Malaria Rapid Diagnostic Test Performance

Results of WHO product testing of malaria RDTs: Round 2 (2009)

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FIND foundation for innovative new diagnostics
CDC
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ABBREVIATIONS

ACT	Artemisinin-based combination therapy
AMI	Army Malaria Institute
AusAID	Australian Agency for International Development
CDC	United States Centers for Disease Control and Prevention
CLIA	Clinical Laboratory Improvement Amendments
FIND	Foundation for Innovative New Diagnostics
HRP2	Histidine-rich protein 2
HTD	Hospital for Tropical Diseases
ISO	International Organization for Standardization
PCR	Polymerase chain reaction
PDS	Panel detection score
pLDH	*Plasmodium* lactate dehydrogenase
Pf	*Plasmodium falciparum*
Pv	*Plasmodium vivax*
p/µL	Parasites per microlitre
QA	Quality assurance
QC	Quality control
QMS	Quality management systems
RDT	Rapid diagnostic test (for the purposes of this report, this refers to immunochromatographic lateral flow devices for the detection of malaria parasite antigens)
SOP	Standard Operating Procedure
TDR	Special Programme for Research and Training in Tropical Diseases sponsored by UNICEF, UNDP, World Bank and WHO
UN	United Nations
USA	United States of America
USAID	United States Agency for International Development
WPRO	Western Pacific Regional Office
WHO	World Health Organization

1. SUMMARY PEFORMANCE OF MALARIA RDTS: WHO PRODUCT TESTING: ROUNDS 1 AND 2

1.1. Introduction

The World Health Organization estimates that half the world's population are at risk of malaria, with 243 million people developing clinical malaria last year (86% in Africa), with nearly 863,000 deaths (89% in Africa, most being children). Malaria remains endemic in 108 countries, and while parasite-based diagnosis is increasing, most suspected cases of malaria are still not properly identified, resulting in over-use of anti-malarial drugs and poor disease monitoring.[1]

WHO recommends that malaria case management be based on parasite-based diagnosis in all cases[2]. The use of antigen-detecting rapid diagnostic tests (RDTs) forms a vital part of this strategy, forming the backbone of expansion of access to malaria diagnosis as they provide parasite-based diagnosis in areas where good quality microscopy can not be maintained. The number of RDTs available, and the scale of their use, has rapidly increased over the past few years. However, limitations of comparative field trials and the heterogeneous nature of malaria transmission and epidemiology has limited the availability of good quality performance data that national malaria programmes require to make informed decisions on procurement and implementation, and limits the ability to extrapolate results of field trials to different populations and time periods. To this end in 2006, the World Health Organization (WHO), Special Programme for Research and Training in Tropical Diseases (TDR) and the Foundation for Innovative New Diagnostics (FIND) launched an evaluation programme to assess the comparative performance of commercially available malaria RDTs. This data will guide procurement decisions and help drive improvement in the quality of manufacturing. The results of the first round of Product Testing were published in April 2009, and now form the basis of procurement criteria of WHO and UN agencies and national governments.

This Summary presents an overview of the results of the first and second rounds of WHO product testing of malaria antigen-detecting RDTs completed in 2008 and 2009 respectively, and is published in conjunction with the release of the results of Round 2. The results of the two rounds of testing should be considered as a single data set, and the full reports of both Rounds 1 and 2 consulted for further detail on product performance, and on the interpretation and use of these results.

1.2. The WHO Product Testing Programme

The RDT evaluations summarized here were performed as a collaboration between WHO, TDR, FIND, the US Centers for Disease Control and Prevention (CDC) and other partners[3]. All companies manufacturing under ISO 13485:2003 Quality System Standard were invited to submit up to 3 tests for evaluation under the programme. In the first round of testing, 41 products from 21 manufacturers were evaluated against prepared blood panels of cultured *Plasmodium falciparum* parasites, while 29 products from 13 manufacturers were evaluated in Round 2. Of these products, 68 progressed to testing against panels of patient-derived *P. falciparum* and *P. vivax* parasites, and a parasite-negative panel. Thermal stability was assessed after two months of storage at elevated temperature and humidity, and a descriptive ease of use assessment was recorded. Of the 68 products, 22 detect *P. falciparum* alone, 39 detect and differentiate *P. falciparum* from non-*P. falciparum* malaria (either pan-specific or species-specific), 6 detect *P. falciparum* and non-*P. falciparum* malaria without distinguishing between them, and 1 product was designed to detect *P. vivax* only. Manufacturers submitted two lots of each product for evaluation.

The Phase 1, *P. falciparum* cultured-parasite panel was derived from the same *P. falciparum* cultures in Rounds 1 and 2. However, the *P. falciparum* and *P. vivax* wild-type (clinical samples) panels were expanded in Round 2. More specifically, the *P. falciparum* panel was increased from 79 in Round 1 to 100 in Round 2, with 76 *P. falciparum* samples common to both rounds of testing. The *P. vivax* panel increased from 20 in Round 1 to 40 samples in Round 2, and the parasite-negative panel from 42 clean-negative samples and 48 disease or immune-factor positive samples in Round 1 to 50 of each in Round 2. The distribution of culture and wild-type sample antigen concentrations for *P. falciparum*-HRP2, *P. falciparum*-pLDH and P. vivax-pLDH were compared between the two rounds of testing to ensure consistency. The median *P. falciparum*-HRP2 and *P. falciparum*-pLDH levels were marginally lower in the Round 2 panel compared to that for Round 1; however, the difference was not statistically significant for either antigen (P>0.2; Mann-Whitney test). The median antigen concentration for *P. vivax*-pLDH, was higher

[1] *World Malaria Report 2009.* Geneva, World Health Organization, 2009.

[2] *Guidelines for the Treatment of Malaria, Second Edition.* Geneva, World Health Organization, 2010.

[3] See full reports of Rounds 1 and 2 for full list of collaborating partners.

in the Round 2 panel, but this difference was not statistically significant (P=0.68; Mann-Whitney test). The results of Round 1 and 2 are, therefore, comparable and should be viewed as a single data set for procurement purposes.

The evaluation is designed to provide comparative data on the performance of the submitted production lots of each product. Such data will be used to guide procurement decisions of WHO and other UN agencies and national governments. Product testing is part of a continuing programme of work to improve the quality of RDTs that are used, and to support broad implementation of reliable malaria diagnosis in areas where malaria is prevalent. A third round of product testing began in April 2010.

1.3. Results of the Evaluation

The results (summarized in Figures S1 and S2 and Tables S1 and S2) provide comparative data on two lots of products against a panel of parasite samples diluted to a low parasite density (200 parasites/µL) and a higher parasite density (2000 or 5000 parasites/µL). The former is below the mean parasite density found in many populations with endemic malaria, and considered close to the threshold that tests must detect to reliably identify clinical malaria in many settings.[1] For the purposes of this report, the main measure of performance is the 'panel detection score (PDS)'[2]; the percentage of malaria samples in the panel giving a positive result by two RDTs per lot at the lower parasite density, and a single RDT per lot at the higher parasite density. Thus, it is not a measure of RDT clinical sensitivity, or positivity rate against the panel but rather a combined measure of positivity rate, along with inter-test and inter-lot consistency. The figures also show the false-positive rates against blood samples containing no malaria parasites or known markers of other diseases, and the rate at which invalid results occurred.

The clinical sensitivity of an RDT to detect malaria is highly dependent on the local conditions, including parasite density in the target population, and so will vary between populations with differing levels of transmission. The results in this report show comparative performance between RDTs, and give an idea of which products are likely to provide higher sensitivity in the field, particularly in populations with low-density infections. In general, as countries reduce malaria prevalence and even move towards malaria elimination, detection of low parasite densities becomes increasingly important in case management. As the detection rate at 2000 parasites/µL indicates, the sensitivity of many of these products will be similar in populations with higher parasite densities, although a subset of any population will include vulnerable individuals who may develop illness at low parasite densities (e.g. young children, pregnant women, those well protected by bed nets) and must always be taken into account when interpreting RDT results.

Heat stability (summarized in Table S2) is vital to maintaining sensitivity of the test in the field. As a result, for procurement, it is essential that careful consideration be given to stability results to ensure that products to be used in areas with high temperatures of transport and storage have demonstrated stability in the product testing programme. Requirements will vary between countries: for example, if tests are to be deployed in areas where temperatures rarely rise above 30°C, less emphasis needs to be placed on stability at high temperatures.

Ease of use requirements will also vary, depending on the extent of training and the work environment of the end-users. Particularly in primary health care settings, the simpler the tests, the easier it will be to avoid errors in preparation and interpretation.

Detailed results of the evaluations can be found in the reports of each evaluation,[3] and at www.wpro.who.int/sites/rdt

[1] *WHO Technical Consultation on Parasitological Confirmation of Malaria Diagnosis. Report.* Geneva, World Health Organization, 2010. (Unpublished)

[2] Termed 'Detection Rate' in the full report of Round 1, published in 2009. See the Round 2 report for a full explanation of the panel detection score (PDS).

[3] *Malaria Rapid Diagnostic Test Performance : Results of WHO product testing of malaria RDTs: Round 1 (2008).* Geneva, World Health Organization, 2009. ISBN 978 92 4 1598071

1.4. Summary of outcomes

This laboratory-based evaluation provides a comparative measure of RDT performance in a standardized way to distinguish between well and poorly performing tests to inform procurement decisions of malaria control programmes and guide UN procurement policy.

Several RDTs from Rounds 1 and 2 demonstrated consistent detection of malaria at low parasite densities (200 parasites/µl), have low false positive rates, are stable at tropical temperatures, are relatively easy to use, and can detect *P. falciparum*, *P. vivax* infections, or both.

Performance between products varied widely at low parasite density (200 parasites/µl); however, most products showed a high level of detection at 2000 or 5000 parasites/µl.

P. falciparum tests targeting HRP2 antigen demonstrated the highest detection rates, but some tests targeting pLDH also exhibited high detection rates.

Test performance varied between lots, and widely between similar products, confirming the advisability of lot-testing post purchase and prior to use in the field.

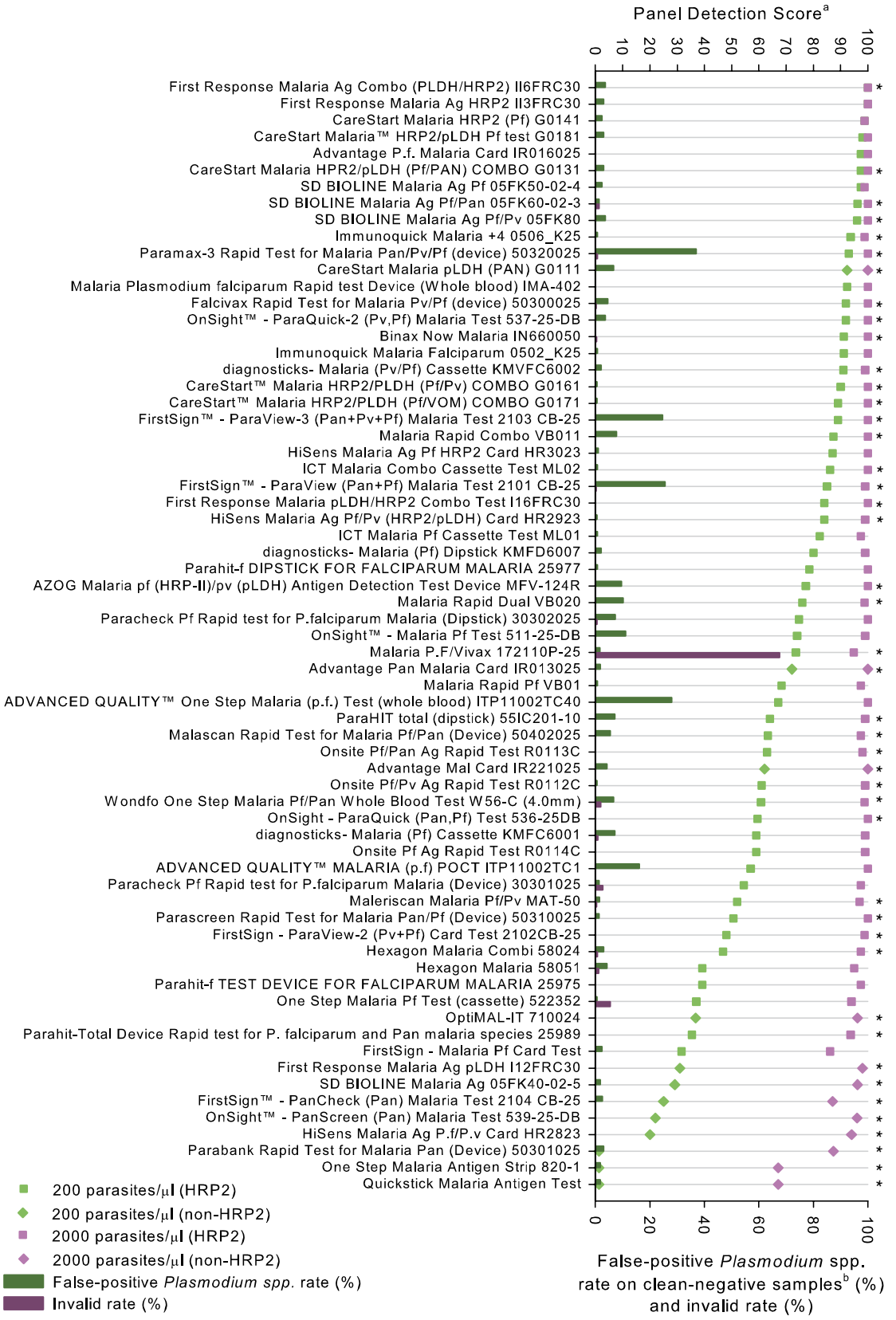
The results underscore the need for manufacturers to have adequate reference materials for product development and lot-release. The WHO-FIND malaria RDT evaluation programme, in collaboration with the CDC, offers quality standard panels to manufacturers to assist in this process.

1.5. Use of these Results

Ultimately, it is imperative that procurement decisions based on these results take into consideration local conditions of malaria transmission and illness where the tests will be used (e.g. *Plasmodium* species, target antigen variation, parasite densities, climate). Accurate diagnosis is vital to good malaria case management, whether based on microscopy or RDTs. These results should be used to short-list products for procurement for use in cases where good microscopy is not available of appropriate. Other considerations, including training and retraining requirements, are also essential components of product selection. It is recommended that each lot of RDTs is also tested in a standardized way prior to dispersal to the field, to ensure that the high performance demonstrated by the lots evaluated in the product testing programme is maintained.[1] Procurement of RDTs must not occur without programmatic and infrastructure preparation for proper use, including supply chain management, training on test usage and disposal, and training on patient management in response to results. Both reports provides an algorithm to assist in this decision-making process (Rounds 1 and 2: Annex 5).

[1] The WHO-FIND Malaria RDT Evaluation Programme provides lot-testing capacity in a number of regional laboratories free of charge, and can be accessed through *mal-rdt@wpro.who.int* and *info@finddiagnostics.org*.

Figure S1: Malaria RDT performance in Phase 2 of Rounds 1 and 2 against wild type (clinical) samples containing *P. falciparum* at low (200) and high (2000 or 5000) parasite densities (parasites/µl) and clean-negative samples

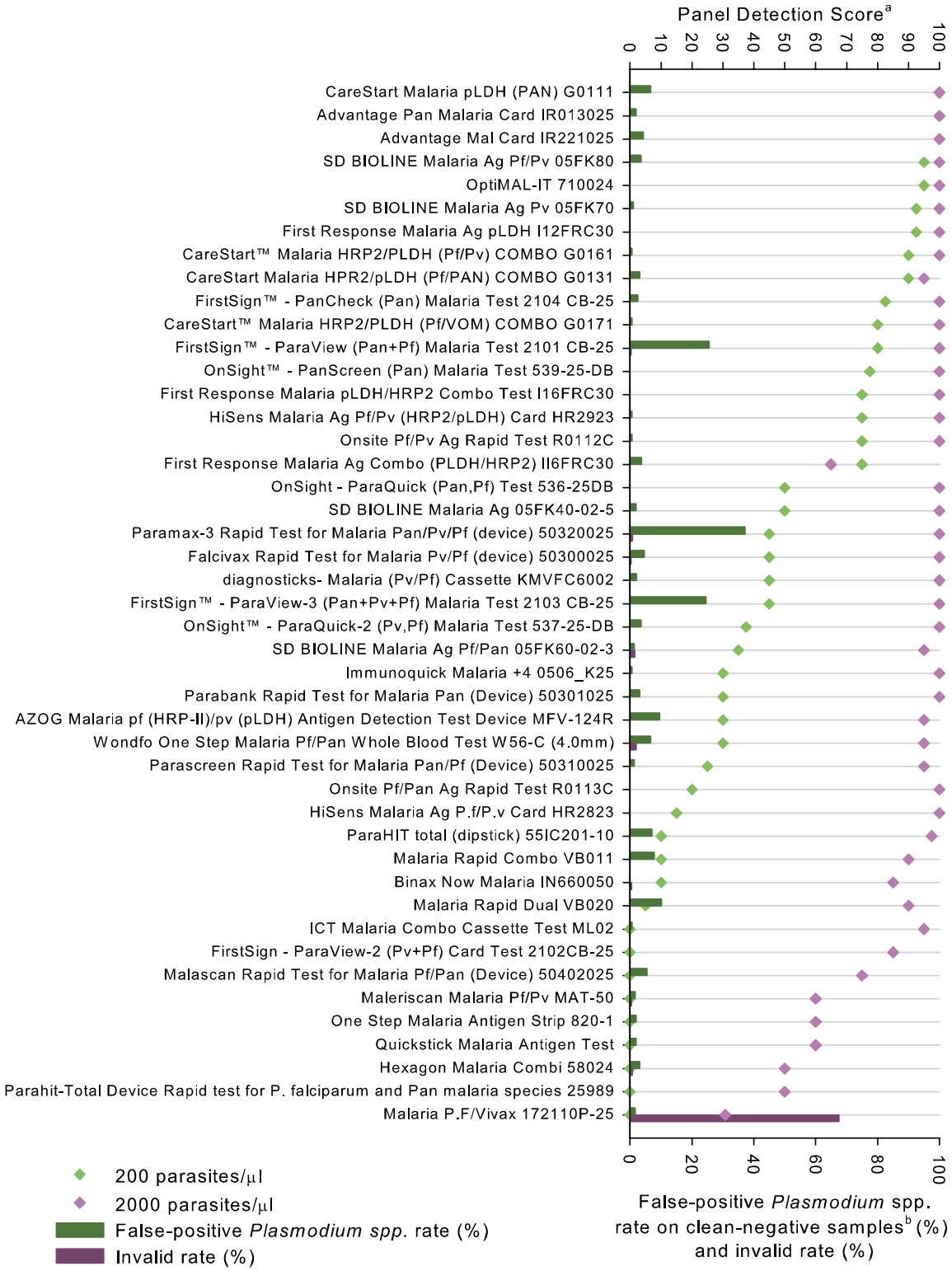


[a] panel detection score - A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive.;

[b] clean-negative - blood samples from healthy volunteers with no known current illness or blood abnormality.

* indicates tests that also detect other non-*P. falciparum* parasites. (see Figure S2)

Figure S2: Malaria RDT performance in Phase 2 of Rounds 1 and 2 against wild type (clinical) samples containing *P. vivax* at low (200) and high (2000 or 5000) parasite densities (parasites/µl)) and clean-negative samples



[a] panel detection score - A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive.

[b] clean-negative - blood samples from healthy volunteers with no known current illness or blood abnormality.

