Discussions (FGDs) and individual structured key interviews were conducted to collect qualitative information on the health workers' experience and perception on the use of the HS-RDTs. Participants were purposely selected to obtain a representation of both the facility and village health workers with differences in the range of abilities. A total of five interviews were conducted of which three were focus group discussions (FGDs) conducted among the facility-based health workers, one FGD for VHTs and one structured interview for the key informant interview (KII) with the District malaria focal point-person. The discussions were conducted by a team of three study personnel (including a moderator and a note taker), following topic guides that had been specifically developed for this study. The discussions were conducted either in English and/or the local language (Luganda), depending on which was convenient for the health workers. The audio files were transcribed into texts, which were then thematically analysed.

Results

A total of 28 private and government-sponsored health facilities providing routine antenatal and curative out-patient care were selected as study sites. Patient enrolment and testing was conducted between April and December 2019 in Mpigi district. A total of 312 health workers were trained during the whole course of the study, among whom 217 were VHTs and 95 were facility health workers during eight training sessions. The trained health workers were issued with HS-RDTs and android phones as data loggers based on the patient load estimates for 3 months. Of these, 217 (70%) were VHW, and two-thirds were female. The study team screened and enrolled 29,333 clients within the three cohorts. The demographic details of the participants enrolled are summarised in Table 1.

Symptomatic participants at OPD: Out of the 11,511 patients screened, 8,585 (74.6%) were tested, 1,553 in a serial procedure (cRDT first, then HS-RDT for the initial negative). Out of the 1,553, only 165 (11%) were positive with cRDT. The subset of negative cRDT patients were tested again with HS-RDTs. Out of 1,386 (1,553-165) patients, 167 (12.1%) were positive on HS-RDTs, documented as the undetected magnitude of low-density infections with two missing results.

Of the 7,032 patients tested with both cRDTs and HS-RDTs performed by the concurrent testing approach, a 7% difference was observed, with 447 (6.4%) cRDT and 933 (13.3%) HS-RDT positive for malaria infection. The calculated level of agreement between the two test kit results was 91.2% (6,410/7,032), kappa statistics = 0.51 ($p<0.001$). The details are shown in Table 2.

Asymptomatic pregnant participants at ANC: A total of 3,446 women tested at ANC were tested with HS-RDT, of whom 13% (67/514) were positive. When cRDTs and HS-RDTs were performed using the

concurrent testing approach, 10.4% (310/2,976) (95% CI 9.3-11.6) were HS-RDTs positive compared to 7.0% (209/2,976) (95% CI 6.2-8.0) for cRDTs. The percentage increment detected due to low-density infections was 48% (310-209/209) (95% CI: 41.6-55.1). The level of agreement between the two tests was 96.1%, kappa statistics = 0.758 ($p<0.001$).

Febrile children among Community cohort: Seventy-three percent (3,009/ 4,131) of the febrile children tested for malaria using cRDTs in the VHT provider cohort were children under five years of age. The number of cRDTs that tested positive among the children were 2,397 (79.7%), who were treated and denoted as index cases. When asymptomatic households of these children were tested with HS-RDTs, 21.1% (1,877/8,888) were found to be positive. The proportions of the asymptomatic infections were: (1) 26.3% (672/2,555) under-five years of age, (2) 25% (546/2,192) 6-12 years of age (school going age group), and (3) 16.3% (653/4,010) 13 years of age and above. One hundred and one (101) participants had missing age information and were removed from further stratified analysis (Table 3A and B). Invalid results at all testing points were repeated so that a decision for treatment was made. At the community level, invalid results were five out of the 4,131 (0.12%) for cRDTs and fourteen out of 8,888 (0.16%) for the HS-RDT test kits.

Polymerase Chain Reaction (PCR): Out of the 10,008 parallel tests performed at OPD (7,032) and ANC (2,976) a the subset of 497 paired samples analysed for *P. falciparum* infection using qPCR. The samples at OPD were from febrile symptomatic patients and xx from OPD were asymptomatic women seeking routine care, For the total PCR tests done, 52.2% (95% CI 46.3-58.0) had detectable *P. falciparum* infection, with wide variability as shown by the CT values(Appendix 1). The sensitivity was 75.5% (95% CI: 69.7-80.7) for HS-RDTs compared to 45.8% (95% CI: 39.6-52.2) for the cRDTs for any detected parasite for the symptomatic cases at OPD. The specificity, positive and negative predictive values are shown in Table 4.

Acceptability and ease of use of new technology:

All 250 health workers were able to provide data throughout the study period. Each user of the smartphone device required an operator account. The data logger was used to transmit test results for both cRDT and HS-RDTs and associated patient demographic information, as well as a tracked sample number (ticket). Thirty-seven (37) of 217 (17%) community health workers did not enter the test results in the data logger, even when they were testing and keeping the hard-copy records. The majority, (75%) of the data transfers were transmitted and received during the normal working time (8:00am-5:00pm) and the remaining data was sent at the end of the day or at night. (Figure 3).