Table S1: Malaria RDT Phase 2 performance in Rounds 1 and 2 against wild type (clinical) samples containing *P. falciparum* and *P. vivax* at low (200) and high (2000 or 5000) parasite densities (parasites/µl) and clean negative samples

Product	Catalogue number	Manufacturer	Panel Detection Score[a]				False positive rates (%)				Total false positive rates[b] (%)	Invalid rate (%)	Round
			200 parasites/µl		2000 or 5000 parasites/µl		200 parasites/µl		2000 or 5000 parasites/µl		Clean negative samples		
							Pf samples	Pv samples	Pf samples	Pv samples			
			Pf samples[c]	Pv samples[d]	Pf samples[c]	Pv samples[d]	False positive non Pf infection[e]	False positive Pf infection[f]	False positive non Pf infection[g]	False positive Pf infection[h]	False positive Plasmodium spp. Infectioni		
Pf only													
ADVANCED QUALITY™ MALARIA (p.f) POCT	ITP11002TC1	InTec Products, Inc.	57.0	N/A	100.0	N/A	N/A	12.5	N/A	17.5	16.1	0.0	1
ADVANCED QUALITY ™ One Step Malaria (p.f.) Test (whole blood)	ITP11002TC40	InTec Products, Inc.	67.1	N/A	100.0	N/A	N/A	48.8	N/A	45.0	28.0	0.0	1
Advantage P.f. Malaria Card	IR016025	J. Mitra & Co. Pvt. Ltd.	97.5	N/A	100.0	N/A	N/A	1.3	N/A	2.5	0.0	0.0	1
CareStart™ Malaria HRP2 (Pf)	G0141	Access Bio, Inc.	98.7	N/A	98.7	N/A	N/A	5.0	N/A	7.5	2.4	0.0	1
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	98.0	N/A	100.0	N/A	N/A	0.6	N/A	1.3	3.0	0.0	2
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	59.0	N/A	99.0	N/A	N/A	1.9	N/A	2.6 (77)	7.0	0.9	2
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	80.0	N/A	99.0	N/A	N/A	2.5	N/A	3.8	2.0	0.0	2
First Response Malaria Ag HRP2	II3FRC30	Premier Medical Corporation Ltd.	100.0	N/A	100.0	N/A	N/A	0.0	N/A	0.0	3.0	0.0	1
FirstSign™ – Malaria Pf Card Test	--	Unimed International, Inc.	31.7	N/A	86.1	N/A	N/A	12.5	N/A	15.0	2.4 (166)	0.0	1
Hexagon Malaria	58051	Human GmbH	39.2	N/A	94.9	N/A	N/A	7.9 (76)	N/A	2.5	4.2(167)	1.2	1
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	87.0	N/A	100.0	N/A	N/A	0.0	N/A	0.0	1.0	0.1	2
ICT Malaria Pf Cassette Test (ML01)	ML01	ICT Diagnostics	82.3	N/A	97.5	N/A	N/A	1.3(79)	N/A	2.5	0.6	0.0	1
Immunoquick Malaria *Falciparum*	0502_K25	Biosynex	91.1	N/A	100.0	N/A	N/A	0.0	N/A	0.0	0.6	0.0	1
Malaria Plasmodium falciparum Rapid test Device (Whole blood)	IMA-402	ACON Laboratories, Inc.	92.4	N/A	100.0	N/A	N/A	0.0	N/A	0.0	0.0	0.0	1
Malaria Rapid Pf	VB01	Vision Biotech (Pty) Ltd.	68.4	N/A	97.5	N/A	N/A	0.0	N/A	0.0	0.6	0.0	1
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	37.0	N/A	94.0	N/A	N/A	1.3 (153)	N/A	0.0 (77)	0.5 (186)	5.4	2
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	74.0	N/A	99.0	N/A	N/A	8.1	N/A	2.5	11.0	0.0	2
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	59.0	N/A	99.0	N/A	N/A	0.0	N/A	0.0	0.0	0.0	2
Paracheck Pf Rapid test for *P. falciparum* Malaria (Device)	30301025	Orchid Biomedical Systems	54.4	N/A	97.5	N/A	N/A	3.9 (76)	N/A	5.0	1.2 (160)	2.7	1
Paracheck Pf Rapid test for *P. falciparum* Malaria (Dipstick)	30302025	Orchid Biomedical Systems	74.7	N/A	100.0	N/A	N/A	16.5 (79)	N/A	10.0	7.2 (167)	0.0	1
Parahit-f DIPSTICK FOR FALCIPARUM MALARIA	25977	Span Diagnostics Ltd.	78.5	N/A	100.0	N/A	N/A	0.0	N/A	0.0	0.6	0.0	1
Parahit-f TEST DEVICE FOR FALCIPARUM MALARIA	25975	Span Diagnostics Ltd.	39.2	N/A	97.5	N/A	N/A	0.0	N/A	0.0	0.0	0.0	1
SD BIOLINE Malaria Ag Pf	05FK50-02-4	Standard Diagnostics, Inc.	97.5	N/A	98.7	N/A	N/A	0.0	N/A	0.0	2.4	0.0	1
Pf and Pan													
Advantage Mal Card	IR221025	J. Mitra & Co. Pvt. Ltd.	62.0	100.0	100.0	100.0	2.5	0.0	0.0	0.0	4.2	0.0	1
AZOG Malaria pf (HRP-II) /pv (pLDH) Antigen Detection Test Device	MFV-124R	AZOG, Inc.	77.2	30.0	100.0	95.0	1.9	0.0	0.0	2.5	9.5	0.0	1
Binax Now Malaria Test	IN660050	Inverness Medical Innovations, Inc.	91.1	10.0	100.0	85.0	0.3	3.8 (79)	0.0 (157)	5.0	0.0	0.3	1
CareStart™ Malaria HRP2/pLDH (Pf/PAN) COMBO	G0131	Access Bio, Inc.	97.5	90.0	100.0	95.0	0.3	1.3	0.0	2.5	3.0	0.0	1
First Response Malaria Ag Combo (PLDH/HRP2)	II6FRC30	Premier Medical Corporation Ltd.	100.0	75.0	100.0	65.0	0.0	3.8	0.0	20.0	3.6	0.0	1
First Response® Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	84.0	75.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	2
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	85.0	80.0	99.0	100.0	0.0	0.6 (159)	0.5 (199)	0.0	25.5	0.2	2
Hexagon Malaria Combi	58024	Human GmbH	46.8	0.0	97.5	50.0	0.0	0.0 (79)	0.0 (157)	2.6 (38)	3.0 (167)	0.7	1
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	20.0	15.0	94.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	2
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	84.0	75.0	99.0	100.0	0.0	0.0	0.0	0.0	0.5	0.0	2
ICT Malaria Combo Cassette Test (ML02)	ML02	ICT Diagnostics	86.1	0.0	100.0	95.0	0.3	3.8	0.0	5.0	0.6	0.0	1
Immunoquick Malaria +4	0506_K25	Biosynex	93.7	30.0	98.7	100.0	0.0 (314)	0.0	0.0 (157)	0.0	0.6	0.0	1
Malaria P.F/Vivax	17211OP-25	Diagnostics Automation/Cortez Diagnostics, Inc.	73.6 (53)	0.0 (15)	94.9 (39)	30.8 (13)	1.0 (97)	0.0 (30)	2.1 (48)	0.0 (18)	1.6 (64)	67.5	1
Malaria Rapid Combo	VB011	Vision Biotech (Pty) Ltd.	87.3	10.0	100.0	90.0	0.3	7.5	0.0	7.5	7.7	0.0	1
Malaria Rapid Dual	VB020	Vision Biotech (Pty) Ltd.	76.0	5.0	98.7	90.0	1.3	0.0	0.0	0.0	10.1	0.0	1

Product	Catalogue number	Manufacturer	Panel Detection Score[a]				False positive rates (%)				Total false positive rates[b] (%)	Invalid rate (%)	Round
			200 parasites/µl		2000 or 5000 parasites/µl		200 parasites/µl		2000 or 5000 parasites/µl		Clean negative samples		
			Pf samples[c]	Pv samples[d]	Pf samples[c]	Pv samples[d]	Pf samples	Pv samples	Pf samples	Pv samples			
							False positive non Pf infection[e]	False positive Pf infection[f]	False positive non Pf infection[g]	False positive Pf infection[h]	False positive Plasmodium spp. Infectioni		
Malascan Rapid Test for Malaria Pf/Pan (Device)	50402025	Zephyr Biomedicals	63.3	0.0	97.5	75.0	0.6	6.3	0.0	17.5	5.4	0.0	1
One Step Malaria Antigen Strip	820-1	IND Diagnostic Inc.	1.3	0.0	67.1	60.0	2.2	3.8	1.9	0.0	1.8 (167)	0.0	1
OnSight™ – ParaQuick (Pan, Pf) Test	536-25DB	Amgenix International, Inc.	59.5	50.0	100.0	100.0	0.0	1.3	0.0	0.0	0.0	0.0	1
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	63.0	20.0	98.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	2
OptiMAL-IT	710024	DiaMed AG	36.7	95.0	96.2	100.0	3.8	0.0	0.6	0.0	0.0 (167)	0.0	1
ParaHIT® total (dipstick)	55IC201-10	Span Diagnostics Ltd	64.0	10.0	99.0	97.5	0.0	0.0	0.0	0.0	7.0	0.0	2
Parahit-Total Device Rapid test for *P. falciparum* and Pan malarial species.	25989	Span Diagnostics Ltd.	35.4	0.0	93.7	50.0	0.0 (315)	0.0	0.0	2.5	0.0	0.2	1
Parascreen Rapid Test for Malaria Pan/Pf (Device)	50310025	Zephyr Biomedicals	50.6	25.0	100.0	95.0	0.6	3.8 (79)	0.0	0.0	1.2	0.2	1
Quickstick Malaria Antigen Test	--	Innovatek Medical Inc.	1.3	0.0	67.1	60.0	2.2	3.8	1.9	0.0	1.8 (167)	0.0	1
SD BIOLINE Malaria Ag	05FK40-02-5	Standard Diagnostics, Inc.	29.1	50.0	96.2	100.0	0.0	1.3	0.0	0.0	1.8	0.0	1
SD BIOLINE Malaria Ag Pf/Pan	05FK60-02-3	Standard Diagnostics, Inc.	96.2	35.0	100.0	95.0	0.0 (310)	0.0 (79)	0.0	2.6 (39)	1.2	1.4	1
Wondfo One Step Malaria Pf/Pan Whole Blood Test	W56-C(4.0mm)	Guangzhou Wonfo Biotech Co., Ltd	60.8	30.0	98.7	95.0	3.5 (312)	1.3 (77)	0.0 (155)	2.6 (39)	6.6 (167)	1.9	1
Pf and Pv													
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	90.0	90.0	100.0	100.0	0.3	0.6	0.0	0.0	0.5	0.0	2
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	89.0	80.0	100.0	100.0	1.3	0.0	0.5	0.0	0.5	0.0	2
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	91.0	45.0	99.0	100.0	0.2 (399)	0.6	0.0	0.0	2.0	0.1	2
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	92.0	45.0	100.0	100.0	0.0	1.3	0.0	0.0 (79)	4.5	0.2	2
FirstSign – ParaView-2 (Pv + Pf) Card Test	2102CB-25	Unimed International, Inc.	48.1	0.0	98.7	85.0	1.0	3.8	N/A	5.0	4.8 (167)	0.0	1
Maleriscan® Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	52.0	0.0	97.0	60.0	1.7 (399)	2.5	32.5	2.5 (79)	1.5 (199)	0.4	2
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	92.0	37.5	100.0	100.0	0.5	1.9	0.0	0.0	3.5	0.1	2
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	61.0	75.0	99.0	100.0	0.3	0.0	0.5	0.0	0.5	0.0	2
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	96.0	95.0	100.0	100.0	0.0	0.0 (159)	0.0 (199)	0.0	3.5	0.2	2
Pf, Pv and Pan													
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	89.0	45.0	100.0	100.0	0.0 (399)	2.5	0.0	0.0	24.5	0.1	2
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	93.0	45.0	100.0	100.0	0.0 (396)	0.0 (159)	0.0 (199)	0.0	37.0 (198)	0.7	2
Pan only													
Advantage Pan Malaria Card	IR013025	J. Mitra & Co. Pvt. Ltd.	72.2	100.0	100.0	100.0	N/A	N/A	N/A	N/A	1.8	0.0	1
CareStart™ Malaria pLDH (PAN)	G0111	Access Bio, Inc.	92.4	100.0	100.0	100.0	N/A	N/A	N/A	N/A	6.6	0.0	1
First Response® Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	31.0	92.5	98.0	100.0	N/A	N/A	N/A	N/A	0.0	0.0	2
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	25.0	82.5	87.0	100.0	N/A	N/A	N/A	N/A	2.5	0.2	2
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	22.0	77.5	96.0	100.0	N/A	N/A	N/A	N/A	2.5	0.2	2
Parabank Rapid Test for Malaria Pan (Device)	50301025	Zephyr Biomedicals	1.3	30.0	87.3	100.0	N/A	N/A	N/A	N/A	3.0 (167)	0.0	1
Pv only													
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	92.5	N/A	100.0	0.3	N/A	1.0	N/A	1.0	0.0	2

Pf:*Plasmodiumfalciparum*-Pv:*Plasmodiumvivax*-pan:*Plasmodiumspecies*

[a] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive

[b] The total number of times a positive result for malaria was generated when it should not have been

[c] Round 1, n=79; Round 2, n=100

[d] Round 1, n=20; Round 2, n=40

[e] For combination tests, Pan or Pv line, only, positive indicates a false positive *P. falciparum* infection (Round 1 n=316; Round 2, n=400)

[f] Pf line positive indicates a false positive *P. falciparum* infection (Round 1, n=80; Round 2, n=160)

[g] For combination tests, Pan or Pv line, only, positive indicates a false positive *P. falciparum* infection (Round 1, n=158, Round 2, n=200)

[h] Pf line positive indicates a false positive *P. falciparum* infection (Round 1, n=40; Round 2, n=80)

[i] Round 1, n=168; Round 2, n=200

Detection rate (%)	≥95	85-94	50-84	< 50
False positive rate (%)	<2	2-5	6 -10	>10
Invalid rate (%)	<1% of tests conducted	1-2% of tests conducted	2-5% of tests conducted	>5% of tests conducted

Table S2: Malaria RDT Rounds 1 and 2 heat stability results on a cultured *P. falciparum* sample at low (200) and high (2000) parasite density (parasites/µl). Positivity rate at baseline, and after 60 days incubation at 35°C and 45°C

Product	Catalogue number	Manufacturer	Positive test results for *P. falciparum* (Pf line)			Positive test results for *P. falciparum* (Pf line)			Positive test results for *P. falciparum* (Pan line)			Positive test results for *P. falciparum* (Pan line)			Round
			200 parasites/µl			2000 parasites/µl			200 parasites/µl			2000 parasites/µl			
			Baseline	35°C	45°C	Baseline	35°C	45°C	Baseline	35°C	45°C	Baseline	35°C	45°C	
			Number of tests positive (max. 20)			Number of tests positive (max. 20)			Number of tests positive (max. 20)			Number of tests positive (max. 20)			
			Lots 1 and 2 combined			Lots 1 and 2 combined			Lots 1 and 2 combined			Lots 1 and 2 combined			
Pf only															
ADVANCED QUALITY™ MALARIA (p.f) POCT	ITP11002TC1	InTec Products, Inc.	16	19	18	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
ADVANCED QUALITY ™ One Step Malaria (p.f.) Test (whole blood)	ITP11002TC40	InTec Products, Inc.	16	17	9	20	19	20	N/A	N/A	N/A	N/A	N/A	N/A	1
Advantage P.f. Malaria Card	IR016025	J. Mitra & Co. Pvt. Ltd.	19	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
CareStart™ Malaria HRP2 (Pf)	G0141	Access Bio, Inc.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	2
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	19	14	11	19	19	19	N/A	N/A	N/A	N/A	N/A	N/A	2
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	2
First Response Malaria Ag HRP2	II3FRC30	Premier Medical Corporation Ltd.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
FirstSign™ – Malaria Pf Card Test	--	Unimed International, Inc.	4	3	0	20	18	19	N/A	N/A	N/A	N/A	N/A	N/A	1
Hexagon Malaria	58051	Human GmbH	10	7	12	19	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	2
ICT Malaria Pf Cassette Test (ML01)	ML01	ICT Diagnostics	20	20	19	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
Immunoquick Malaria Falciparum	0502_K25	Biosynex	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
Malaria Plasmodium falciparum Rapid test Device (Whole blood)	IMA-402	ACON Laboratories, Inc.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
Malaria Rapid Pf	VB01	Vision Biotech (Pty) Ltd.	20	20	17	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
One Step Malaria Pf Test (cassette)	522352	BlueCross Bio-Medical (Beijing) Co., Ltd	6	3	2	16	18	16	N/A	N/A	N/A	N/A	N/A	N/A	2
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	20	19	18	20	20	13	N/A	N/A	N/A	N/A	N/A	N/A	2
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	20	18	13	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	2
Paracheck Pf Rapid test for *P. falciparum* Malaria (Device)	30301025	Orchid Biomedical Systems	18	14	10	20	17	20	N/A	N/A	N/A	N/A	N/A	N/A	1
Paracheck Pf Rapid test for *P. falciparum* Malaria (Dipstick)	30302025	Orchid Biomedical Systems	19	20	17	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
Parahit-f DIPSTICK FOR FALCIPARUM MALARIA	25977	Span Diagnostics Ltd.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
Parahit-f TEST DEVICE FOR FALCIPARUM MALARIA	25975	Span Diagnostics Ltd.	14	10	8	20	19	19	N/A	N/A	N/A	N/A	N/A	N/A	1
SD BIOLINE Malaria Ag Pf	05FK50-02-4	Standard Diagnostics, Inc.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	1
Pf and Pan															
Advantage Mal Card	IR221025	J. Mitra & Co. Pvt. Ltd.	20	20	11	19	20	19	11	9	8	20	20	20	1
AZOG Malaria pf (HRP-II) /pv (pLDH) Antigen Detection Test Device	MFV-124R	AZOG, Inc.	12	13	7	20	20	20	8	7	5	18	14	4	1
Binax Now Malaria Test	IN660050	Inverness Medical Innovations, Inc.	20	20	20	20	20	19	1	0	0	19	19	15	1
CareStart™ Malaria HRP2/pLDH (Pf/PAN) COMBO	G0131	Access Bio, Inc.	20	19	20	20	20	20	20	19	20	20	20	20	1
First Response Malaria Ag Combo (PLDH/HRP2)	II6FRC30	Premier Medical Corporation Ltd.	20	20	20	20	20	20	19	14	20	20	20	20	1
First Response® Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	20	20	20	20	20	20	17	11	11	10	10	10	2
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	20	20	20	20	20	20	19	8	8	10	10	10	2
Hexagon Malaria Combi	58024	Human GmbH	13	11	10	20	17	19	0	0	0	0	0	0	1
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	7	0	1	20	20	20	0	0	0	7	0	0	2
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	20	20	20	20	20	20	20	20	19	20	20	20	2
ICT Malaria Combo Cassette Test (ML02)	ML02	ICT Diagnostics	20	20	20	20	20	20	1	1	0	18	15	15	1
Immunoquick Malaria +4	0506_K25	Biosynex	20	20	20	20	20	20	0	0	0	20	16	16	1
Malaria P.F/Vivax	172110P-25	Diagnostics Automation/Cortez Diagnostics, Inc.	13	3	4	13	9	1	0	0	0	0	0	0	1
Malaria Rapid Combo	VB011	Vision Biotech (Pty) Ltd.	20	20	20	20	20	20	3	6	0	19	20	15	1