Health workers scanned the digital Near Field Communication (NFC) feature that provided the Lot and Batch numbers as well as expiration dates for 4,753 tests. Such details were useful for logistics management, quality control, and tracking activities. Analysis of the data showed that all the kits were utilised within the shelf life-time before April 2020 (Figure 4).

In Mpigi, 20 participants, 8 VHTs and 14 clinic staff participated in the FGDs and KII. Overall, the users of HS-RDTs found them easy to manipulate, given their routine use of malaria RDTs, and experienced

minimal challenges transmitting test results on the android mobile phones. The skill improved with time as they used the phones and additional training for those who were struggling.

Acceptability of new technology was observed and demonstrated by the users. In general, health workers reported that using the new technology gave them an opportunity to have peer discussions on the quality of the results generated. The summary of responses and views are summarized in Table 5. Participation in the study restored confidence among health workers (VHTs), and raised their motivation to serve their communities (quote 2a, 2b). The health workers made personal adjustments to accommodate the work requirement by transmitting data at a convenient time during the study (Quote 2c)

The tools were a useful demonstration of quality of the diagnostic process at all levels. When both cRDTs and HS-RDTs were used in the community, VHTs used kits as a way to confirm RDT quality and restore confidence in RDT results in situations where doubt existed. For example, when health workers encountered a discrepancy between their clinical impression (that a patient had malaria) and a negative RDT result, HS-RDT use was reported to help resolve the uncertainty (Quotes 3 and 4). Some health workers mentioned that HS-RDTs were useful in detecting asymptomatic malaria infection among ANC women. (Quote 4)

Health workers had previously been trained to check RDT expiration dates and desiccant packets as a means of quality control. When HS-RDTs and Data Loggers (SMAPP) were introduced, the same information was obtained by scanning the near field communications (NFC) tag, and imbedded method similar to reading a bar code. This introduced a new quality-control option which was integrated into routine practice, reported as useful and fully embraced. (Quote 5). Use of SMAPP phones to transmit data was time-saving, improved the quality of the data collection, reporting, analysis, and tracking of commodities (Quotes 6, 6b and 7).

Discussion

Local magnitude of undetected malaria infections:

Rapid diagnostic tests (RDTs) are the only practical method to provide parasite-based early diagnosis in remote and poorly resourced areas, where most malaria cases and malaria mortality occur[2]. Evidence from the study demonstrated that the use of HS-RDTs yielded a two-fold increase in the number of malaria detections among patients seeking care for febrile illness at OPD in health facilities compared to conventional tests. This was consistent for the two testing approaches. The reported positive cases were twice as high with HS-RDTs across the different study populations of clinical symptomatic patients and asymptomatic pregnant women (6.4% with cRDT, compared to 13.3% for HS-RDT). When tests were done on the same patients, the HS-RDT detected twice the number of positives as cRDT. Similarly, HS-RDTs testing in asymptomatic pregnant women attending ANC showed a 13% prevalence. The magnitude of undetected malaria infections in symptomatic patients at OPD and non-febrile patients giving negative results using the conventional test kit was higher. The positive cases that had been missed by the cRDT but detected by the HS-RDT may represent undetected malaria infections with low parasite densities. This

evidence correlates with results from earlier studies in Uganda [25] and Colombia [26] that demonstrated that HS-RDTs may offer an improved parasitological diagnosis of malaria with a significantly lower LOD. The first of these is the NxTek™ Eliminate Malaria Pf. RDT hsRDT) for Pf HRP2. This test has a ten-fold improvement in Limit of Detection (LOD) from 800 pg/mL to 80 pg/mL Pf HRP2, as demonstrated with recombinant Pf HRP2 and several native Pf HRP2 types from axenic parasite cultures [25]. Further validation of the HS-RDTs with a panel of clinical specimens from asymptomatic study participants in Myanmar and Uganda showed that the order of magnitude of improvement in LOD indicates: (1) the ability of the test to reliably detect infections with as low as 1 pg/µL as compared to the 50-200 pg/µL detected by microscopy or current RDTs, and (2) the detection of Pf HRP2 as low as 25 pg/mL as compared to 800 pg/mL by current RDTs [25]. The results indicate a significant increase in sensitivity for *P. falciparum* detection in low-density sub-clinical infections with the HS-RDTs compared to the current commercial RDTs. Additionally, asymptomatic cases were detected in Uganda and Myanmar[25].

The school-going age group had a disproportionately higher malaria positivity rate among asymptomatic individuals.

The positive cases that had been missed by the conventional RDT (cRDT) but detected by the HS-RDT may represent undetected malaria infections with low parasite densities.

The majority of the services offered at OPD were utilised by women and school going children. Prevention emphasis should now focus on the new vulnerable groups observed in the study population of school going children. The disease burden seems to be shifting to the school age group, and therefore, routine testing and prevention approaches to cover this group needs to be introduced under school health care programmes.

Improved diagnosis is the first step in providing appropriate treatment. The use of a more robust test kit will ensure that all malaria cases receive the appropriate treatment, and the negative cases are provided alternative care. Effective diagnosis and treatment result in reduced disease burden.