Product	Catalogue number	Manufacturer	Positive test results for *P. falciparum* (Pf line)			Positive test results for *P. falciparum* (Pf line)			Positive test results for *P. falciparum* (Pan line)			Positive test results for *P. falciparum* (Pan line)			Round
			200 parasites/µl			2000 parasites/µl			200 parasites/µl			2000 parasites/µl			
			Baseline	35°C	45°C	Baseline	35°C	45°C	Baseline	35°C	45°C	Baseline	35°C	45°C	
			Number of tests positive (max. 20)			Number of tests positive (max. 20)			Number of tests positive (max. 20)			Number of tests positive (max. 20)			
			Lots 1 and 2 combined			Lots 1 and 2 combined			Lots 1 and 2 combined			Lots 1 and 2 combined			
Malaria Rapid Dual	VB020	Vision Biotech (Pty) Ltd.	20	20	20	20	20	20	0	3	0	12	5	3	1
Malascan Rapid Test for Malaria Pf/Pan (Device)	50402025	Zephyr Biomedicals	19	18	17	20	20	20	0	1	0	13	11	3	1
One Step Malaria Antigen Strip	820-1	IND Diagnostic Inc.	3	0	0	13	10	0	3	0	0	13	10	1	1
OnSight™ – ParaQuick (Pan, Pf) Test	536-25DB	Amgenix International, Inc.	20	18	12	20	20	20	0	0	0	20	20	19	1
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	20	18	8	20	20	20	1	0	0	20	14	9	2
OptiMAL-IT	710024	DiaMed AG	6	2	0	20	19	0	6	3	0	20	20	2	1
ParaHIT® total (dipstick)	55IC201-10	Span Diagnostics Ltd	11	17	11	20	20	19	2	0	0	10	9	14	2
Parahit-Total Device Rapid test for *P. falciparum* and Pan malarial species.	25989	Span Diagnostics Ltd.	13	15	5	19	20	20	1	0	0	0	0	0	1
Parascreen Rapid Test for Malaria Pan/Pf (Device)	50310025	Zephyr Biomedicals	19	16	9	20	20	19	1	0	0	17	14	16	1
Quickstick Malaria Antigen Test	--	Innovatek Medical Inc.	3	0	0	13	10	0	3	0	0	13	10	1	1
SD BIOLINE Malaria Ag	05FK40-02-5	Standard Diagnostics, Inc.	7	12	15	20	20	20	0	9	15	11	20	19	1
SD BIOLINE Malaria Ag Pf/Pan	05FK60-02-3	Standard Diagnostics, Inc.	20	20	20	20	20	19	0	1	16	18	9	18	1
Wondfo One Step Malaria Pf/Pan Whole Blood Test	W56-C(4.0mm)	Guangzhou Wonfo Biotech Co., Ltd	20	19	20	19	20	20	14	18	14	19	20	20	1
Pf and Pv															
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	20	20	19	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	2
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	2
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	20	19	19	20	20	19	N/A	N/A	N/A	N/A	N/A	N/A	2
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	2
FirstSign – ParaView-2 (Pv + Pf) Card Test	2102CB-25	Unimed International, Inc.	19	14	0	20	19	15	N/A	N/A	N/A	N/A	N/A	N/A	1
Maleriscan® Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	20	12	6	20	18	19	N/A	N/A	N/A	N/A	N/A	N/A	2
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	20	20	20	20	20	17	N/A	N/A	N/A	N/A	N/A	N/A	2
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	20	19	9	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A	2
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	20	20	20	20	20	19	N/A	N/A	N/A	N/A	N/A	N/A	2
Pf, Pv and Pan															
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	20	20	20	20	20	20	12	10	3	20	18	20	2
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	20	10	10	20	20	20	20	5	6	20	19	20	2
Pan only															
Advantage Pan Malaria Card	IR013025	J. Mitra & Co. Pvt. Ltd.	N/A	N/A	N/A	N/A	N/A	N/A	10	13	14	20	20	20	1
CareStart™ Malaria pLDH (PAN)	G0111	Access Bio, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	20	20	18	20	20	20	1
First Response® Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	N/A	N/A	N/A	N/A	N/A	N/A	10	16	11	20	20	20	2
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	N/A	N/A	N/A	N/A	N/A	N/A	5	1	2	20	20	20	2
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	1	7	3	20	20	20	2
Parabank Rapid Test for Malaria Pan (Device)	50301025	Zephyr Biomedicals	N/A	N/A	N/A	N/A	N/A	N/A	1	0	0	17	18	14	1
Pv only															
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

2. WHO MALARIA RDT PRODUCT TESTING: ROUND 2 EXECUTIVE SUMMARY

2.1. Introduction

The World Health Organization estimates that half the world's population are at risk of malaria, with 243 million people developing clinical malaria last year (86% in Africa), with nearly 863,000 deaths (89% in Africa, most being children). Malaria remains endemic in 108 countries, and while parasite-based diagnosis is increasing, most suspected cases of malaria are still not properly identified, resulting in over-use of anti-malarial drugs and poor disease monitoring (*1*).

WHO recommends that malaria case management be based on parasite-based diagnosis in all cases (*2*). The use of antigen-detecting rapid diagnostic tests (RDTs) forms a vital part of this strategy, providing the possibility of parasite-based diagnosis in areas where good quality microscopy can not be maintained. The number of RDTs available, and the scale of their use, has rapidly increased over the past few years. However, limitations of comparative field trials and the heterogeneous nature of malaria transmission and epidemiology has limited the availability of good quality performance data that national malaria programmes require to make informed decisions on procurement and implementation, and limits the ability to extrapolate results of field trials to different populations and time periods. To this end in 2006, the World Health Organization (WHO), Special Programme for Research and Training in Tropical Diseases (TDR) and the Foundation for Innovative New Diagnostics (FIND) launched an evaluation programme to assess the comparative performance of commercially available malaria RDTs. This data will guide procurement decisions and help drive improvement in the quality of manufacturing. The results of the first round of Product Testing were published in April 2009, and now form the basis of procurement criteria of WHO, other UN agencies and national governments (*3*).

This Report provides data on Round 2 of Product Testing, performed at the United States Centers for Disease Control and Prevention, Division of Malaria and Parasitic Diseases (CDC) in 2009. It provides performance data on 29 products (3). This evaluation should be seen as additive to the Round 1 evaluation published in 2009, and in no way replaces it; the two reports should be viewed together. The evaluation panels were essentially equivalent, and the same testing protocols were followed. This report expands the data set from Round 1, and therefore increases the number of RDTs available for procurement that have detailed comparative data on aspects of performance relevant to field use.

2.2. The WHO Product Testing Programme

Product Testing is part of the WHO-FIND Malaria RDT Evaluation Programme aiming to develop methods for evaluation, and provide relevant data on, antigen-detecting malaria rapid diagnostic tests. The programme is a collaboration of many institutions in malaria-endemic and non-endemic countries, with the global specimen bank maintained, and the testing performed, at CDC (Figure2). All companies manufacturing under ISO 13485:2003 Quality System Standard were invited to submit up to three tests for evaluation under the programme. The 29 products from 13 manufacturers were evaluated against prepared blood panels of cultured *Plasmodium falciparum* parasites and patient-derived, wild-type *P. falciparum* and *P. vivax* parasites, and a parasite-negative panel. Thermal stability was assessed after two months of storage at elevated temperature and humidity, and a descriptive ease of use assessment was recorded. As in Round 1, RDTs are grouped in the result tables and figures into those detecting *P. falciparum* only, combination tests, and those that have only a pan-specific (or *P. vivax*-specific) line. Manufacturers submitted two lots of each product for evaluation.

The evaluation is designed to provide comparative data on the performance of the submitted production lots of each product. Such data will be used to guide procurement decisions of WHO and other UN agencies and national governments. Product testing is part of a continuing programme of work to improve the quality of RDTs that are used, and to support broad implementation of reliable malaria diagnosis in areas where malaria is prevalent. A third round of product testing began in April 2010, and results will be published in 2011.

2.3. Results of the Evaluation

The results (summarized in Tables 3, 4, 5 and Figures S1 and S2) provide comparative data on two lots of products against a panel of parasite samples diluted to a low parasite density (200 parasites/μL), considered close to the threshold that tests must detect to reliably identify clinical malaria in many settings (4), and a higher parasite density (2000 (or 5000) parasites/μL). For the purposes of this report, the main measure of performance is the 'panel detection score (PDS)'; the percentage of malaria samples in the panel giving a positive result by two RDTs per lot at the lower parasite density, and a single RDT per lot at the higher parasite density. Thus,

it is not a measure of RDT clinical sensitivity, or positivity rate against the panel but rather a combined measure of positivity rate, along with inter-test and inter-lot consistency.

Consistent with the performance of products included in Round 1 of Product Testing in 2008, the PDS varies widely between products, with some products showing high performance in detecting parasites, in thermal stability and other performance measures. Overall, there is no obvious trade-off seen between PDS (or positivity rate) and false-positive rate, these being surrogates for sensitivity and specificity in the field, respectively. ,Furthermore, a number of tests showed good outcomes on both of these indicators. However, high false-positive rates are seen in some products, particularly against the blood samples containing specific immunological abnormalities. The number of samples evaluated was small and the clinical significance of these results is limited, but may become important in certain populations with very low parasite prevalence. Some products show a variation in performance indicators between the two lots evaluated, underlining the advisability of lot-testing before field use. Heat (thermal) stability varies widely, with some products retaining high positivity rates after two months storage at 45°C in 75% humidity.

The clinical sensitivity of an RDT to detect malaria is highly dependent on the local conditions, including parasite density in the target population, and so will vary between populations with differing levels of transmission. The results in this report show comparative performance between RDTs, and give an idea of which products are likely to provide higher sensitivity in the field, particularly in populations with low-density infections. In general, as countries reduce malaria prevalence and even move towards malaria elimination, detection of low parasite densities becomes increasingly important in case management. As the detection rate at 2000 parasites/μL indicates, the sensitivity of many of these products will be similar in populations with higher parasite densities, although a subset of any population will include vulnerable individuals who may develop illness at low parasite densities (e.g. young children, pregnant women, those well protected by bed nets) and must always be taken into account when interpreting RDT results.

Heat stability (summarized in Table 5) is vital to maintaining sensitivity of the test in the field. As a result, for procurement, it is essential that careful consideration be given to stability results to ensure that products to be used in areas with high temperatures of transport and storage have demonstrated great stability in the product testing programme. Requirements will vary between countries: for example, if tests are to be deployed in areas where temperatures rarely rise above 30°C, less emphasis needs to be placed on stability at high temperatures.

Ease of use requirements will also vary, depending on the extent of training and the work environment of the end-users. Particularly in primary health care settings, the simpler the tests, the easier it will be to avoid errors in preparation and interpretation.

2.4. Use of these Results

The results included here should be considered together with those of Round 1 (2008) (*3*). Ultimately, it is imperative that procurement decisions based on these results take into consideration local conditions of malaria transmission and illness where the tests will be used (e.g. *Plasmodium* species, target antigen variation, parasite densities, climate). Procurement of RDTs must not occur without programmatic and infrastructure preparation for proper use, including supply chain management, training on test usage and disposal, and training on patient management in response to results. This report provides an algorithm to assist in this decision-making process (Annex 5).

3. BACKGROUND

In 2006, WHO estimated that 3.3 billion persons were at risk of acquiring malaria. Of these, 243 million were infected (86% in Africa) and nearly 863,000 (mostly African children) died of the infection. In 2009, malaria was still endemic in 109 countries worldwide, 45 of them in Africa. WHO estimates that approximately 1.1 million persons were still dying of malaria that year (*1*).

In the past decade, major new opportunities for the control of malaria have emerged, including implementation of long-lasting insecticidal nets, indoor residual spraying of insecticides and artemisinin-based combination therapy (ACT). These tools, in combination with increased coverage of malaria control programs, are likely to reduce the burden of malaria infection in countries where they are adequately implemented. In turn, the proportion of febrile episodes attributable to malaria is likely to decrease substantially.

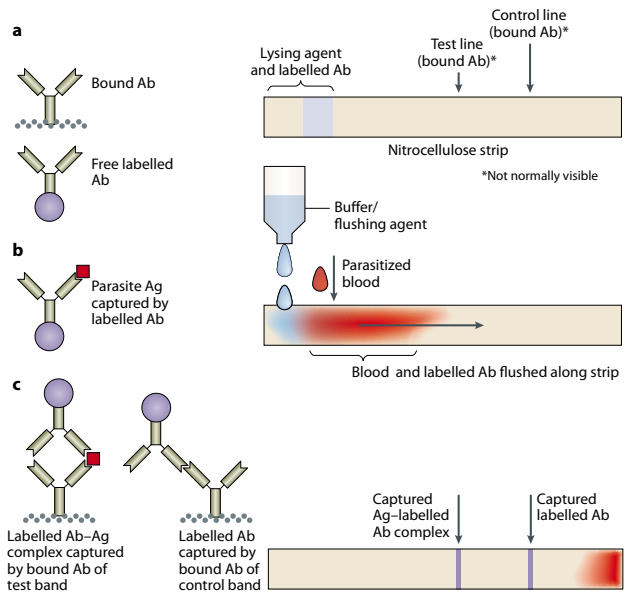
Despite WHO recommendations for laboratory-confirmed diagnosis of malaria infections prior to treatment in all cases (*2*), diagnosis is often made on clinical grounds (*4*). However, in most endemic areas malaria makes up a minority of 'malaria-like' febrile illness. Microscopy has been the cornerstone of diagnosis and is recommended for malaria diagnosis where its quality can be maintained, but the need for trained personnel, adequate reagents and equipment limit its availability and accessibility to many people in malaria-endemic areas. Rapid, accurate and accessible diagnostic tools are becoming increasingly important, as programmes expand parasite-based diagnosis and the prevalence of malaria decreases. In recent years, rapid diagnostic tests (RDTs), which detect *Plasmodium*-specific antigens (proteins) in whole blood of infected people, have emerged as an attractive alternative to microscopy. Currently available RDTs come in various formats (dipstick, cassette or card) and contain bound antibodies to specific antigens such as histidine-rich protein-2 (HRP2) (specific to *P. falciparum*), pan-specific or species-specific *plasmodium* lactate dehydrogenase (pLDH) or aldolase (specific to all the major *Plasmodium* species: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* (Figure 1).

To be widely useful, a RDT must have high sensitivity to ensure all clinically-significant malaria infections are detected; high specificity to enable monitoring of low malaria prevalence and appropriate management of non-malarial fever; and high stability to allow transport and storage in ambient conditions in malaria-endemic areas. Published field trials of RDTs show high variability in performance, likely due to inadequate quality of manufacture, incorrect storage and handling, poor preparation and interpretation, and sometimes poor study methods, analysis and reporting (*5-13*). In general, diagnostic testing (by microscopy or RDT) to a level of 200 parasites/µL will reliably detect nearly all clinically relevant infections in malaria-endemic areas (*4*).

The number of RDTs available on the market has grown rapidly since their introduction in the late 1990s. It is estimated that there are 60 brands and over 200 tests commercially available today, with an estimated 50-70 million tests used in 2008[1]. However, regulatory oversight of diagnostics is often weak, and procurement agencies have faced considerable problems in selecting appropriate RDTs and ensuring quality. In view of the inconsistency in field study results and the inherent difficulties in assessing large numbers of products in a standardized way through field trials, WHO and various partners embarked on a Malaria Rapid Diagnostic Test Product

[1] WHO Unpublished data.

Figure 1: Mode of action of antigen-detecting malaria RDTs



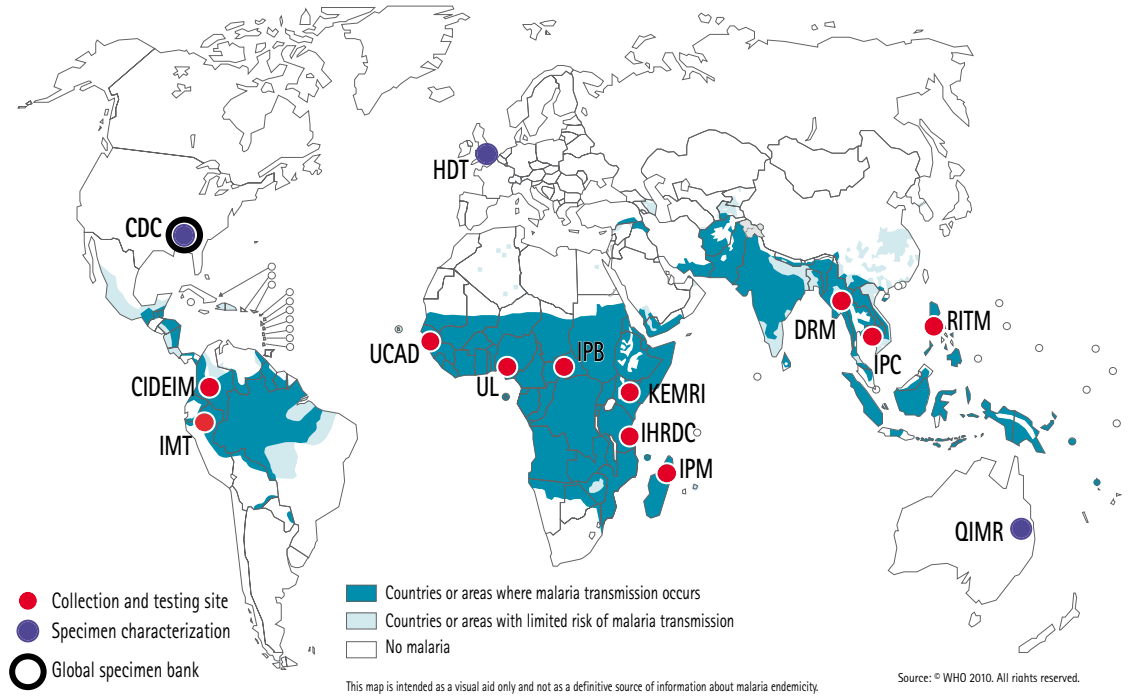
Mode of action of common malaria RDT format:

(a) Dye-labeled antibody (Ab), specific for target antigen, is present on the lower end of the nitrocellulose strip or in a well provided with the strip. Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line, and either antibody specific for the labeled antibody, or antigen, is bound at the control line.

(b) Blood and buffer, which have been placed on the strip or in the well, are mixed with the labeled antibody and are drawn up the strip across the lines of bound antibody.

(c) If antigen is present, some labeled antibody will be trapped on the test line. Other labeled antibody is trapped on the control line.

Figure 2: Network of specimen collection, characterization and testing sites



Abbreviations: CDC Centers for Disease Control and Prevention (Atlanta, United States of America); CIDEIM Centro Internacional de Entrenamiento y Investigaciones Médicas (Cali, Colombia); DMR Experimental Medicine Research Division (Department of Medical Research, Yangon, Myanmar); HTD Hospital for Tropical Diseases (London, United Kingdom of Great Britain and Ireland); IHRDC Ifakara Health Research and Development Center (Bagamoyo, The United Republic of Tanzania); IMT Instituto de Medicina Tropical (Universidad Peruana Cayetano Heredia, Lima, Peru); IPB Institut Pasteur de Bangui (Bangui, Central African Republic); IPC Institut Pasteur du Cambodge (Phnom Penh, Cambodia); IPM Institut Pasteur de Madagascar (Antananarivo, Madagascar); KEMRI: Kenya Medical Research Institute (Kisumu, Kenya); QIMR Queensland Institute of Medical Research (Brisbane, Australia); RITM Research Institute of Tropical Medicine (Manila, The Philippines); UCAD: Université Cheikh Anta DIOP (Dakar, Senegal); UL University of Lagos (Lagos, Nigeria).

Evaluation Programme in 2002 to develop and employ standardized assessment of malaria RDT performance, and to guide procurement decisions and regulatory mechanisms. The Programme has been overseen by WHO and TDR in partnership with FIND, and has been guided by a Steering Committee and technical consultations from 2003 to 2010 overseeing the development of standard operating procedures (SOPs) for the programme (*14*). A network of specimen collection sites was established to contribute specimens to a global bank at the CDC and to facilitate local quality control activities (Figure 2).

The report of the first round of Product Testing was released in 2009 *(3)*, and this second report adds performance data on 29 RDTs. Testing for Round 2 was conducted against a slightly expanded evaluation panel with new samples with similar characteristics in terms of overall antigen concentration, parasite origin, and parasite-negative blood samples. The results should be considered together with those from Round 1 *(3)*.

4. OBJECTIVE

Evaluate malaria RDTs to produce performance data to guide procurement of RDTs for use in the field in malaria-endemic countries.

5. MATERIALS AND METHODS

5.1. Test selection

In October 2008, the WHO-FIND Malaria RDT Evaluation Programme issued a call for expression of interest to manufacturers of malaria RDTs along with information regarding the requirements for submission of a product to Round 2 of the Product Testing programme and the conditions for participation in the Evaluation Programme.[1] Requirements included: ISO 13485:2003 certification, supply of sufficient quantities of products (1100 tests from each of 2 lots), and compliance with in-house real-time stability testing protocol(*14*).