ANC Cohort. In Uganda, pregnant women are provided IPTp with the assumption that they will not have any parasites. In this study, the introduction of testing in ANC identified asymptomatic pregnant women who benefitted from the appropriate timely treatment. For the asymptomatic pregnant women visiting ANC, the magnitude of undetected malaria infections was 13%. Antenatal parasitaemias are frequently very low and a testing tool for this population is yet to be defined. A nested analysis of 198 women, showed that close to half of these women in the low to medium transmission setting had detectable malaria infection. HS-RDTs showed a sensitivity of 75% (13% positive) against PCR. The higher sensitivity of the HRP2-detecting RDT may reflect the detection of circulating antigen from placenta sequestered malaria parasites, or recent spikes in parasite density remaining as persistent antigenemia. While it is reasonable to postulate that higher parasite densities in circulation may cause greater harm to mother and child, a greater understanding of the clinical significance of the third of PCR-positive women, who were undetectable by the most sensitive RDT and so would not receive treatment under a RDT-based

testing approach, is needed. These findings are not similar to earlier studies performed in Eastern Uganda and Burkina Faso[14].

Currently, achieving greater sensitivity is possible with the next-generation highly sensitive RDTs, supplementing the use of a nucleic acid amplification test (NAAT) assay. . However, development of pan-species malaria are yet to be developed. As IPTp is reduced, programmes will have to make this cost-benefit trade-off of introducing sensitive tests like HS-RDTs. A previous study showed that improving the diagnostic sensitivity to 20 parasites per microlitre increased the proportion of detection of infection by 49% in Burkina Faso[27].

The updated WHO recommendation is that all pregnant women in malaria endemic countries must receive at least three doses of IPTp. However, the coverage of IPTp in most of sub-Saharan Africa remains below international targets. Given the low coverage and other factors that limit the effectiveness of IPTp, it is not unusual to find pregnant women with malaria infection during ANC testing surveys in endemic areas[14, 28]. These findings showed a malaria prevalence of up to 13% among ANC clients, justifying the need to test and treat. This difference between the two testing tools is expected among the asymptomatic pregnant women in this setting because of low parasite densities.

ICCM treatment: CHWs, their supervisors at the district, and study facilitators commended the reactive case detection (RCD) system for improving access to malaria services, and significantly detecting a number of cases in their areas. The main implementation barriers included lack of supplies such as rain gear, transportation such as bicycles, communication (e.g. difficulties in maintaining cell phone charge to transmit data by phone), and inconsistent supply chain (e.g., inadequate numbers of RDT kits and anti-malarial drugs to test and treat uncomplicated cases).

Future efforts to reduce the spread of malaria further will require moving beyond the treatment of clinical infections to targeting malaria transmission more broadly in the community. As such, the accurate identification of asymptomatic human infections, which can sustain a large proportion of transmission, is becoming a vital component of control and elimination programmes[29].

The relative importance of these very low parasite infections has been debated in relation to their contribution to malaria transmission[29-35]. In areas working towards malaria elimination, the low undetected infections act as a reservoir of infection, even when they do not contribute to the burden of malaria cases[32].

To our knowledge, this is the first study to conduct a RCD, using the existing structures of the ICCM, where the index cases of a positive under five years of age child was the basis for tracking household members (contacts). RCD seeks to enhance malaria surveillance and control, by identifying and treating parasitaemic individuals residing near index cases.

Many VHTs found the RDC feasible and effective, similar to other places in Zambia[36]

One in every five household members tested using the HS-RDT was found to have malaria parasites antigens. The majority of these were in the school going age group. This may call for targeted intervention at schools like mass drug administration (MDA).

Training and capacity development:

Our study showed that health workers across different cadres and with different levels of pre-service training were able to learn how to effectively perform the HS-RDT, from a one-day training session. Because the HS-RDTs have the same design and usability features as the current cRDTs, they were easily adopted in health facilities and community settings and detected a high proportion of people with low-density *P. falciparum* infections. The only additional training needed beyond the one-day training was specifically for the data logger usage and this was done through continuous mentorship where need was identified.

The HS-RDTs exhibited excellent specificity. The advantage of this study is that it was performed with clinical specimens from an endemic region, in real-life resource-limited settings. The results depict performance under field conditions. This validates the findings from laboratory studies of high accuracy of the new tools[37]

Data logger: To our knowledge, the smartphone loaded with the data logger mobile app was used by POC clinicians to log patient results in real-time, and for the first time, piloted in Uganda during the HS-RDT malaria study. There was no damage due to a fall or mishandling for all 250 devices deployed for 6 months of the study, which can be attributed to the sturdy protection casing. Both the hardware and software are secure in that and it allows only the app and associated phone system settings to run.

Routine review of data submitted using the data logger, guided identification of individuals whose phones were not visible on the server and/or had data errors, received additional mentorship and support. The data logger allowed for the capture of GPS coordinates, as the results were transmitted to the central server. The accurate GPS coordinate elements captured along with each transmitted result allowed the application to create geospatial outputs for analysis of the disease. When this was utilised, interventions could be targeted to the identified locations and populations.

The feature of data cost savings enables only the data logger to access internet data for sending results, while all the other background apps are disabled. Charging of mobile devices has been one of the main challenges of implementing real-time electronic datasolutions in rural areas.

Limitations: The three tests were not compared against microscopy. In different transmission settings, where parasitemia and HRP-2 distribution vary, the improvement in the detection of HS-RDTs versus cRDTs is not consistent. Future studies should be conducted in varied settings to validate the importance of improvement in the detection of asymptomatic infections. However, many other studies have shown that the performance of RDTs is superior to routine microscopy, both in symptomatic and asymptomatic populations [27, 29].

This study did not consider the whole spectrum of case management beyond diagnostic results, and therefore recommends further research on how to handle cases of low parasitemia among asymptomatic patients in the community.

The recent development and distribution of *P. falciparum* pfhrp2 and/or pfhrp3 gene deletion is not fully characterised in this part of the country. The deletions, if present, may have reduced the specificity of both HS-RDTs and cRDTs. In other studies, HS-RDTs were found to have the same threshold of cross-reactivity with HRP3 similar to cRDTs [38]. HS-RDTs was found to have a 10 fold lower parasitaemia limit of detection (LOD) than SD-RDTs [37] .

Conclusion

This study was conducted in an operational manner, putting the tools in the hands of many and diverse health caregivers in their routine settings, to demonstrate feasibility and acceptability of HS-RDTs both at health facilities and community care providers (VHTs). This can be translated into routine practice, using new technology of data collection and reporting among all health workers, including ICCMs and reactive case detection for malaria surveillance.

The health workers did not require additional training to perform the HS-RDTs except for a single demonstration and provision of a chart on how to conduct the test. The HS-RDTs have the same design and usability features as the current RDTs, and hence was easily adopted. In comparison to conventional RDTs, the HS-RDT was able to detect low parasite densities among both symptomatic and asymptomatic patients.

This study demonstrated the ability to detect asymptomatic cases, increased case findings using HS-RDT, leading to the elimination of a malaria reservoir through treatment of asymptomatic patients, active case detection and linking the community households to care, which is the actual goal of malaria elimination. This may help identify active case detection strategies that will successfully reduce the parasite reservoirs.

Previous concerns about overtreatment, incorrect diagnosis of febrile patients, increased burden to malaria control programmes due to identification of more malaria cases may be valid and need to be better understood in large scale out implementation pilots. Further, research is needed to better understand how a more sensitive test can be implemented appropriately in a manner that is cost-effective. This evidence supports the earlier propositions by Das *et. al.* that the HS-RDT is an effective diagnostic tool for malaria control and elimination programmes [37].

Declarations

Ethical Approval and consent to participate

The study protocol was reviewed and approved by the Uganda National Council for Science and Technology and institutional review board of Mildmay Uganda Research Ethics Committee (REF#:0402-2019). Informed consent was obtained from all participants and parents or guardians of the participating children. Additional consent was obtained from household members from the community, in which an index case is identified by village health teams (VHT).

Consent for publication

Not applicable

Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

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Competing interests

There authors declare on competing interest. The study sponsors did not have any role in the study design, conduct or interpretation of the results.

Authors' contributions

DJK, DK, JO, SN and DR conceived and participated in designing the study. PMN, BB, BN CO and MK directed the field study. IS, PMN, MK, MA, DR, BKB, and TM participated in the data acquisition and the on-site supervisor. DJK, SN, DK and TM verified data, DK, BN and PNM analysed the data. DJK, DK and SN drafted the manuscript and all others authors contributed equally in the preparation. All authors read and approved the final manuscript.

Authors' information

Not applicable

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Tables

Table 1. Overall demographics of patients screened for eligibility to participate in the diagnostic study

<i>Variable</i>	<i>Category</i>	<i>Number N</i>	<i>Percent (%)</i>
<i>Outpatient Department (OPD) (N=11,511)</i>			
<i>Sex</i>	Female	7,158	62.2
	Male	4,346	37.7
	Missing	7	0.06
<i>Age groups</i>	<5 years	1,645	14.3
	5-17 years	3,672	31.9
	18 years and above	6,182	53.7
	Missing	12	0.1
<i>Antenatal care clinic (ANC) (N=5,151)</i>			
<i>Age groups</i>	<5 years	42	0.8
	15-49 years	5078	98.6
	50 years and above	22	0.4
	Missing	9	0.2
<i>Community (N=12,671)</i>			
<i>Sex</i>	Female	6,910	54.5
	Male	5,615	44.3
	Missing	146	1.2
<i>Age groups</i>	Less than 5 years	5,119	40.4
	5-17 years	4,054	31.9
	18 years and above	3,365	26.6
	Missing	133	1.1

Table 2: Table showing the proportion of patients tested at OPD with a positive result using both the HS-RDTs and cRDTs stratified for age in Mpigi district, Uganda between May (April? – December 2019