After an initial call for expressions of interest, 13 manufacturers submitted a total of 29 products to be included in Round 2. After initial evaluation against the *P. falciparum* culture-derived panel (Phase 1), 27 products met minimum performance requirements and proceeded to the full evaluation.

In summary, of the 27 products fully evaluated: 6 are designed to detect *P. falciparum* alone, 17 to detect and differentiate *P. falciparum* from non-*P. falciparum* malaria,[2] 3 to detect *P. falciparum* and non-*P. falciparum* malaria without distinguishing between them, and one to detect *P. vivax* only. Annexes 1 and 2 provide a comprehensive overview of product characteristics.

[1] http://www.wpro.who.int/sites/rdt/who_rdt_evaluation/call_for_testing_round2.htm

[2] One is *P. vivax* only

Table 1: Manufacturers and products accepted into Round 2 of WHO Malaria RDT Product Testing Programme

Manufacturer	Product name	Catalogue number[a]	Target antigen(s)
Access Bio, Inc.	CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	pLDH (Pv); HRP2
	CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	pLDH (VOM); HRP2
	CareStart™ Malaria HRP2/pLDH Pf test	G0181	HRP2; pLDH (Pf)
Amgenix International, Inc.	OnSight™ - Malaria Pf Test	511-25-DB	HRP2
	OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	pLDH (Pv); HRP2
	OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	pLDH (pan)
Bhat Bio-Tech India (P) Ltd	Maleriscan® Malaria Pf/Pv	MAT-50	pLDH (VOM); HRP2
Blue Cross Bio-Medical (Beijing) Co., Ltd	One Step Malaria P.f. Test (cassette)	522352	HRP2
CTK Biotech, Inc.	OnSite Pf Ag Rapid Test	R0114C	HRP2
	Onsite Pf/Pan Ag rapid test	R0113C	HRP2; pLDH (pan)
	Onsite Pf/Pv Ag rapid test	R0112C	HRP2; pLDH (Pv)
Guangzhou Wondfo Biotech Co. Ltd.	One Step Malaria P.f. test	W37-C	HRP2
HBI Co., Ltd.	HiSens Malaria Ag P.f/P.v Card	HR2823	pLDH (pan); pLDH (Pf)
	HiSens Malaria Ag P.f./ P.v. (HRP2/pLDH) Card	HR2923	pLDH (pan); HRP2
	HiSens Malaria Ag P.f. HRP2 Card	HR3023	HRP2
Premier Medical Corporation Ltd.	First Response® Malaria pLDH/HRP2 Combo Test	I16FRC30	pLDH (pan); HRP2
	First Response® Malaria Ag pLDH	I12FRC30	pLDH (pan)
Span Diagnostics Ltd	ParaHIT® total (dipstick)	55IC201-10	pLDH (pan); aldolase; HRP2
	ParaHIT® Pan M (dipstick)	55IC301-10	pLDH (pan); aldolase
SSA Diagnostics & Biotech Systems	diagnosticks- Malaria (Pf) Cassette	KMFC6001	HRP2
	diagnosticks- Malaria (Pf) Dipstick	KMFD6007	HRP2
	diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	pLDH (Pv); HRP2
Standard Diagnostics, Inc.	SD BIOLINE Malaria Ag Pv	05FK70	pLDH (Pv)
	SD BIOLINE Malaria Ag Pf/Pv	05FK80	pLDH (Pv); HRP2
Unimed International Inc.	FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	pLDH (pan)
	FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	pLDH (pan); HRP2
	FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	pLDH (pan); pLDH (Pv); HRP2
Zephyr Biomedicals	Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	pLDH (Pv); HRP2
	Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	pLDH(pan); pLDH (Pv); HRP2

a Some products may include different catalogue numbers for different box sizes, contact manufacturers for details.

5.2. Outline of the Product Testing Protocol

The testing process is outlined in Figure 3 and in the Methods Manual for Product Testing of Malaria Rapid Diagnostic Tests - Version Two (*14)*. In brief, RDTs from each of two lots of each product were evaluated against a panel of parasite-positive and parasite-negative cryo-preserved blood samples, and a panel of parasite-negative samples. Both lots were also tested for heat (thermal) stability, evaluated before and after two months' storage at 4°C, 35°C and 45°C. Finally, an ease-of-use description was developed using a standard assessment format .

The testing process and all results were overseen by the specimen bank steering committee, and manufacturers were given 60 days to comment on individual product results prior to publication.

5.3. Evaluation panels

RDTs were evaluated against three panels, specifically:

i) *P. falciparum* culture lines (includes a subset, 'manufacturer's panel') at low (200 parasites/µl) and high parasite densities (2000 parasites/µL).

ii) Wild-type *Plasmodium* species (*P. falciparum*, *P. vivax*) from naturally infected humans and parasite-negative samples at low (200 parasites/µL) and high parasite densities (2000 (or 5000[1]) parasites/µl).

iii) Parasite-negative panel ('clean' samples and disease-specific or blood factor-specific samples).

An overview of the sample collection and characterization process can be found in the methods manuals developed for this purpose *(14-15)*. Characterization results can be found on the WHO/WPRO RDT website.[2]

In summary, each panel specimen was characterized for:

i) Geographical origin

ii) Species by duplicate microscopy (two microscopists) and confirmation by nested PCR of mono-species infection

iii) HRP2 sequence by PCR amplification

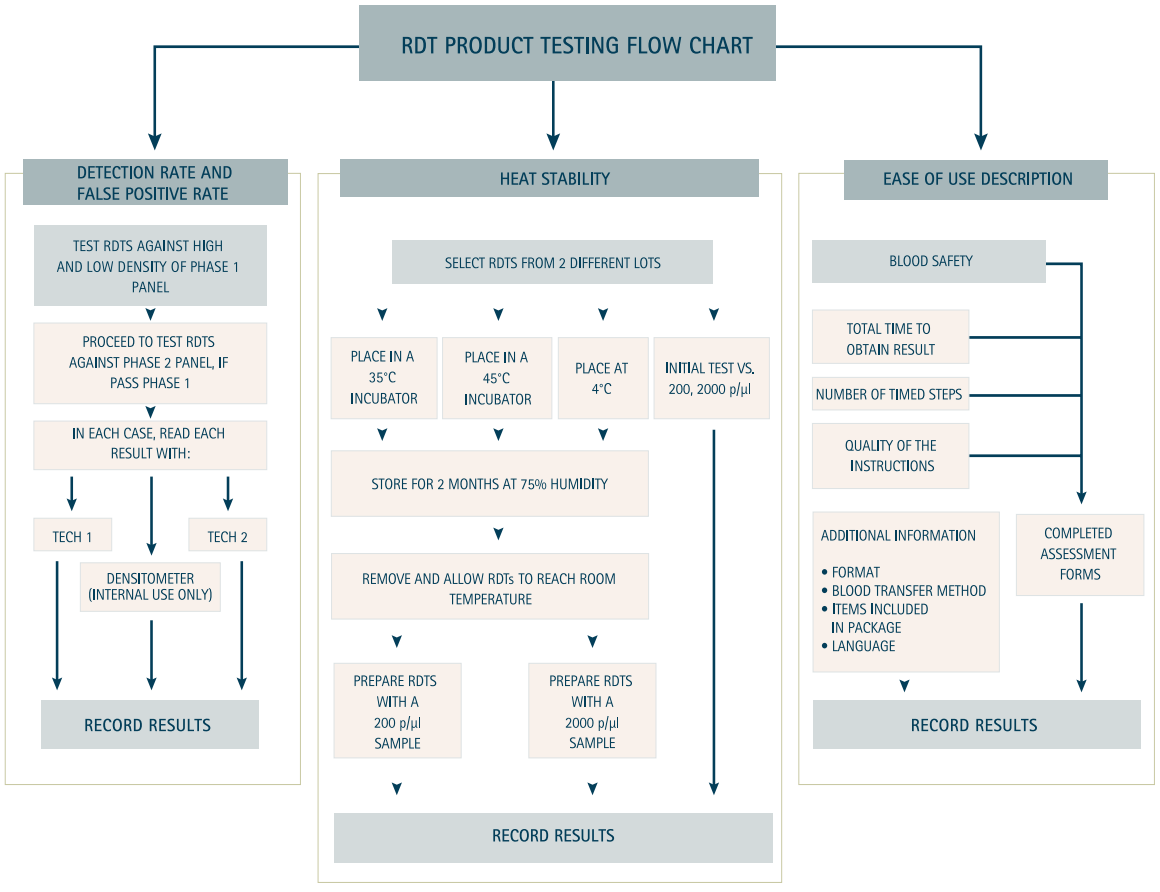
iv) Antigen concentration, determined by quantitative ELISA for HRP2, pLDH, aldolase

v) PCR for malaria and confirmatory testing for other pathology in the case of parasite-negative samples

[1] Six (6%) of the 100 *P. falciparum* dilution samples sets were 200 and 5000 parasites/µl and 2 (5%) of the 40 *P. vivax* dilution sample sets were 200 and 5000 parasites/µl.

[2] http://www.wpro.who.int/sites/rdt/who_rdt_evaluation/call_for_testing_round2.htm

Figure 3: Malaria RDT Product Testing Overview



Panel composition

P. falciparum–cultured parasites panel

Twenty culture-adapted strains of *P. falciparum* of varied geographical origin were selected, including 15 strains with type B HRP2 sequence, 3 with Type A, and 2 with Type C HRP2 sequence. All specimens were derived from the culture bank of CDC, and diluted in O+ USA donor blood *(14)*.

Wild-type parasite panel

The parasite-positive wild-type (clinical) panel consisted of samples from 100 cases of *P. falciparum* and 40 cases of *P. vivax*, derived from 10 collection sites in Asia, Africa and South America (Figures 2, 4a and 4b). 15 *P. falciparum* strains were type B HRP2 sequence, 59 with Type A, 10 with Type C and 16, while expressing HRP2, had inconclusive sequences (probably due to multi-clone infections).

Samples were collected from febrile patients and processed according to standardized methods designed to preserve target antigen concentration.*(15)* After dilutions and cryo-preservation, samples were transferred to the global bank at CDC for further characterization. The distribution of concentration of HRP2, aldolase and pLDH were determined on a larger sample, and a test panel developed that excluded samples with extremes of high or low antigen concentration.

Negative blood samples

The negative panel consisted of 'clean' parasite-negative samples from donor-derived blood banks in non-endemic areas of the Philippines, Madagascar, USA, Senegal and Nigeria, and parasite-negative samples from donors with diseases that may potentially be in the differential diagnoses of malaria, or with specific blood factors known to be common in the community or known to have the potential to cause false-positive reactions on immunochromatographic tests (Table 2). Further details of the parasite-negative panel are found at *http://www.wpro.who.int/sites/rdt.*

Table 2: Characteristics of *Plasmodium spp.* negative samples

Nature of negative sample[a]	No.
Clean-negative[b]	50
Anti-nuclear antibody positive (sera)	13
Anti-mouse antibody positive (plasma)	3
Rheumatoid factor positive (whole blood and sera)	4
Rapid plasma reagin positive (sera)	9
Chagas' disease antibody positive (plasma)	2
Dengue antibody positive (whole blood and sera)	4
Leishmaniasis antibody positive (sera)	5
Schistosomiasis antibody positive (whole blood and sera)	10

[a] Whole blood unless indicated. Sera and plasma samples were reconstituted packed cells
[b] Healthy volunteers with no known current illness or blood abnormality

5.4. RDT registration

The receipt of each shipment of RDTs at the evaluation centre was recorded in a dedicated RDT register. Temperature monitoring devices were offered to manufacturers free of charge, to accompany RDTs shipments to CDC. All RDTs were stored at ≤ 25°C immediately and temperature monitors were labelled with receipt date and forwarded for downloading, when applicable.

5.5. Specimen panel registration

All panel specimens were assigned unique identification numbers at the collection sites and stored in aliquots of 50µL at -70°C until the time of testing. All data pertaining to specimen identification, storage location and characterization results are stored in a secure, dedicated database.

Figure 4a: Origin of Phase 2 *P. falciparum* wild type (clinical) samples (n= 100)

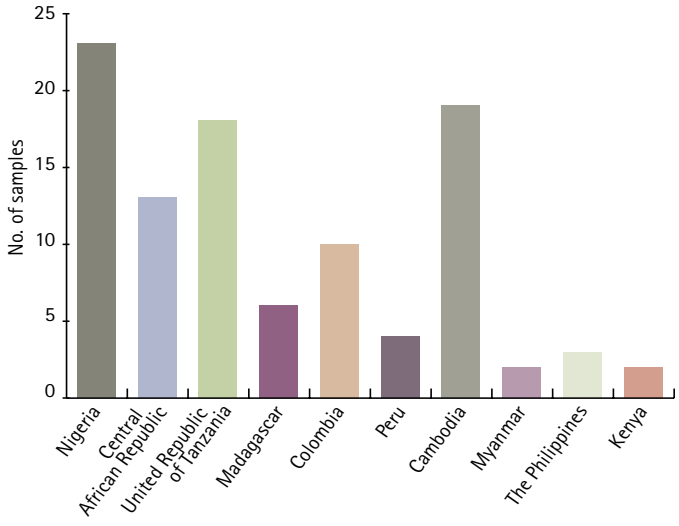
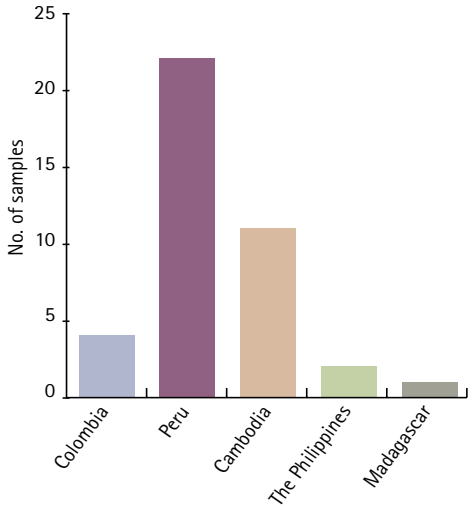


Figure 4b: Origin of Phase 2 *P. vivax* wild type (clinical) samples (n=40)



5.6. Test phases

The evaluation was divided into two testing phases:

Phase 1 – A screening step to allow the selection of RDTs meeting minimal quality requirements. Products from two lots were evaluated against a panel of 20 culture-derived *P. falciparum* samples at high (2000 parasites/µL) and low (200 parasites/µL) parasite densities. Products not designed to detect *P. falciparum* were excluded from Phase 1. To move to the full evaluation (Phase 2), a product evaluated in Phase 1 must have achieved an 80% panel detection score (PDS) against the 2000 parasite/µL samples (Figures 5 and 6)

Phase 2 – Products from two lots were evaluated against a panel of diluted clinical blood samples containing wild-type parasites and a parasite-negative panel, evaluated for heat (thermal) stability, and assessed for ease of use.

a. The parasite-positive and parasite-negative panel was comprised of 100 *P. falciparum*, 40 *P. vivax* at two parasite densities (200 parasites/µL and 2000 (or 5000)[1] parasites/µL), and 100 parasite-negative controls.

b. Heat stability evaluation: Baseline testing of 10 RDTs from each of two lots against a single culture-derived *P. falciparum* isolate (Nigeria XII strain, Pf HRP2 sequence type B with a typical antigen concentration) at 200 parasites/µL and 2000 parasites/µL and 4 RDTs from each lot against a negative sample. This procedure was repeated after RDTs were maintained for 60 days at 4°C, 35°C and 45°C at 75% humidity.

c. Ease of use assessment: After becoming familiar with the test device, technicians jointly described the test for blood safety characteristics, quality of instructions, number of timed steps and total time to result, using a standard reference guide *(14).*

d. A stability assessment was also required to be conducted by manufacturers at the manufacturing site. Manufacturers were requested to assess real-time heat stability at three month intervals against high and low parasite densities supplied by WHO at the upper limit of their recommended storage temperature throughout shelf-life and at the end of shelf-life. Results are submitted to WHO at regular intervals, for internal use, only.

[1] Six (6%) of the 100 *P. falciparum* dilution samples sets were 200 and 5000 parasites/µl and 2 (5%) of the 40 *P. vivax* dilution sample sets were 200 and 5000 parasites/µl.

5.7. Performing rapid tests

All RDTs were brought to room temperature prior to first use. Desiccant was inspected for colour changes and products were discarded if present. RDTs were labelled with sample identification number, dilution, and the date when test was performed. Performance of rapid tests was in accordance with manufacturer's instructions, with the exception that blood transfer was carried out by micro-pipette from the sample tube. The result was recorded by a technician at the minimum specified reading time. A second technician re-read the result within 1 hour for internal monitoring purposes and for information for manufacturers. Technicians were rotated, and blinded to sample type and to each other's results during Phase 2. Annexes 1 and 2 contain a descriptive and illustrated summary of the test characteristics, steps and guide to interpretation of results.

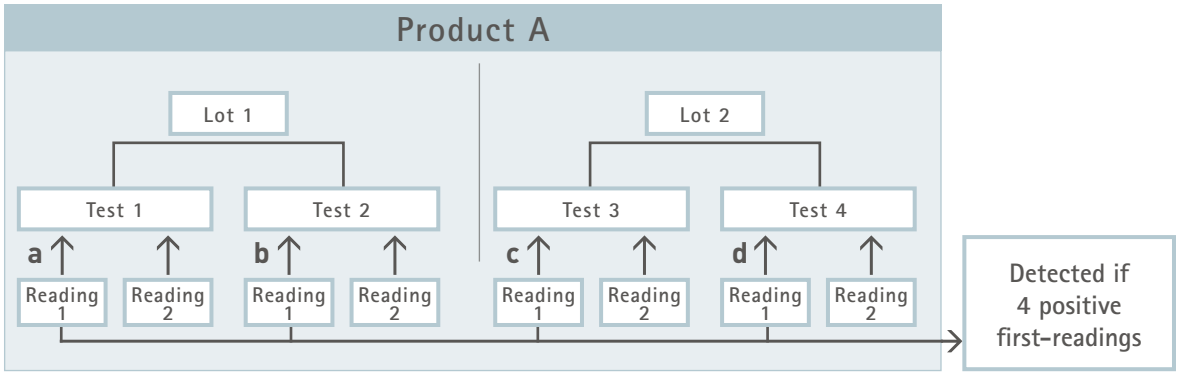
5.8. Interpretation of results

Results of control and test lines were recorded as negative or positive by each technician. Each test was read against a standard colour chart and the band intensity graded as 0 (no visible band), 1, 2, 3 or 4. If the control line is recorded as absent by either technician, the test is recorded as invalid.

Figures 5 and 6 illustrate the testing sequence at low and high parasite densities.

Figure 5: Testing procedure and calculation of 'panel detection score' and band intensity for Product A against a sample density of 200 parasites/µl

The first reading was at the minimum time specified by the manufacturer; the second reading was up to one hour later[a]. A sample is considered detected only if all first test readings, from both lots, are positive ie. Readings a, b, c and d must be positive.



Based on the positive results of first test reading (2 tests per lot), the mean band intensity score =a+b+c+d/4 (excluding negative results).

[a] second reading results are for internal use only

6. DATA MANAGEMENT

The receipt of products was hand recorded in an RDT register at the CDC as per Standard Operating Procedures (SOPs). Data associated with specimen collection and characterization was recorded first on hard copy report forms as per the SOPs at the collection sites (Figure 2), HTD (ELISA reporting) and CDC (PCR) and then entered directly into formatted excel spreadsheets that were subsequently imported into a specially developed database.

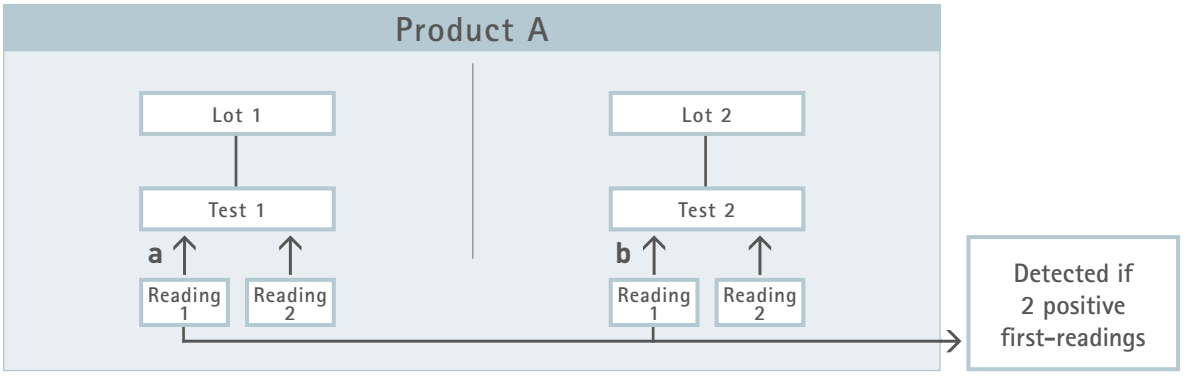
The results of the product panel testing and heat stability testing conducted at the CDC were recorded on report forms by each technician individually, as per the SOP. These results were double-data entered, and analysed for discrepancies.

All source documents and electronic records of study data are maintained in secure storage until the conclusion of the evaluation, data analysis and report publication.

Individual product testing reports and accompanying raw data were distributed to manufacturers' for review 60 days prior to publication of the final report.

Figure 6: Testing procedure and calculation of 'panel detection score' and band intensity for Product A against a sample density of 2000 parasites/µl

The first reading was at the minimum time specified by the manufacturer; the second reading was up to one hour later[a]. A sample is considered detected only if all first test readings, from both lots, are positive ie. Readings a and b must be positive.



Based on positive results of first test reading (2 tests per lot), in each lot, the mean band intensity score =a+b/2

[a] second reading results are for internal use only

7. QUALITY ASSURANCE

Product testing follows SOPs developed through prior testing experience and are based on recommendations of expert consultations, with minor modifications made on recommendation of the Steering Committee prior to Round 2 *(14)*. The quality of critical steps was controlled, as follows:

i) Quality of the malaria RDTs and their use:

All RDTs were stored in a controlled environment at ≤ 25°C; the pouch was opened and desiccant checked immediately before use; manufacturer instructions were followed with the exception of use of the blood transfer device provided by the manufacturer (a micropipette was used to ensure correct blood volume).

A temperature-monitoring device was offered to be included with the RDTs for shipment to the testing site. Logs were analysed for any temperatures exceeding manufacturers recommended storage conditions.

ii) Quality and objectivity of the RDT reading results:

Results were read in good lighting by trained technicians tested for visual acuity, and doubly entered into the database. Technicians were rotated. Readings of a second technician were used for internal monitoring purposes, and summarized results reviewed in detail and potential discrepancies identified and cross-checked against source laboratory report forms.

All wild-type parasite samples were randomized with parasite-negative samples and re-labelled for blinded reading of the RDT results.

iii) Quality of the specimen bank samples:

SOPs were established for the preparation of all specimen bank samples.*(15)*. Culture lines of parasites and wild-type samples were selected taking into account previous evidence and data from specifically conducted studies. All diluted parasite samples were stored and transported at -70°C, and were used only once within 8 hours of thawing.

iv) Quality of the product testing site:

The Division of Malaria and Parasitic Diseases, CDC, is one of the major operating components of the Department of Health and Human Services (HHS) of the USA. The laboratory holds Clinical Laboratory Improvement Amendments (CLIA) accreditation and is monitored by internal quality management systems (QMS) programmes.

8. ETHICAL CONSIDERATIONS

Each specimen collection site obtained approval from a WHO Research Ethics Review Committee and local institutional review board for specimen collection, transport and archiving of blood samples for the purpose of product testing, lot testing and quality assurance procedures.

9. DATA ANALYSIS

9.1. Measures of parasite detection: parasite detection score and positivity rates

Malaria RDTs detect parasite-derived antigen. The relationship of the concentration of antigen available from the blood sample (after lysis of red cells and parasites) to the peripheral parasite density varies highly due to a series of host and parasite factors. In addition, the population frequency of specific factors that can result in false-positive results may vary. Therefore, field sensitivity and specificity of an RDT may change in different epidemiological situations. The evaluation reported here does not predict sensitivity or specificity in a given field situation. It reports comparative detection of target antigens and false-positive rates of RDTs against a standardized panel, in a controlled, repeatable manner. As the panel is developed to be a close approximation of field samples, the comparative detection rates between products are expected to be reflected by similar comparative detection rates in the field. As the panel is designed to include a large number of samples close to the limits of detection of RDTs (200 parasites/µL), the panel is likely to discriminate more clearly than a field trial. It follows that in some settings, such as where parasite density is very high, differences in the panel detection score (PDS) and positivity rates between tests observed against the WHO evaluation panel may not be observed in patient populations, or may be much smaller. Furthermore, where parasite densities are very low, detection rates may be lower than those reported here.

Referring to Figure 5, a product must return four positive test results at the manufacturers' recommended minimum reading time (two from Lot One, two from Lot Two at initial reading time) when tested against a parasite density of 200 parasites/µL to contribute to its PDS. When tested against 2000 or 5000 parasites/µl (Figure 6) the product must return two positive tests at the manufacturers' recommended minimum reading time (one from each lot). Thus, the PDS is a measure of inter-test and inter-lot consistency, as well as the ability to detect antigen. The PDS for *P. falciparum* indicates an RDT result confirming the presence of *P. falciparum,* when tested against cultured and wild-type *P. falciparum* samples, while the non-*P. falciparum* PDS (*P. vivax* detection in this Report) indicates *Plasmodium*-positive/*P. falciparum*-negative results when tested on wild-type *P. vivax* samples.

The positivity rate is the percentage of all tests of a particular product that returned a positive test result,at manufacturers' recommended minimum reading time, when tested against a *P. falciparum* or *P. vivax* sample.

9.2. False-positive results

False-positive results are analysed and reported as two separate groups; those that had incorrect species identification, and those that returned a positive result for samples not containing *Plasmodium* spp. parasites. Specifically, the false-positive rate is the percentage of all tests of a particular product that returned a positive test result when it shouldn't have, based on results at the manufacturers recommended minimum reading time.

9.2.1. Incorrect species identification

A test is considered as returning an incorrect species result if a positive *P. falciparum* test line appears on testing against a sample containing non-*P. falciparum (P. vivax)* parasites. *P. falciparum* samples resulting in only a visible pan-specific (or non-*P. falciparum*-specific) test line on combination tests are also considered to be false-positives.

9.2.2. False-positives from *Plasmodium*-negative samples

Any test that produces a positive reading to samples with no *Plasmodium* parasites is considered a false-positive. In Phase 2, parasite-negative samples consist of clean-negative samples and also samples containing other infectious agents (e.g. Dengue, Leishmania, Chagas) and immunological factors (eg. rheumatoid factor, anti-nuclear antibodies, anti-mouse antibodies) (Table 2).

9.3. Band intensity

All positive tests results were recorded according to the band intensity against a standard reference chart, matched closely to line colour. Based on the first reader results, the distribution of band intensity results is presented as the mean band intensity of positive results. In addition, the intensity was expressed for each possible result (0, 1, 2, 3 or 4)[1] as the percentage recorded at that level.

9.4. Lot agreement

Disagreement between test lots is calculated from the number of samples that returned a positive result on both RDTs tested in that lot against parasite-positive samples at 200 parasites/µl, and on the single RDT from each lot tested against samples at 2000 (or 5000) parasites/µl. Thus, high inter-lot agreement indicates consistency in detecting malaria parasites.

[1] A standard intensity comparison chart is used which allows matching to the closest of four common colour variants of labelled antibodies used on RDTs, each at four levels of intensity.

9.5. Invalid tests

The total number of tests that were deemed invalid during testing of both lots, using samples at 200 parasites/µl and 2000 (or 5000) parasites/µl.

9.6. Heat (thermal) stability

The results of heat stability testing are reported as the number of positive tests (maximum 20)[1] and mean band intensity (for positive tests only) at baseline and after lots were stored at 4°C, 35°C and 45°C for two months against one *P. falciparum* parasite sample at 200 and 2000 parasites/µl.

10. LABORATORY VERSUS FIELD-BASED MALARIA RDT EVALUATIONS

Despite the strengths of the product testing programme, the evaluation is not completely analogous to field testing of malaria RDTs. In order to compose a panel that could be reproducibly used to evaluate RDTs, blood samples were diluted, frozen and stored below –70°C. Blood that has undergone a freeze thaw process may not have exactly the same characteristics as fresh blood, but as red cell lysis occurs as a first step on RDTs, the effect of this is limited. A further variation from field equivalence is the use of a micro-pipette to supply blood to the RDT device rather than the blood transfer device provided by the manufacturer. This was necessary because blood is collected from a cryo-tube rather than a finger-prick, and the blood transfer devices provided with a particular product can vary. This technique also ensured consistency of testing by reducing the likelihood of operator error.

Field trials have a place in product selection, particularly in determining which of a short-list of products is most appropriate for the technicians and situation of its intended use by a programme (e.g. ease-of-use characteristics). Such trials should have carefully-defined objectives and procedures designed to achieve these. Trials to determine the likely field sensitivity and specificity of a product also have a place, but require large sample sizes and populations with low parasite densities to determine significant differences between well-performing products, they need to be tightly controlled, and are therefore expensive. They do not allow comparison of a large number of products. WHO has produced recommendations on good practice for malaria field trials which should be followed to improve the repeatability and quality of results *(16)*.

[1] Ten tests per lot, with invalid results excluded from analysis.

11. RESULTS

11.1. Summary

In Round 2 of the WHO Malaria RDT Product Testing, 29 products were evaluated against *P. falciparum* culture samples, and 27 proceeded to evaluation against wild-type samples collected from parasitaemic patients from three continents and a large panel of parasite-negative samples. Heat stability was assessed at temperatures commonly encountered in malaria endemic countries. Thirteen research institutes were engaged in either sample collection or sample characterization to establish the evaluation panels. Between April and November 2009, in total, over 38,000 tests were performed at the CDC.

The results of the evaluation reveal the following key outcomes:

i) The overall range of results including PDS [formerly 'Detection Rate'], positivity rate, false-positive rates and heat stability, were similar to those reported in Round 1 *(3)*.

ii) A number of RDTs demonstrated consistent detection of malaria at low parasite densities (200 parasites/µl), have low false-positive rates, are stable at tropical temperatures, are relatively easy to use, and can detect *P. falciparum*, *P. vivax* infections, or both, adding to the number of available well-performing tests included in Round 1.

iii) Performance between products varied widely at low parasite density (200 parasites/µl); however, most products showed a high level of *P. falciparum* and *P. vivax* detection at 2000 (or 5000) parasites/µl.

iv) *P. falciparum* tests targeting HRP2 antigen demonstrated the highest PDS for *P. falciparum*, but some tests targeting pLDH also detected *P. vivax* with high consistency.

v) Test performance varied between lots of some products.

Tables 3 and 4 summarize the performance of malaria RDTs against *P. falciparum* cultured parasites (Table 3) and blood containing wild-type *P. falciparum* and *P. vivax* parasites and *Plasmodium* spp. negative samples (Table 4). Data is colour coded according to arbitrary categories, to ease the interpretation of results, and these do not imply limits of acceptable or unacceptable performance. Detailed information pertaining to product testing Phase 1 and Phase 2 results is included in Annex 3 and Annex 4, respectively. A graphical representation of this data follows in Figures 7-15.

Table 3: Summary Phase 1 performance of 29 malaria RDTs against 20 cultured *P. falciparum* lines at low (200) and high (2000) parasite densities (parasites/µl)

Product	Catalogue number	Manufacturer	Panel Detection Score[a] (n=20)		False positive non-Pf infection[b] (%)		Invalid rate (%) (n=120)
			200 parasites/µl	2000 parasites/µl	200 parasites/µl (n=80)	2000 parasites/µl (n=40)	
Pf only							
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	100.0	100.0	N/A	N/A	0.0
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	35.0	100.0	N/A	N/A	0.8
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	75.0	100.0	N/A	N/A	0.0
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	100.0	100.0	N/A	N/A	0.0
One Step Malaria P.f. Test	W37-C	Guangzhou Wondfo Biotech Co. Ltd.	15.0	77.8 (18)	N/A	N/A	36.7
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	15.0	80.0	N/A	N/A	15.0
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	70.0	100.0	N/A	N/A	0.0
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	30.0	100.0	N/A	N/A	0.0
Pf and Pan							
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	85.0	100.0	1.3	0.0	0.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	70.0	100.0	1.3	0.0	0.0
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0.0	100.0	0.0	0.0	0.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	100.0	100.0	0.0	0.0	0.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	25.0	95.0	0.0	0.0	0.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	65.0	100.0	5.0	0.0	0.0
Pf and Pv							
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	80.0	100.0	1.3	0.0	0.0
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	75.0	100.0	0.0	0.0	0.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	85.0	100.0	0.0	0.0	0.0
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	85.0	100.0	0.0	0.0	0.0
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	50.0	95.0	0.0	2.5	0.0
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	90.0	100.0	1.3 (79)	0.0	0.8
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	25.0	100.0	0.0	0.0	0.0
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	90.0	100.0	0.0	0.0	0.0
Pf, Pv and Pan							
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	80.0	100.0	2.5 (79)	0.0	0.8
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	80.0	100.0	3.8 (79)	2.5	0.8
Pan only							
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	5.0	100.0	N/A	N/A	0.0
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	0.0	100.0	N/A	N/A	0.0
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	5.0	100.0	N/A	N/A	0.8
ParaHIT Pan M (dipstick)	55IC301-10	Span Diagnostics Ltd	0.0	50.0	N/A	N/A	0.0
Pv only							
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	0.0

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive

[b] Pan or Pv line only positive indicates a false positive non *P. falciparum* infection

Detection rate (%)	≥95	85-94	50-84	< 50
False positive rate (%)	<2	2-5	6 - 10	>10
Invalid rate (%)	<1% of tests conducted	1-2% of tests conducted	2-5% of tests conducted	>5% of tests conducted

Table 4: Summary Phase 2 performance of 27 malaria RDTs against wild type (clinical) *P. falciparum* and *P. vivax* samples at low (200) and high (2000[a]) parasite density (parasites/µl) and *Plasmodium* spp. negative samples

Product	Catalogue number	Manufacturer	Panel Detection Score[b]				False positive rates (%)				Total false positive rates[e] (%)	Invalid rate (%) (n=1240)
			200 parasites/µl		2000 parasites/µl		200 parasites/µl		2000 parasites/µl		Clean negative samples	
							Pf samples	Pv samples	Pf samples	Pv samples		
			Pf samples (n=100)	Pv samples (n=40)	Pf samples (n=100)	Pv samples (n=40)	False positive non Pf infection[c] (n=400)	False positive Pf infection[d] (n=160)	False positive non Pf infection[c] (n=200)	False positive Pf infection[d] (n=80)	False positive *Plasmodium* spp. Infection (n=200)	
Pf only												
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	98.0	N/A	100.0	N/A	N/A	0.6	N/A	1.3	3.0	0.0
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	59.0	N/A	99.0	N/A	N/A	1.9	N/A	2.6 (77)	7.0	0.9
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	80.0	N/A	99.0	N/A	N/A	2.5	N/A	3.8	2.0	0.0
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	87.0	N/A	100.0	N/A	N/A	0.0	N/A	0.0	1.0	0.1
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	37.0	N/A	94.0	N/A	N/A	1.3 (153)	N/A	0.0 (77)	0.5 (186)	5.4
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	74.0	N/A	99.0	N/A	N/A	8.1	N/A	2.5	11.0	0.0
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	59.0	N/A	99.0	N/A	N/A	0.0	N/A	0.0	0.0	0.0
Pf and Pan												
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	84.0	75.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	85.0	80.0	99.0	100.0	0.0	0.6 (159)	0.5 (199)	0.0	25.5	0.2
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	20.0	15.0	94.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	84.0	75.0	99.0	100.0	0.0	0.0	0.0	0.0	0.5	0.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	63.0	20.0	98.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	64.0	10.0	99.0	97.5	0.0	0.0	0.0	0.0	7.0	0.0
Pf and Pv												
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	89.0	80.0	100.0	100.0	1.3	0.0	0.5	0.0	0.5	0.0
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	90.0	90.0	100.0	100.0	0.3	0.6	0.0	0.0	0.5	0.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	91.0	45.0	99.0	100.0	0.2 (399)	0.6	0.0	0.0	2.0	0.1
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	92.0	45.0	100.0	100.0	0.0	1.3	0.0	0.0 (79)	4.5	0.2
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	52.0	0.0	97.0	60.0	1.7 (399)	2.5	32.5	2.5 (79)	1.5 (199)	0.4
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	92.0	37.5	100.0	100.0	0.5	1.9	0.0	0.0	3.5	0.1
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	61.0	75.0	99.0	100.0	0.3	0.0	0.5	0.0	0.5	0.0
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	96.0	95.0	100.0	100.0	0.0	0.0 (159)	0.0 (199)	0.0	3.5	0.2
Pf, Pv and Pan												
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	89.0	45.0	100.0	100.0	0.0 (399)	2.5	0.0	0.0	24.5	0.1
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	93.0	45.0	100.0	100.0	0.0 (396)	0.0 (159)	0.0 (199)	0.0	37.0 (198)	0.7
Pan only												
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	31.0	92.5	98.0	100.0	N/A	N/A	N/A	N/A	0.0	0.0
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	25.0	82.5	97.0	100.0	N/A	N/A	N/A	N/A	2.5	0.2
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	22.0	77.5	96.0	100.0	N/A	N/A	N/A	N/A	2.5	0.2
Pv only												
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	92.5	N/A	100.0	0.3	N/A	1.0	N/A	1.0	0.0

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] 6 (6%) of the 100 *P. falciparum* dilution samples sets were 200 and 5000 parasites/µl and 2 (5%) of the 40 *P. vivax* dilution sample sets were 200 and 5000 parasites/µl

[b] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive

[c] For combination tests, Pan or Pv line, only, positive indicates a false positive non *P. falciparum* infection

[d] Pf line positive indicates a false positive *P. falciparum* infection

[e] The total number of times a positive result for malaria was generated when it should not have been

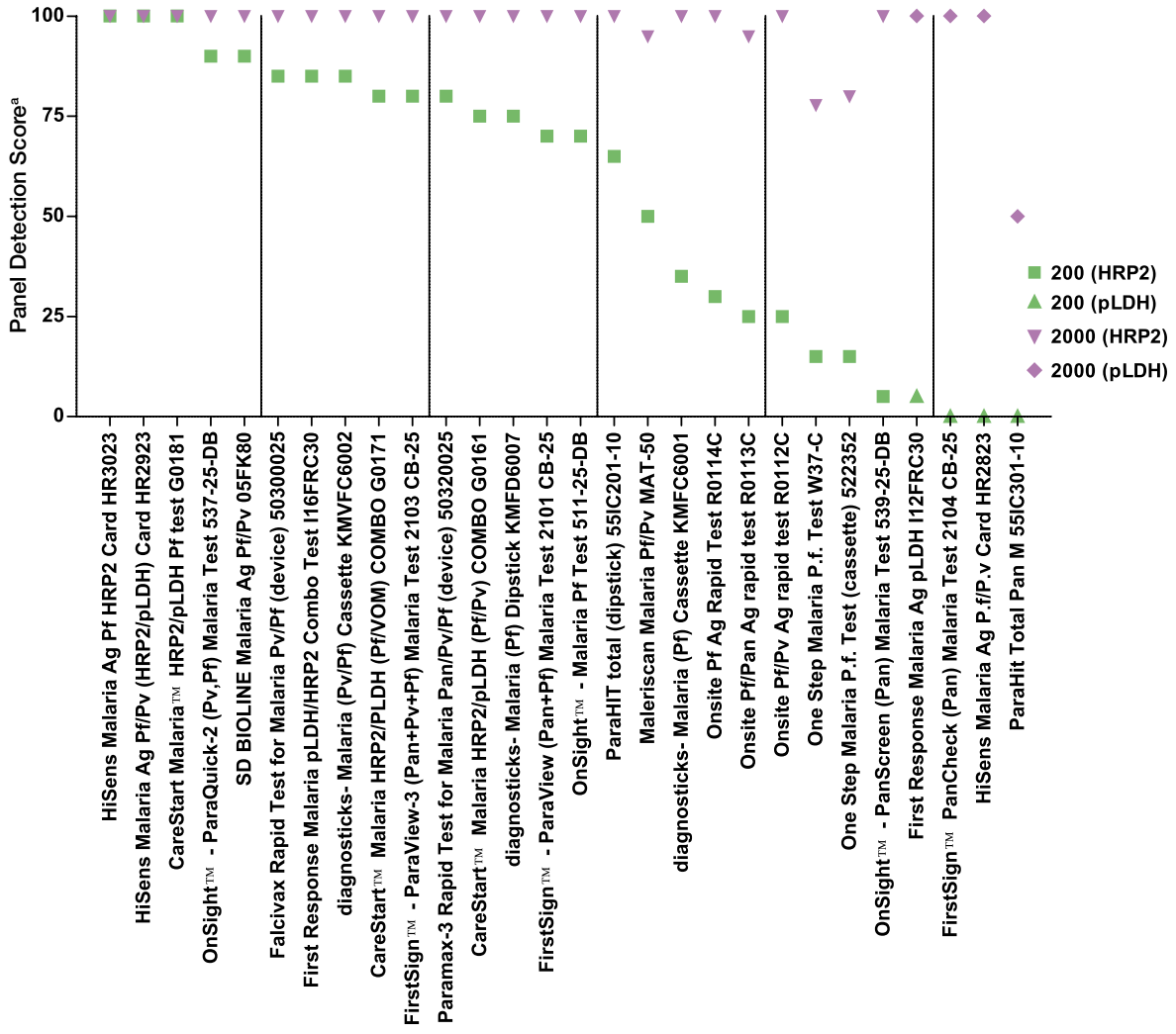
Detection rate (%)	≥95	85-94	50-84	< 50
False positive rate (%)	<2	2-5	6 - 10	>10
Invalid rate (%)	<1% of tests conducted	1-2% of tests conducted	2-5% of tests conducted	>5% of tests conducted

RESULTS

11.2. Phase 1 - *P. falciparum* culture panel

The majority (90%) of tests consistently detected ≥ 95% of *P. falciparum* cultured parasites at high parasite densities (2000 (or 5000) parasites/µl); however, the panel detection score was highly variable (0-100%) at low parasite densities (200 parasites /µl). At low parasite densities, the products with the highest PDS targeted HRP2 (Figure 7).