<i>Stratum</i>	<i>Serial testing*</i>		<i>Parallel testing[‡]</i>	
<i>Age groups</i>	cRDT	HS-RDT	cRDT	HS-RDT
	Proportion positive (n/N)	Proportion positive (n/N)	Proportion positive (n/N)	Proportion positive (n/N)
<5years	11.6% (29/250)	11.3% (25/221)	5.10% (54/1,051)	13.9% (146/1,051)
5-17years	18.1% (92/509)	14.9% (62/416)	8.5% (192/2,267)	16.6% (377/2,267)
>18years	5.60% (44/793)	10.7% (80/748)	5.4% (201/3,713)	11.0% (410/3,713)
Total	10.6% (165/1,553)	12.1% (167/1,386)	6.4% (447/7,032)	13.3% (933/7,032)‡

**Serial Testing (Phase I)*: The patient is offered a cRDT test first test. If the first result is negative, a second test is conducted with HS-RDT.

[‡]*Parallel testing (Phase II)*: In this case, the same patient sample was subjected to both cRDT and HS-RDT test kits simultaneously.

Table 3A and B: Step-wise use of cRDT for index case diagnosis followed by HS-RDTs for entire household screening pilot of active case detection (ACD) of malaria in Mpigi , Uganda 2019

<i>A</i>		<i>B</i>			
<i>Stratum</i>	Number of ICCM/VHT diagnostic tested n(%)	cRDT Positive	Number of Household screened	HS-RDTs Asymptomatic Positive cases n	Positivity rate of age category
<5 year	3,009(72.8)	2,397(79.7%)	2,555	672	26.3%
6-12years	1,122 (27.2)		2,192	546	24.9%*
>13 years			4,010	653	16.3%*
Missing			131	6	4.6%
Total	4,131		8,888	1,877(21%)	21.1%

*statistically significant difference between the two age group populations (proportions by chi-square test)

Table 4: Performance evaluation of HS-RDT and cRDT for the detection of *P. falciparum* in peripheral blood during antenatal and Out-patient department visits with PCR as reference method.

OPD (qPCR positive=155/297)		ANC (qPCR positive=98/198)		Overall (qPCR positive=253 /495)	
Statistic (95% CI) Prev 52.2%		Statistic (95% CI) Prev 49.5%		Statistic (95% CI) Prev 51%	
	HS-HRP2 RDT	c-HRP2 RDT	HS-HRP2 RDT	c-HRP2 RDT	HS-HRP2 RDT
Parameter					
Sensitivity	73.5 (65.9-80.3)	39.4 (31.6-47.5)	78.6 (69.1-86.2)	56.1 (45.7-66.1)	75.5 (69.7-80.7)
Specificity	89.4 (83.2-94.9)	97.9 (94.0-99.6)	91.0 (83.6-95.8)	97.0 (91.5-99.4)	90.1 (85.6-93.5)
PPV (%)	88.4 (81.5-93.3)	95.3 (86.9-99.0)	89.5 (81.1-95.1)	94.8 (85.6-98.9)	88.8 (83.8-92.7)
NPV (%)	75.6 (68.4-81.9)	59.7 (53.1-66.0)	81.2 (72.8-88.0)	69.3 (60.9-76.8)	77.9 (72.5-82.6)

*PPV – Positive Predictive Value

*NPV – Negative Predictive Value

Prev- Prevalence as a proportion positive by qPCR

Table 5: The quotes from health care workers narrations

Quote	Narrative
Training process	
Quote 1	"Some VHT's faced challenges with the data logger in trying to adopt (adapt?) towards using and navigating their way into the app. Many had challenges trying to log in due to exposure gaps, forgetting the log-in information, missing villages in the phone system, some had no electricity for charging the phones but over time they got used, learned the best way to use the phone and they succeeded." KII, District Malaria Focal Person
Acceptability of tools	
Quote 2a	"..... we tested a positive child and then tracked the household members from whom we could also be able to identify positive cases. This bought us a lot of trust and confidence among the village people and actually when they heard that you had recalled the phones, they were so disappointed because they really appreciated the work we were doing." ICCM Buwama
	"More to that they helped improve our image in the society with the way people see us. Whenever you pulled it [data logger] out to send the data then people would look at you in a way that you really know what you are doing, and this has built more trust in us". ICCM 2 Buwama
Quote 2b	"Generally, motivation of VHT's was raised with gained confidence in the use of smart gadgets." KII MFP:
Adaptability	
Quote 2c	"We were sending the results mostly in the evening after work when you have cleared all the day's work and have cleared off all the clients because they are always many and you couldn't get into sending the results as you still have many clients wait in a queue." Health worker from Buwama HCIII
Utility and quality checks	
Quote 3	"Sometimes they would bring you a child who is really sick and when you test at the first attempt the kit shows negative but when you repeat the test like 3 times, then you get a positive (that's using cRDT) but when you go to the household members just one kit of HS RDT would be enough to show you whether one has malaria or not showing that this was really highly sensitive." ICCM Buwama:
Quote 4	"Yes it's really important. Because these mothers need to be tested such that you know exactly which treatment you have to give them for example if you were going to give them Fansidar (Sulfadoxine Pyrimethamine), when you realise they have malaria then you first give them anti-malarial then when they finish the dose you see if you have to give them Fansidar." Mitala Maria HCIII-Health Worker
Quality control	
Quote 5	"Me, initially I didn't know until we came back here for a review and I was told we were supposed to scan it [referring to the NFC tag] then I started scanning it." ICCM Buwama
Data quality reporting	
Quote 6a	"There was increased awareness about malaria especially at the community level through the contact tracing and testing following index patients. The reporting of real time data helped us to locate hotspots and this is going to be a basis for the future decisions". KII - District Malaria Focal Person