Figure 7: Phase 1 *P. falciparum* panel detection score of malaria RDTs at low (200) and high (2000) parasite densities (parasites/µl) according to target antigen type (HRP2 or pLDH)



[a] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive.

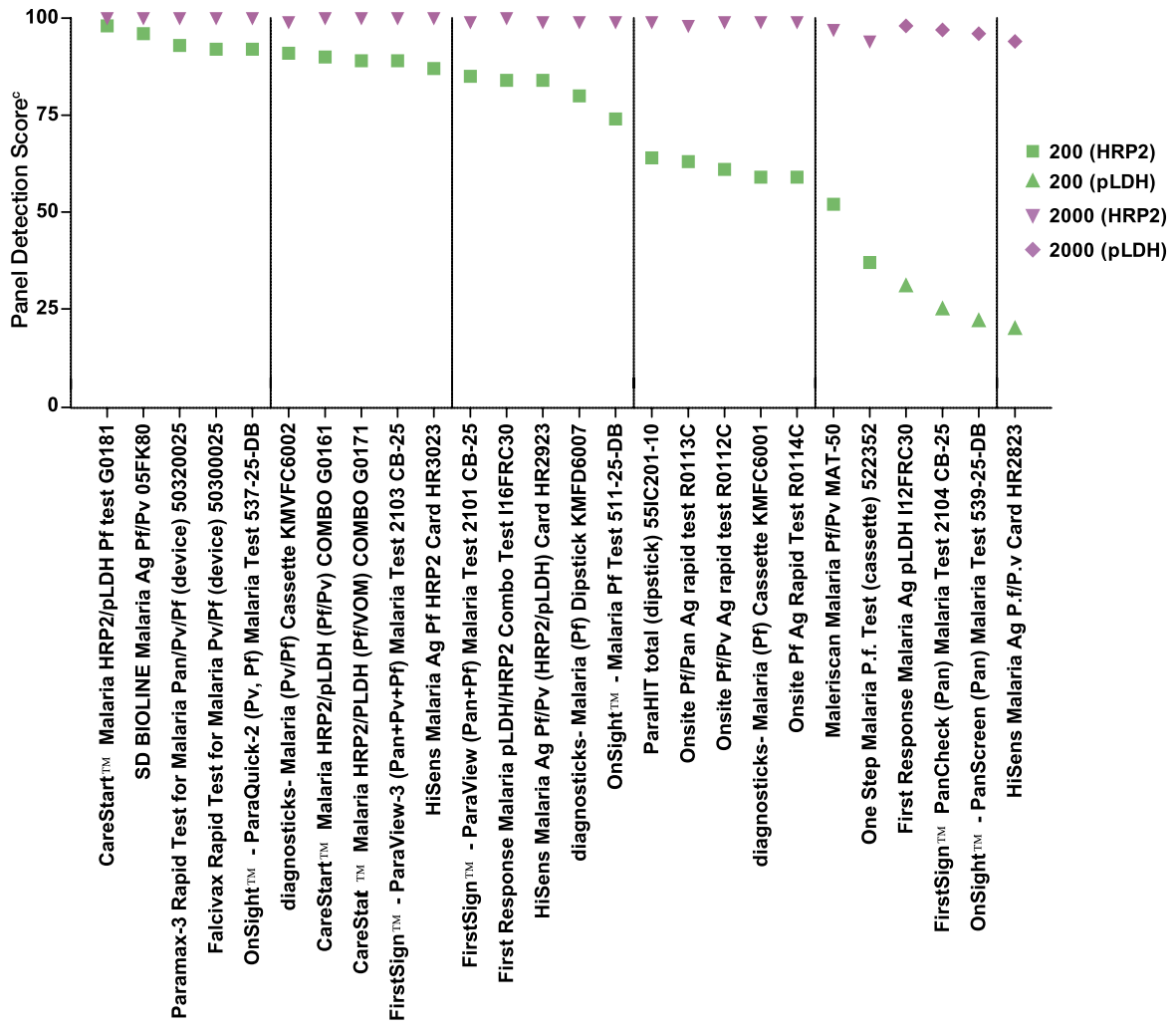
11.3. Phase 2 - Wild-type *P. falciparum* and *P. vivax* and *Plasmodium* spp. negative samples

Results below refer to the 27 products that proceeded to Phase 2.

11.3.1. *P. falciparum* detection

Twenty six of the 27 products in Phase 2 were designed to detect *P. falciparum*. Compared to the *P. falciparum* cultured parasite panel, *P. falciparum* PDS and positivity rates of wild-type samples were generally higher, reflecting the increased antigen content of wild-type samples. As in Phase 1, the majority of tests (25; 92%) had a panel detection score ≥95% of *P. falciparum* samples at high parasite densities but only 2 tests (7%) had this high a PDS at low parasite density (200 parasites/µl). Both of these products targeted HRP2. Six products specific for *P. falciparum* alone achieved PDS of ≥50% (Figure 8).

Figure 8: Phase 2 *P. falciparum* panel detection score of malaria RDTs at low (200) and high (2000[a]) parasite density (parasites/µl) according to target antigen type (HRP2 or pLDH)[b]



[a] 6 (6%) of the 100 *P. falciparum* dilution samples sets were 200 and 5000 parasites/µl and 2 (5%) of the 40 *P. vivax* dilution sample sets were 200 and 5000 parasites/µl

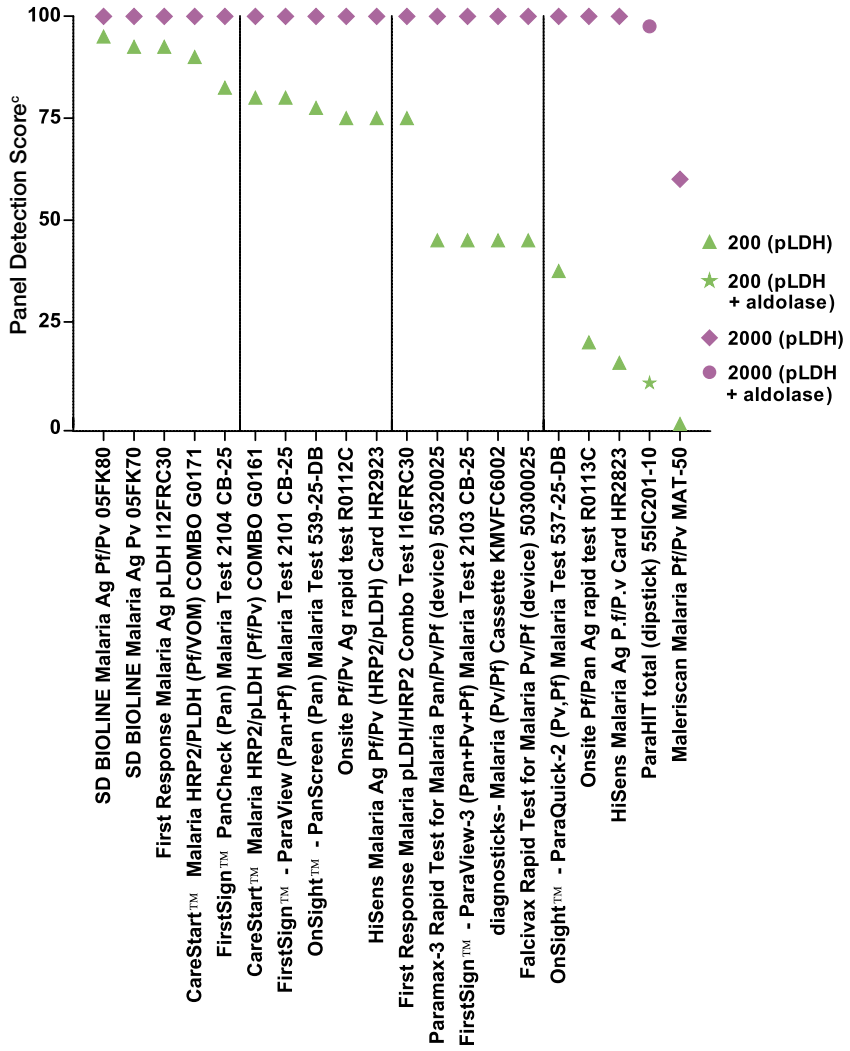
[b] Phase 2 evaluation panel consisted of 100 clinical blood samples containing wild type *P. falciparum*. RDTs performed = 2 tests x 2 lots at 200 p/µl and 1 test x 2 lots at 2000 p/µl

[c] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive.

11.3.2. *P. vivax* detection

Figure 9 illustrates, that of the 20 products designed to detect *P. vivax* most detected high parasite densities (2000 (or 5000) parasites/µL) consistently, and several achieved a high PDS against 200 parasite/µL samples. However, the overall detection of the low parasite density wild-type *P. vivax* samples was lower than that for *P. falciparum*. At low parasite densities (200 parasite/µL), only four products (20%) had panel detection scores ≥ 90% and 11 had a PDS of ≥50%. (Table 4)

Figure 9: Phase 2 *P. vivax* panel detection score of malaria RDTs at low (200) and high (2000[a]) parasite densities (parasites/µl) according to target antigen type (aldolase, pLDH, aldolase + pLDH)[b]



[a] 2 (5%) of the 40 *P. vivax* dilution sample sets were 200 and 5000 parasites/µl

[b] Phase 2 evaluation panel consisted of 40 clinical blood samples containing wild type *P. vivax*; RDTs performed = 2 tests x 2 lots at 200 p/µl and 1 test x 2 lots at 2000 p/µl

[c] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive.

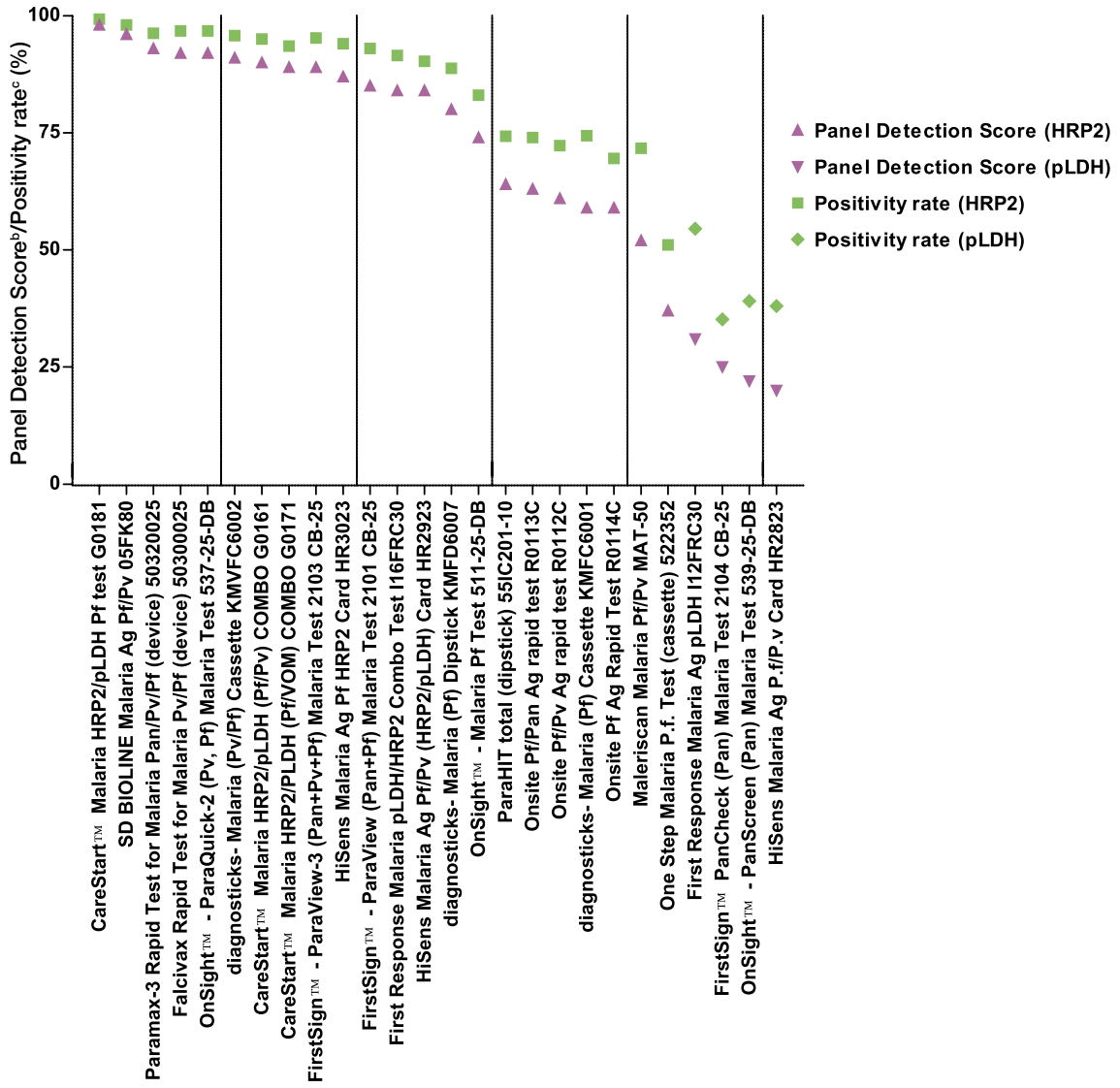
11.3.3. Combined detection of *P. falciparum* and *P. vivax*

Considering the 19 combination tests, 7 (37%) had a PDS of ≥50% for both *P. falciparum* and *P. vivax* at the low parasite density (200 parasites/µL) (Table 4). Several performed well at high parasite densities. The three pan-specific only tests had better panel detection scores for *P. vivax* than *P. falciparum*.

11.3.4. *P. falciparum* and *P. vivax* positivity rate

In addition to the PDS, the positivity rate was also measured. This puts aside test and lot differences captured in the PDS and measures the total number of times a test returned a positive result. As expected, positivity rates were higher than PDS but mirrored PDS against wild-type *P. falciparum* and *P. vivax* samples (Figures 10 and 11).

Figure 10: Phase 2 *P. falciparum* panel detection score and positivity rate at 200 parasites/µl[a]



[a] Phase 2 evaluation panel consisted of 100 clinical blood samples containing wild type *P. falciparum*. RDTs performed = 2 tests x 2 lots at 200 p/µl and 1 test x 2 lots at 2000 p/µl

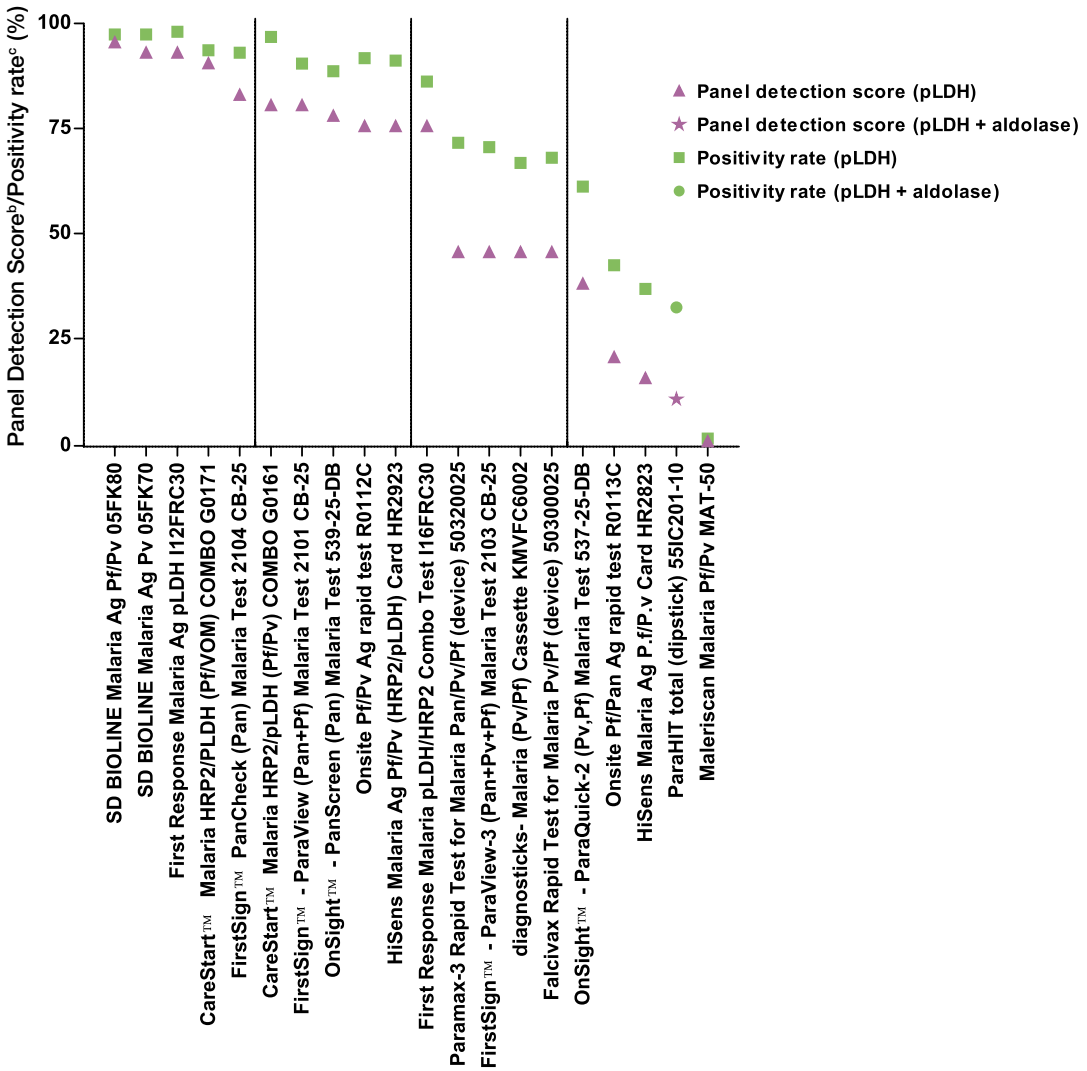
[b] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive

[c] The percentage of all tests performed that returned a positive result.

11.3.5. Band intensity

Although RDTs are not quantitative, technicians did grade positive results according to a standard colour chart and mean band intensity (for positive results) was calculated (Annex 4 - Tables A4.2, A4.3). There was a positive correlation between panel detection score and band intensity and reader agreement; suggesting that, as expected, strong test bands are interpreted more reliably.

Figure 11: Phase 2 *P. vivax* panel detection score and positivity rate at 200 parasites/µl[a]



[a] Phase 2 evaluation panel consisted of 40 clinical blood samples containing wild type *P. vivax*; . RDTs performed = 2 tests x 2 lots at 200 p/µl and 1 test x 2 lots at 2000 p/µl

[b] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive

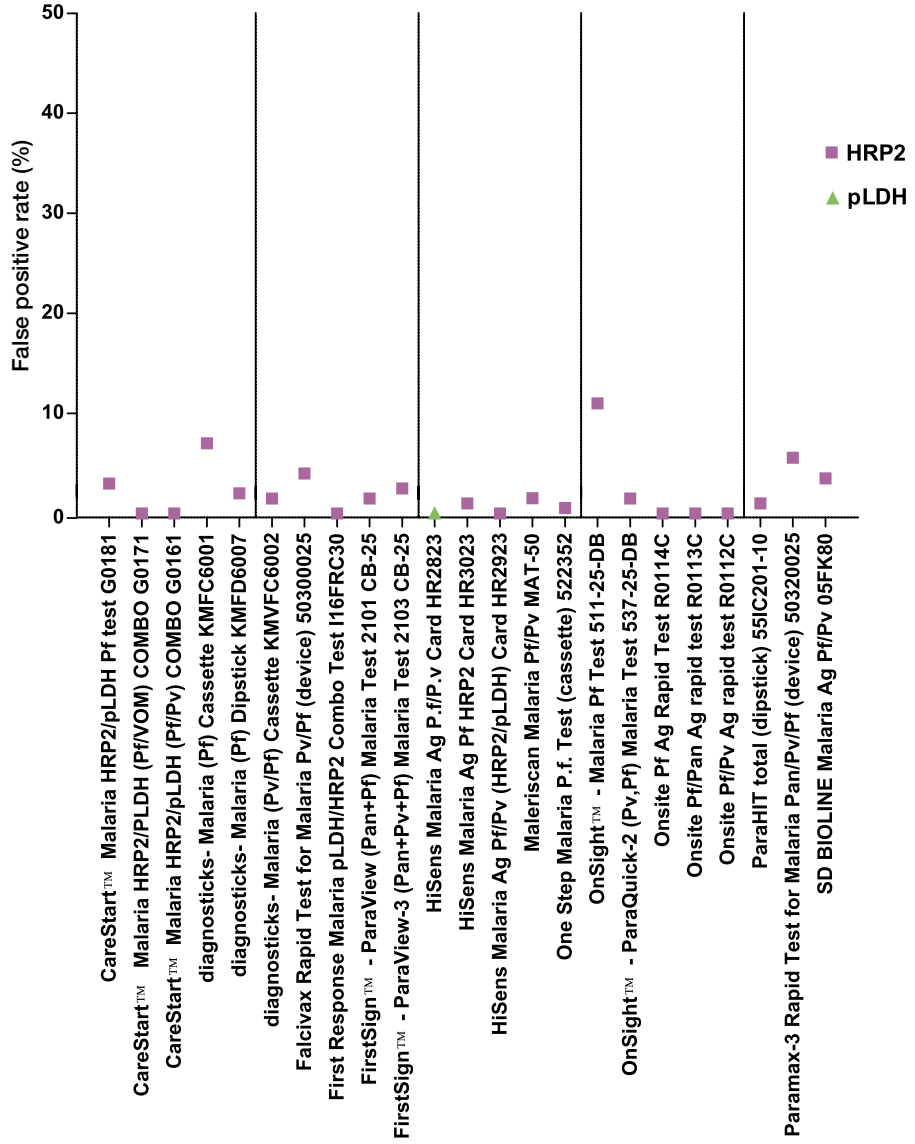
[c] The percentage of all tests performed that returned a positive result.

11.3.6. False-positive rates

Overall false-positive rates were low, with only four tests having rates >10% on clean-negative samples, on any test line (one for the *P. falciparum* test line, three on pan-specific test lines) (Figure 12). High false-positive rates were seen with some tests against parasite-negative blood with all four immunological blood abnormalities, in the panel including RPR, Rheumatoid factor, anti-DNA antibody and human anti-mouse antibody samples (Figure 13). However, sample sizes were small. For detailed information regarding the blood abnormality or pathogen that generated false-positive results for a specific product refer to Annex 4 (Tables A4.8, A4.9).

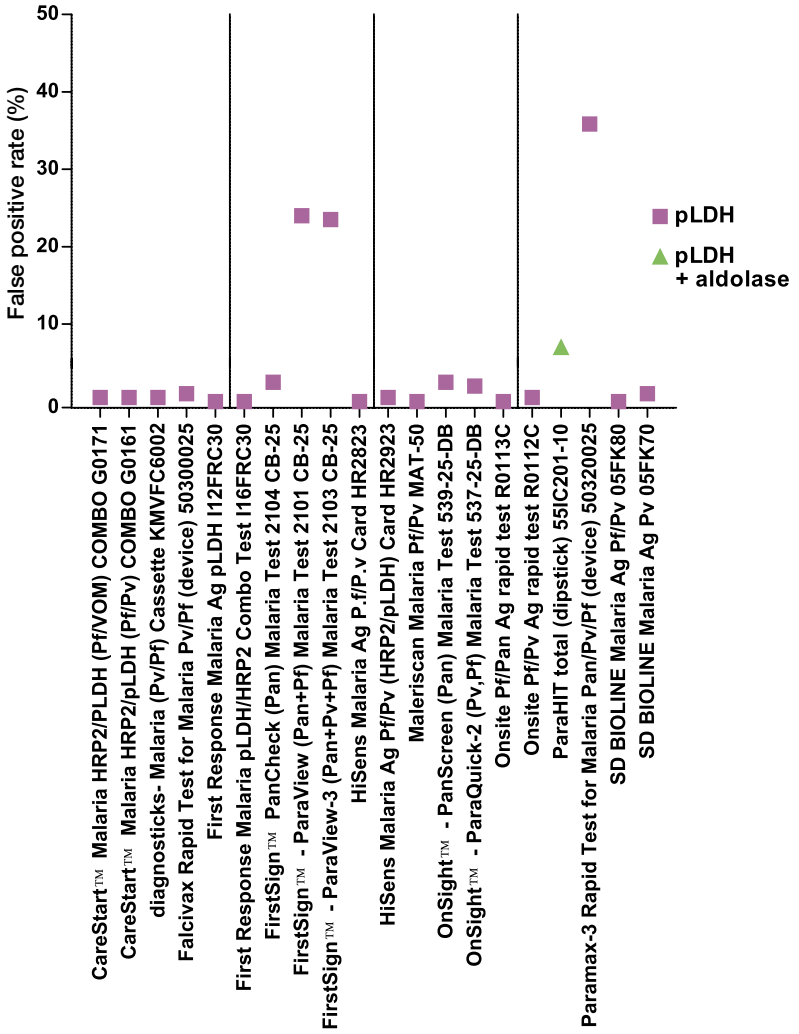
Importantly, there was no clear trend of higher false-positive rates for tests with higher PDS, indicating that there was not a clear trade-off between sensitivity and specificity of tests at these detection thresholds (Figures 14, 15).

Figure 12: Phase 2 *P. falciparum* (*P. falciparum* test line) false positive rate against clean negative samples[a]



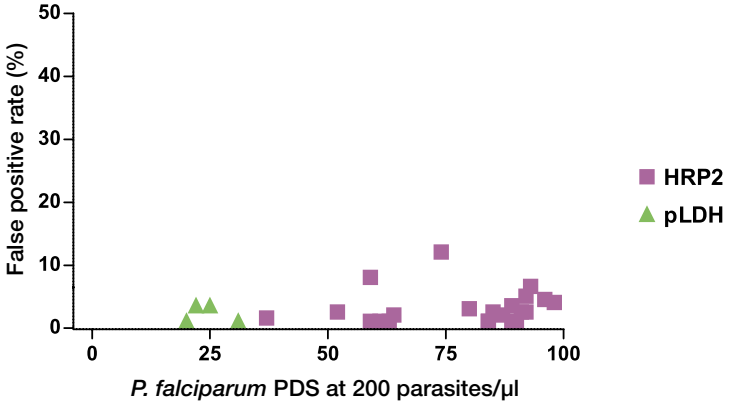
[a] Phase 2 evaluation panel included 100 *Plasmodium spp.* negative samples of which 50 were clean negatives from healthy volunteers with no known current illness or blood abnormality.

Figure 13: Phase 2 *Plasmodium spp.* (pan or *P. vivax* test line) false positive rate against clean negatives[a]



[a] Phase 2 evaluation panel included 100 *Plasmodium spp.* negative samples of which 50 were clean negatives from healthy volunteers with no known current illness or blood abnormality.

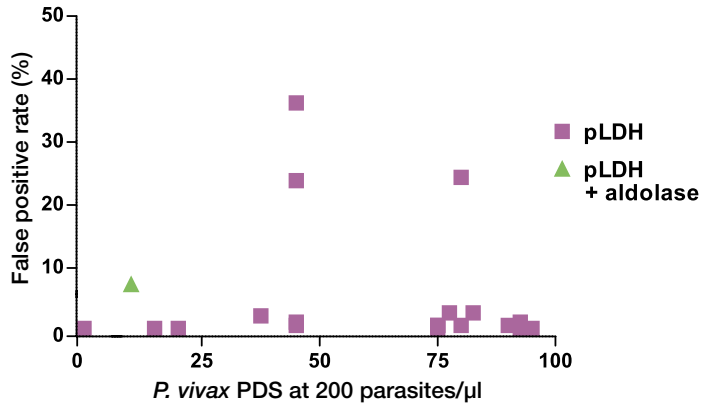
Figure 14: Phase 2 *P. falciparum* false positive rate[a] versus *P. falciparum* panel detection score[b] at low (200) parasite density (parasites/µl)



[a] False positive rate is on clean negatives, only;

[b] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive.

Figure 15: Phase 2 *P. vivax* false positive rate[a] versus *P. vivax* panel detection score[b] at low (200) parasite density (parasites/µl)



[a] False positive rate is on clean negatives, only;
[b] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive.

12. HEAT STABILITY

A single *P. falciparum* culture sample was used as the reference sample for heat stability testing. Variations in baseline performance reflect inter-test variation as the sample at 200 parasites/µL was at the limit of detection of some products.

Several products were stable, meaning that they detected a *P. falciparum* cultured sample the same number of times at baseline and following incubation for two months (75% humidity) at 4°C, 35°C and 45°C. (Table 5). Detailed results are presented in Annex 4 (Tables A4.11-A4,13a) and in Figures 16-23, the results of both lots are combined (maximum score 20; 10 tests per lot).

Overall, products showed greater stability against samples with high (2000 parasites/µL) compared to low (200 parasites/µL) parasite densities, Figures 16, 18, 20, 22 and Figures 17, 19, 21,23, respectively, as small deterioration at these high parasite densities will not be apparent. In a few cases products which had base-line positivity less than 20 of 20 tests showed unpredictable variation in positivity rates on subsequent testing after two months, consistent with test lines on the borderline of visibility. Some test lines showed a high degree of stability at 35°C but lost the ability to detect antigen after incubation at 45°C. As in Round 1, some products showed an improved performance with incubation (Figures 18, 23), but this was less apparent in Round 2. Overall, the stability of pLDH-detecting test lines was lower than that for HRP2-detecting test lines, but as in Round 1, some tests did exhibit good stability of pLDH test lines, indicating heat-stable combination tests.

The summary results of heat/thermal stability testing are presented in Table 5. Note that, as a culture-derived *P. falciparum* sample is used for heat stability testing, it is not possible to provide stability data on test lines that detect only non-*P. falciparum* parasites. Such data, and confirmatory data on the stability of recent production lots of all tests, should always be obtained from manufacturers during product selection processes when procuring RDTs (Annex 5).

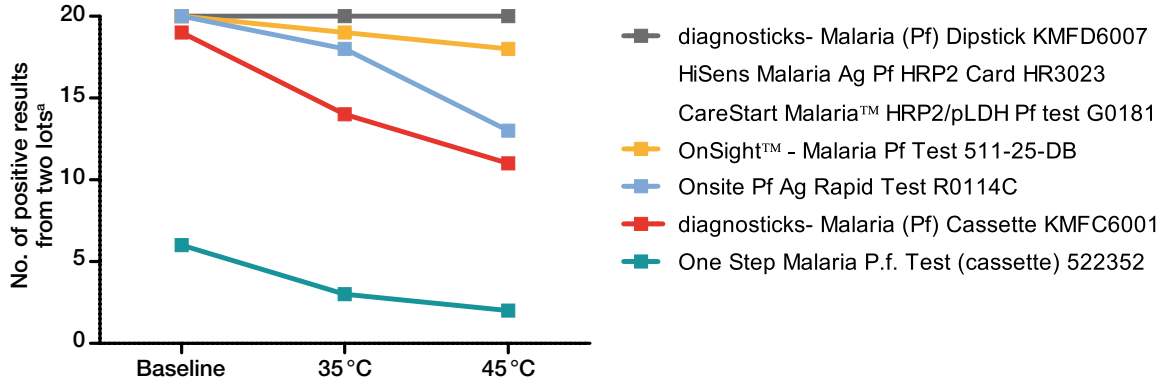
Table 5: Heat stability testing results for 27 malaria RDTs on a cultured *P. falciparum* sample at low (200) and high (2000) parasite density (parasites/µl). Positivity rate at baseline, and after 60 days incubation at 35°C and 45°C

Product	Catalogue number	Manufacturer	Positive test results for *P. falciparum* (Pf line)			Positive test results for *P. falciparum* (Pf line)			Positive test results for *P. falciparum* (Pan line)			Positive test results for *P. falciparum* (Pan line)		
			200 parasites/µl			2000 parasites/µl			200 parasites/µl			2000 parasites/µl		
			Baseline	35°C	45°C	Baseline	35°C	45°C	Baseline	35°C	45°C	Baseline	35°C	45°C
			Number of tests positive (max. 20) Lots 1 and 2 combined			Number of tests positive (max. 20) Lots 1 and 2 combined			Number of tests positive (max. 20) Lots 1 and 2 combined			Number of tests positive (max. 20) Lots 1 and 2 combined		
Pf only														
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	19	14	11	19	19	19	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	6	3	2	16	18	16	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	20	19	18	20	20	13	N/A	N/A	N/A	N/A	N/A	N/A
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	20	18	13	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A
Pf and Pan														
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	20	20	20	20	20	20	17	11	11	10	10	10
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	20	20	20	20	20	20	19	8	8	10	10	10
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	7	0	1	20	20	20	0	0	0	7	0	0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	20	20	20	20	20	20	20	20	19	20	20	20
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	20	18	8	20	20	20	1	0	0	20	14	9
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	11	17	11	20	20	19	2	0	0	10	9	14
Pf and Pv														
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	20	20	19	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	20	19	19	20	20	19	N/A	N/A	N/A	N/A	N/A	N/A
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	20	20	20	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	20	12	6	20	18	19	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	20	20	20	20	20	17	N/A	N/A	N/A	N/A	N/A	N/A
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	20	19	9	20	20	20	N/A	N/A	N/A	N/A	N/A	N/A
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	20	20	20	20	20	19	N/A	N/A	N/A	N/A	N/A	N/A
Pf, Pv and Pan														
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	20	20	20	20	20	20	12	10	3	20	18	20
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	20	10	10	20	20	20	20	5	6	20	19	20
Pan only														
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	N/A	N/A	N/A	N/A	N/A	N/A	10	16	11	20	20	20
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	N/A	N/A	N/A	N/A	N/A	N/A	5	1	2	20	20	20
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	1	7	3	20	20	20
Pv only														
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

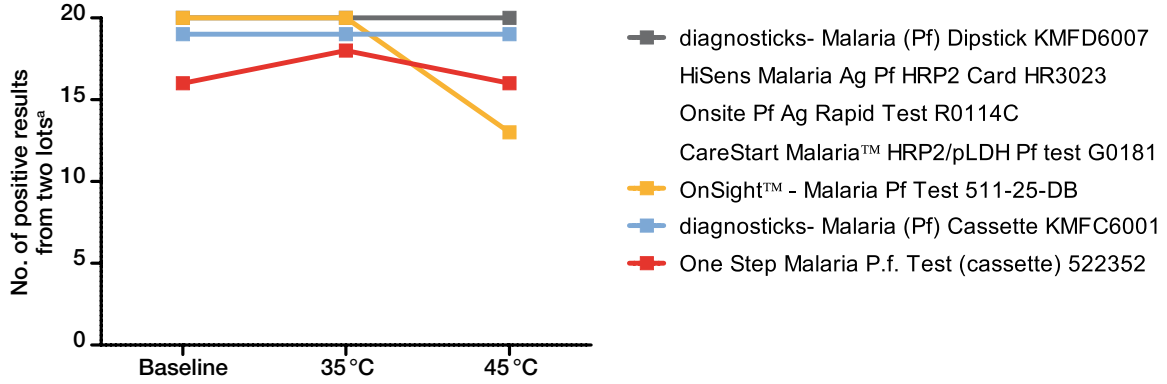
12.1. *P. falciparum* test lines

Figure 16: Heat stability of *P. falciparum* specific test line of *P. falciparum* only tests against a low density *P. falciparum* sample (200 parasites/µl). Positivity rate at baseline, and after 60 days incubation.



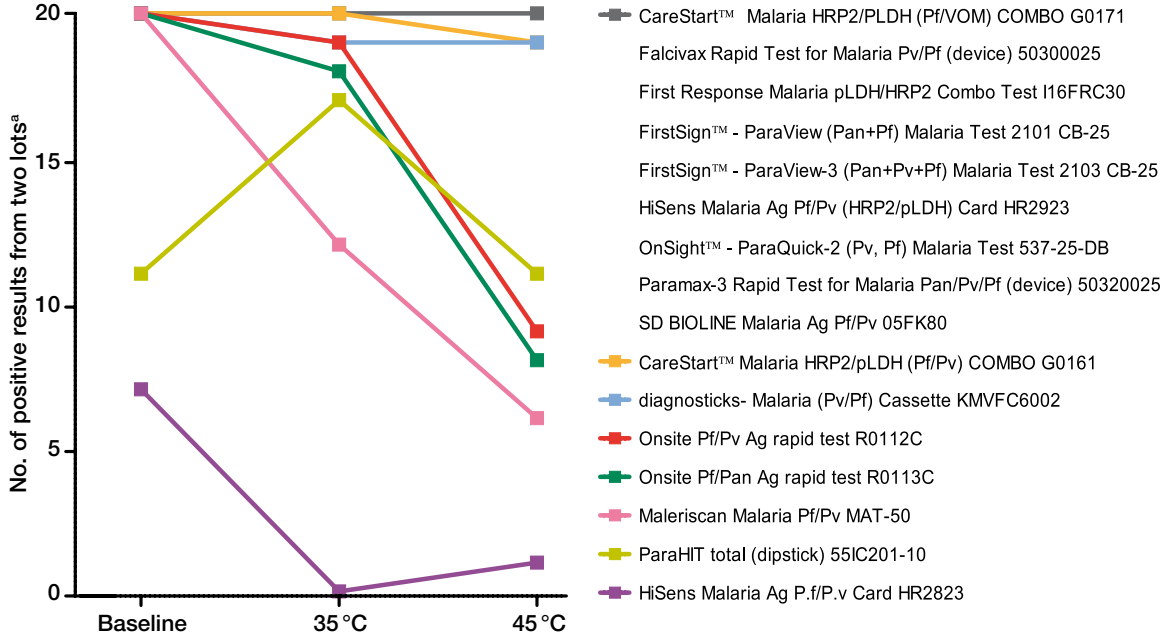
[a] Maximum score is 20 (10 tests x 2 lots)

Figure 17: Heat stability of *P. falciparum* specific test line of *P. falciparum* tests against a high density *P. falciparum* sample (2000 parasites/µl). Positivity rate at baseline, and after 60 days incubation.