Quote	Narrative
Quote 6b	"It helps in monitoring of malaria data without forging it. This will help to monitor the use of testing kits and the anti-malarial which are supposed to be administered to clients that have tested positive. This will help to minimize on the misuse." Buwama HCIII -Health Worker:
Quote 7	"It's quick and new technology. Normally we have to go to the district with a compilation of the data to be sent, but with this you just send data without going to the district." Mitala Maria HCIII-Health worker

Figures

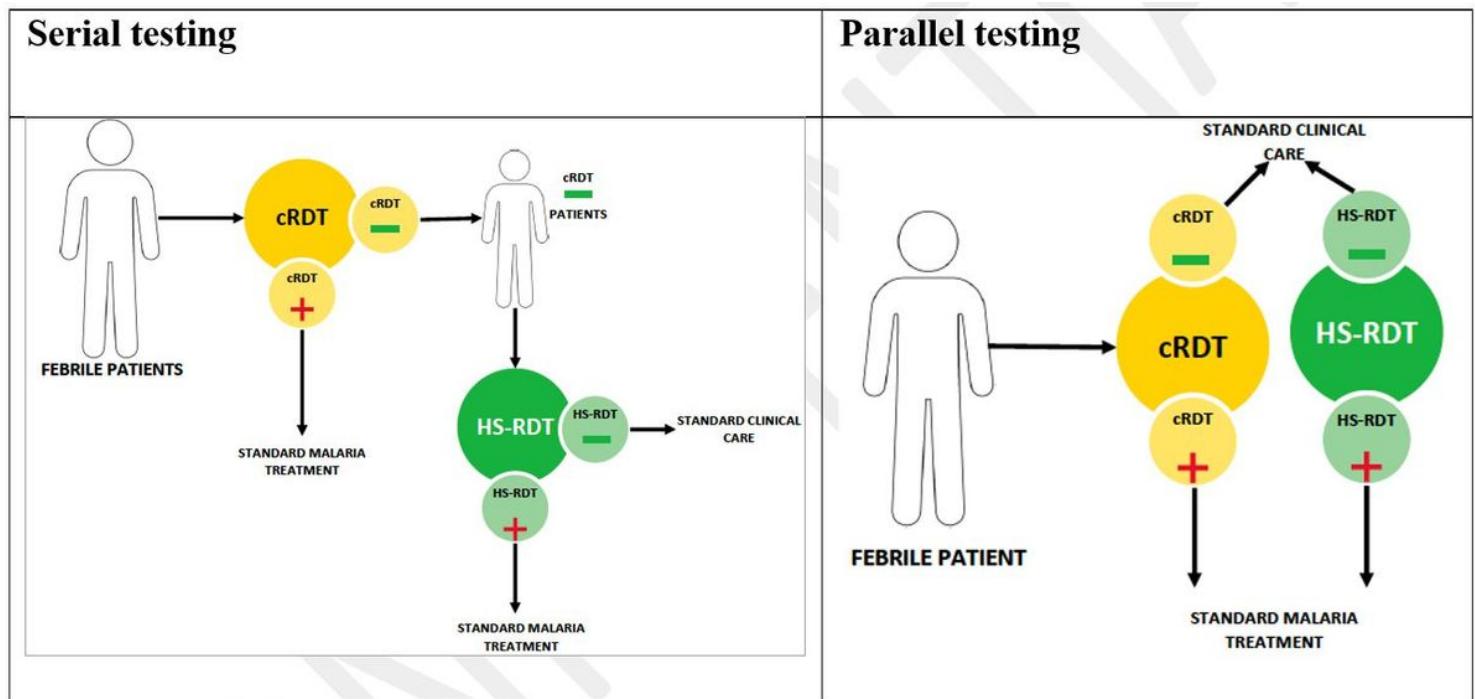


Figure 1

OPD Cohort showing the serial and parallel (concurrence) testing approaches.

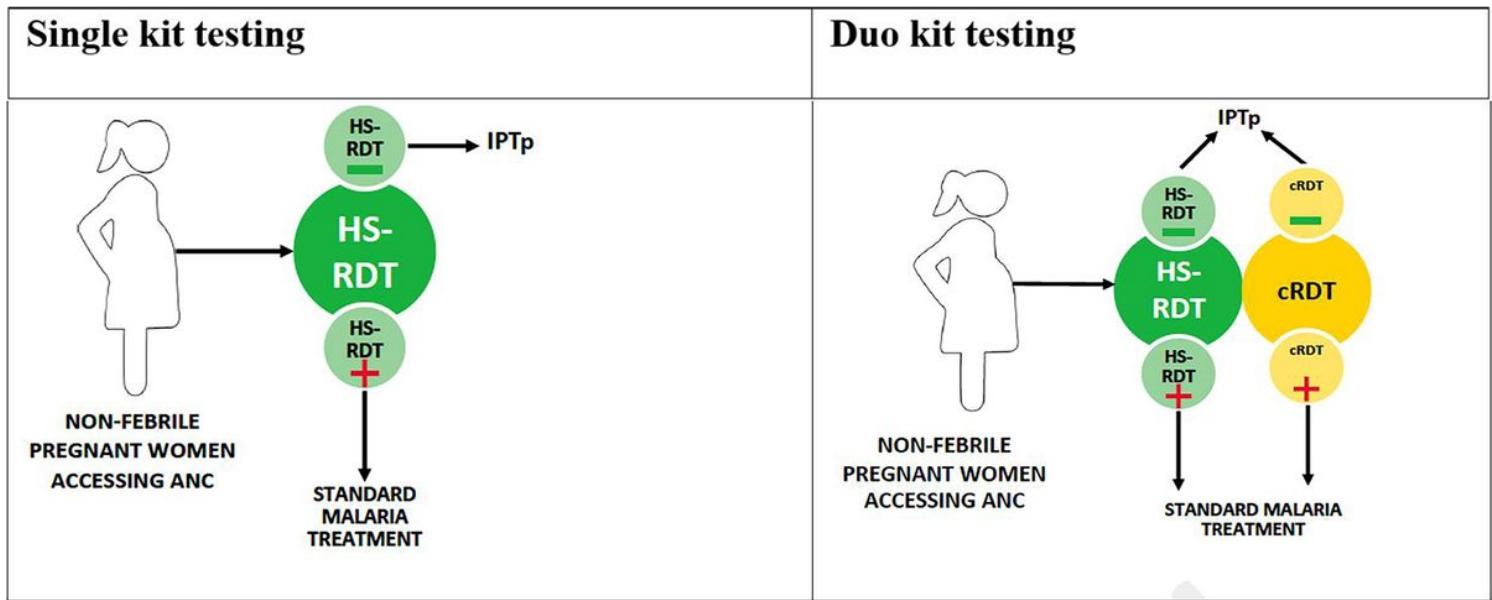


Figure 2

ANC cohort (please also add the description)

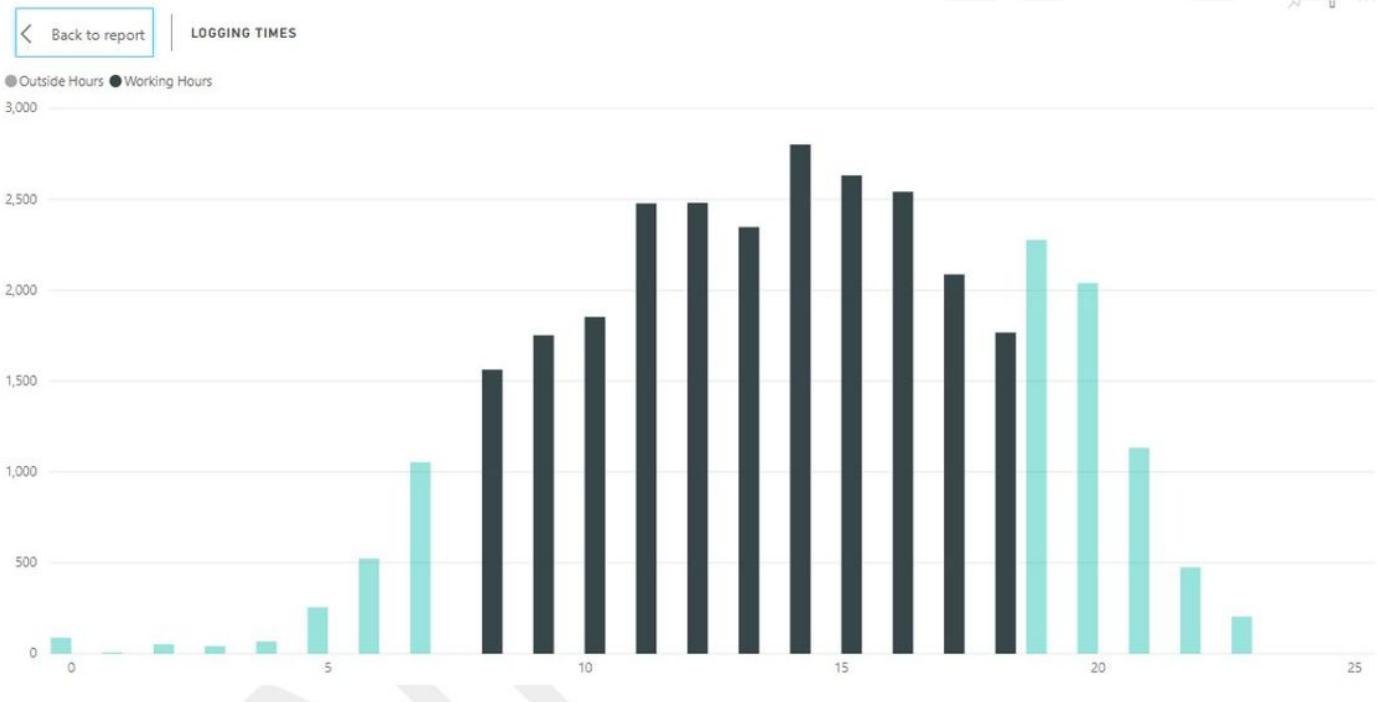


Figure 3

The majority, (75%) of the data transfers were transmitted and received during the normal working time (8:00am-5:00pm) and the remaining data was sent at the end of the day or at night.

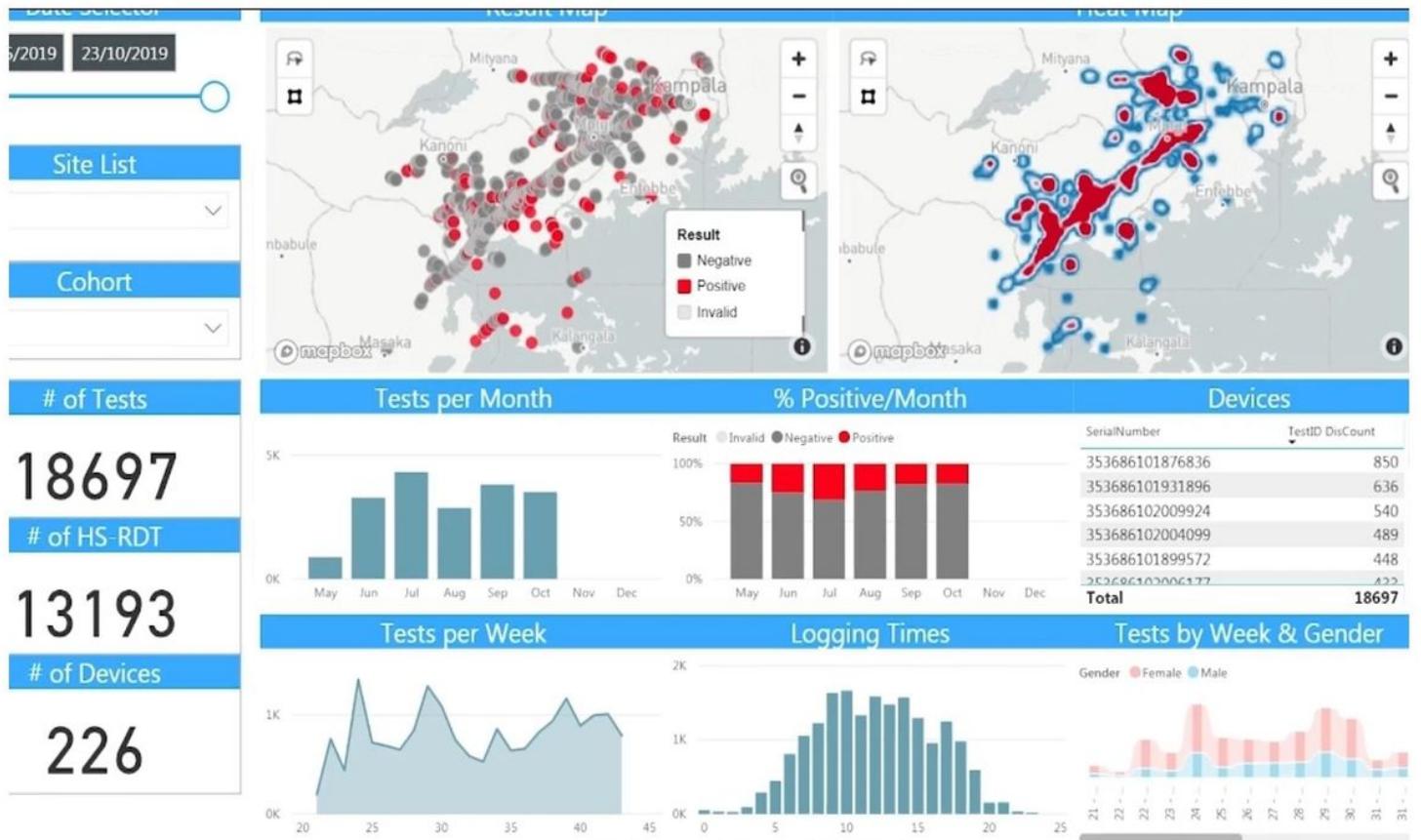


Figure 4

Dashboard showing the transmitted results and summarised outputs