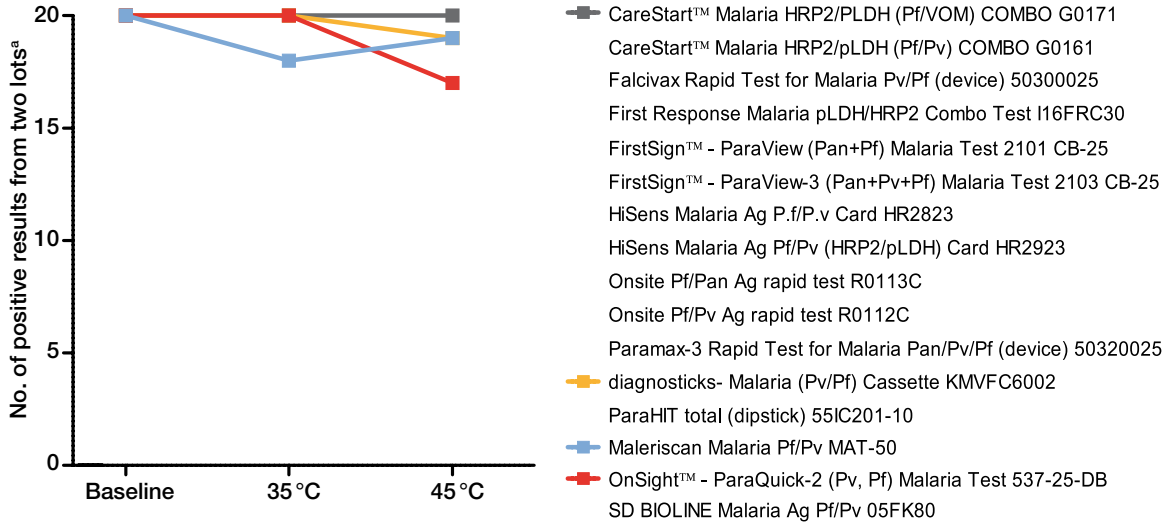
[a] Maximum score is 20 (10 tests x 2 lots)

Figure 18: Heat stability of *P. falciparum* specific test line in combination tests against a low density *P. falciparum* sample (200 parasites/µl). Positivity rate at baseline, and after 60 days incubation.



[a] Maximum score is 20 (10 tests x 2 lots)

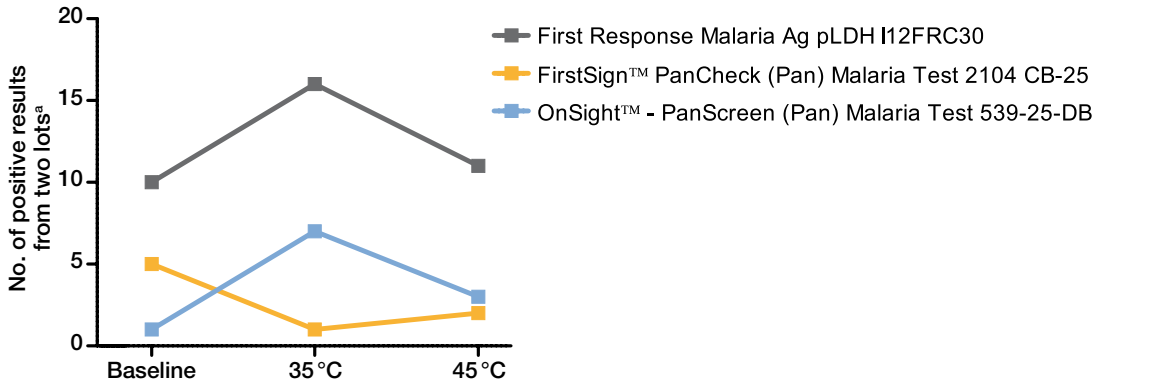
Figure 19: Heat stability of *P. falciparum* specific test line in combination tests against a high density *P. falciparum* sample (2000 parasites/µl). Positivity rate at baseline, and after 60 days incubation.



[a] Maximum score is 20 (10 tests x 2 lots)

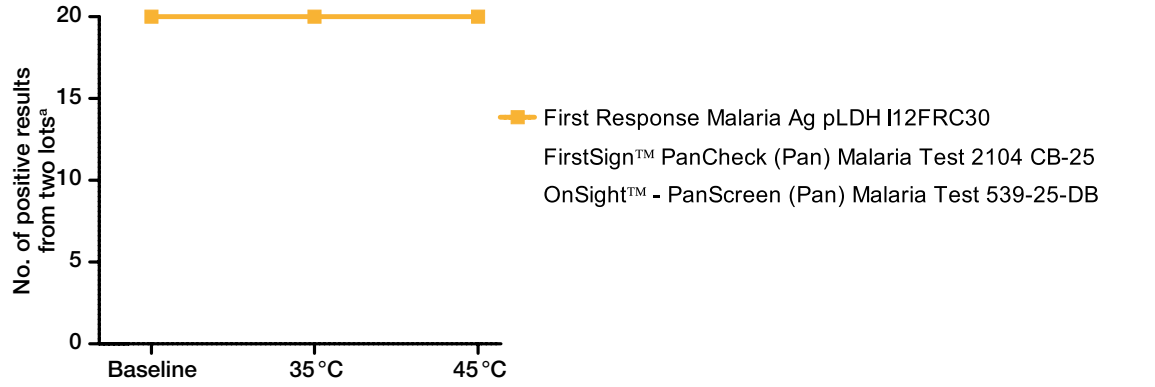
12.2. Pan-specific test lines

Figure 20: Heat stability of pan-line of pan-specific tests against a low density *P. falciparum* sample (200 parasites/µl). Positivity rate at baseline, and after 60 days incubation.



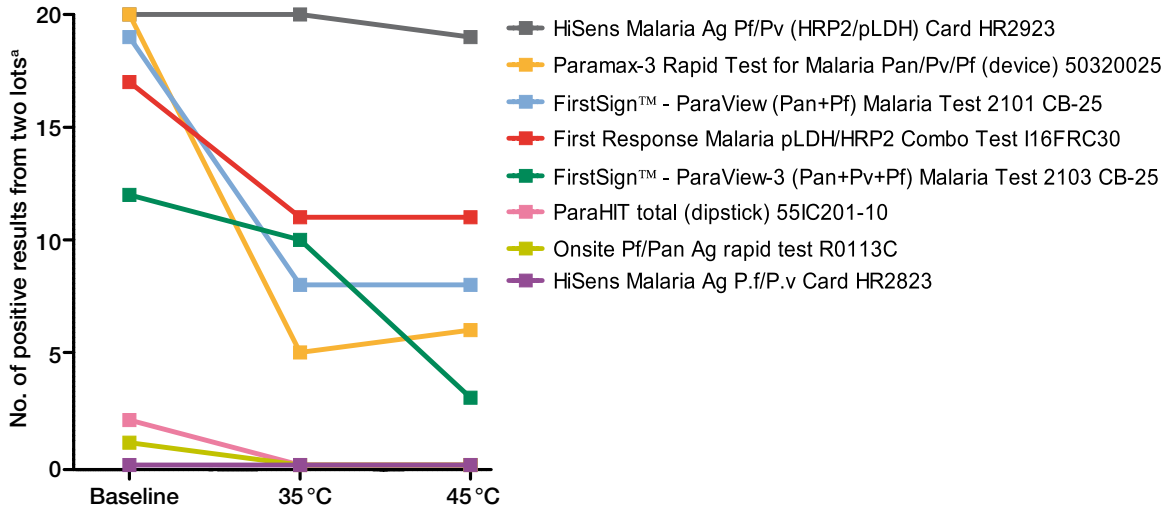
[a] Maximum score is 20 (10 tests x 2 lots)

Figure 21: Heat stability of pan-line of pan-specific tests against a high density *P. falciparum* sample (2000 parasites/µl). Positivity rate at baseline, and after 60 days incubation.



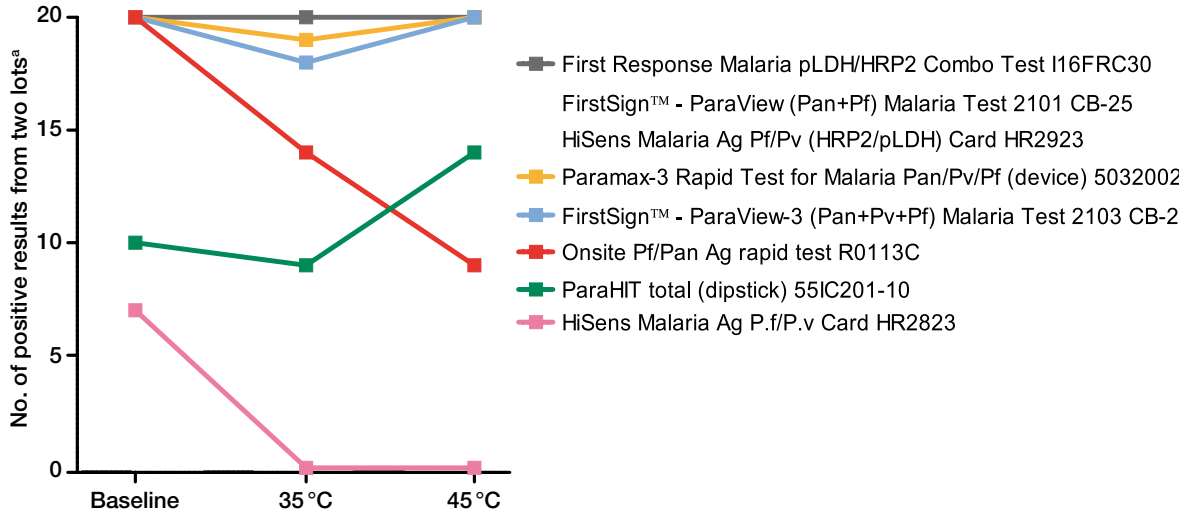
[a] Maximum score is 20 (10 tests x 2 lots)

Figure 22: Heat stability of pan-line of combination tests against a low density *P. falciparum* sample (200 parasites/µl). Positivity rate at baseline, and after 60 days incubation.



[a] Maximum score is 20 (10 tests x 2 lots)

Figure 23: Heat stability of pan-line of combination tests against a high density *P. falciparum* sample (2000 parasites/µl). Positivity rate at baseline, and after 60 days incubation.



[a] Maximum score is 20 (10 tests x 2 lots)

13. EASE OF USE DESCRIPTION

After becoming proficient at using a product, two technicians jointly produced an agreed assessment of product usability. The results, which constitute a description of the product with emphasis on aspects considered of importance to ease of use in a field setting, are presented in Table 6. It is recommended that ease of use also be assessed during product selection processes when procuring RDTs (Annex 5).

Table 6: Ease of use description of 29 malaria RDTs

Product	Catalogue number	Manufacturer	Blood safety[a]				Instruction quality[b]				Combined score (max. 5)	Number of timed steps	Total time to result	Blood transfer device	Language of instruction	Items included in package[c]
			Mixing wells involved	Retractable needle	Strip Exposed	Score (max. 3)	No diagram	Diagram of result	Diagram of result & method	Score (max. 2)						
Pf only																
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	1	0	1	2		1	1	2	4	1	20 min	pipette	English	Cassette/pipette/alcohol swab/lancet/buffer/desiccant (non color change)
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	1	0	1	2		1	0	1	3	1	20 min	loop	English	Cassette/loop/buffer/lancet/alcohol swab/desiccant (color change)
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	1	0	0	1		1	0	1	2	1	20 min	None	English	Dipstick/buffer/lancet/alcohol swab/desiccant (color change
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	1	N/A	1	2		1	1	2	4	1	20 min	None	English	Cassette/buffer/desiccant (non color change)
One Step Malaria P.f. Test	W37-C	Guangzhou Wondfo Biotech Co. Ltd.	1	1	1	3		1	1	2	5	1	15 min	pipette	English	Cassette/buffer/alcohol swabs/retractable needle/pipette/desiccant (color change)
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	1	N/A	1	2		1	1	2	4	1	15 min	None	English	Cassette/buffer/desiccant (non color change)
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	1	N/A	1	2		1	0	1	3	1	20 min	Capillary tube	English	Cassette/buffer/capillary tube/desiccant (color change)
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	1	N/A	1	2		1	1	2	4	1	30 min	pipette	English	Cassette/buffer/pipette/desiccant (non color change)
Pf and Pan																
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	1	0	1	2		1	1	2	4	1	20 min	pipette	English, Portuguese, French, Spanish	Cassette/buffer/lancet/alcohol swabs/desiccant (color change)
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	1	N/A	1	2		1	0	1	3	1	20 min	loop	English	Cassette/buffer/loop/desiccant (color change)
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	1	N/A	1	2		1	1	2	4	1	20 min	none	English	Cassette/buffer/desiccant (non color change)
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	1	N/A	1	2		1	1	2	4	1	20 min	none	English	Cassette/buffer/desiccant (non color change)
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	1	N/A	1	2		1	1	2	4	1	30 min	pipette	English	Cassette/pipette/buffer/desiccant (no color change)
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	1	0	0	1		1	1	2	3	1	15 min	capillary tube	English	Dipstick/buffer/capillary tube/plastic test tube/alcohol swab/lancet/desiccant (color change)
Pf and Pv																
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	1	0	1	2		1	1	2	4	1	20 min	pipette	English	Cassette/pipette/alcohol swab/lancet/buffer/desiccant (non color change)
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	1	0	1	2		1	1	2	4	1	20 min	pipette	English	Cassette/pipette/alcohol swab/lancet/buffer/desiccant (non color change)
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	1	0	1	2		1	0	1	3	1	20 min	loop	English	Cassette/buffer/lancet/loop/desiccant (color change)
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	1	0	1	2		1	0	1	3	1	20 min	loop	English	Cassette/buffer/lancet/loop/desicccant (color change)
Maleriscan® Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	1	N/A	1	2		1	1	2	4	1	20 min	pipette	English	Cassette/pipette/buffer/desiccant (color change)
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	1	N/A	1	2		1	0	1	3	1	20 min	loop	English	Cassette/loop/buffer/desiccant (color change)

Product	Catalogue number	Manufacturer	Blood safety[a]				Instruction quality[b]				Combined score (max. 5)	Number of timed steps	Total time to result	Blood transfer device	Language of instruction	Items included in package[c]
			Mixing wells involved	Retractable needle	Strip Exposed	Score (max. 3)	No diagram	Diagram of result	Diagram of result & method	Score (max. 2)						
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	1	N/A	1	2		1	1	2	4	1	30 min	pipette	English	Cassette/pipette/buffer/desiccant (non color change)
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	1	0	1	2		1	1	2	4	1	15 min	loop	English	Cassette/needle with cap/loop buffer/ desiccant (color change)
Pf, Pv and Pan																
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	1	N/A	1	2		1	0	1	3	1	20 min	loop	English	Cassette/buffer/loop/desiccant (color change)
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	1	0	1	2		1	0	1	3	1	20 min	loop	English	Cassette/buffer/lancet/loop/desiccant (color change)
Pan only																
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	1	0	1	2		1	1	2	4	1	20 min	pippette	English	Cassette/buffer/lancet/alcohol swabs/ desiccant (color change)
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	1	N/A	1	2		1	0	1	3	1	20 min	loop	English	Cassette/loop/buffer/desiccant (color change)
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	1	N/A	1	2		1	0	1	3	1	20 min	loop	English	Cassette/buffer/loop/desiccant (color change)
ParaHIT Pan M (dipstick)	55IC301-10	Span Diagnostics Ltd	1	0	0	1		1	1	2	3	1	15 min	capillary tube	English	Dipstick/buffer/capillary tubes/plastic test tube/alcohol swas/lancet/desiccant (color change)
Pv only																
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	1	0	1	2		1	1	2	4	1	15 min	loop	English	Cassette/buffer/needle with cap/alcohol swab/loop/desiccant (color change)

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

N/A - not applicable

[a] Mixing wells involve: Yes=0; No=1 Retractable needle: Yes=1; No=0 Strip exposed, not written within card or cassette: Exposed=0; Covered=1

[b] No diagrams=0 Diagrams of the result=1 Diagrams of result and method=2

[c] These are not necessarily standard contents. Procurers should verify with the manufacturer what materials accompany test kits and ensure they procure all the required accessories at the same time.

14. DISCUSSION OF KEY FINDINGS

This report describes the performance of many of the available malaria antigen-detecting RDTs manufactured under the ISO 13485:2003 quality standard. Malaria RDTs have the potential to provide a huge step forward in the management of febrile illness in malaria-endemic areas. To be useful in this context, malaria RDTs must have adequate:

i. sensitivity, to detect nearly all clinically-significant cases of malaria;

ii. specificity, to accurately discriminate non-malarial febrile illness from malaria, to ensure appropriate management and accurate disease monitoring;

iii. stability, for accuracy to be maintained after transport and storage in ambient conditions;

iv. ease **of use and safety,** to allow safe and correct preparation, and correct interpretation of results.

In order to assist national malaria control programmes, and other procurement agencies in the selection of products appropriate to their needs, malaria RDTs were evaluated in terms of these four major requirements. The panel used successfully discriminated between the RDTs evaluated, showing a considerable range of performance. Importantly, a number of products demonstrated a high rate of antigen detection combined with a low false-positive rate and good heat (thermal) stability, attributes essential if they are to be relied on as a basis for malaria treatment decisions in most endemic populations.

The principal results in this report are presented in Tables 3 and 4. The tables group the RDTs by type, depending on what they aim to detect, e.g. *P. falciparum* only, *P. falciparum* and non-*falciparum* species, non-*P. falciparum species only,* or all malaria species without discrimination. Panel detection scores at both high and low parasite concentrations are presented, as are false-positive rates, and the percentage of invalid test results. Tests in each category are listed alphabetically, but the results are colour-coded to assist the reader in quick interpretation of the data. These colour codes are intended to be used to quickly compare performance in the different categories and not as performance cut-offs to guide test selection or procurement. When choosing an appropriate product, it is important to also review the stability results (Table 5) in the context of the expected conditions of transport and storage of the RDTs in the field.

This evaluation is performed against a standardized panel of cultured *P. falciparum* and frozen blood samples by experienced technicians in a research laboratory and is not a field evaluation of RDT accuracy in a specific epidemiological context in the hands of intended users. The panel is designed to mimic fresh blood samples from actual cases as closely as possible, while allowing direct comparison of a large number of products simultaneously in a manner that controls for confounding factors and is calibrated to a level likely to discriminate performance differences of various products. In interpreting the results, it is therefore important that the following discussion points are taken into account.

14.1. Panel Detection Score (PDS) and its relationship to sensitivity

Evaluation of the RDTs against the Phase 2 wild-type parasite panel with parasite densities of 200 parasites/µL (Figures 8, 9) revealed a wide range of frequency and consistency of antigen detection between products, recorded as the "panel detection score" (PDS)[1] As expected, testing at higher parasite densities (2000 (or 5000) parasites/µL) results in smaller differences in performance. As two tests each from two different lots were tested at 200 parasites/µL, and as all four results had to be positive for a sample to be considered *detected* by an RDT, a positive result indicated both the ability of a product to detect the target antigen in the sample, and to do this consistently (both tests from both lots). Parasite densities of around 200 parasites/µL should be detected to ensure high field sensitivity for clinically-significant malaria infection in many malaria-endemic populations *(4).*

The PDS against the panels used in this evaluation is expected to differ from the test sensitivity in a specific clinical setting for five main reasons.

i. Performance may vary between lots or batches of the same product. Variability in lot performance is an issue with all diagnostics, and it can not be guaranteed that the results found here will predict results from different RDT lots. It is important to test lots prior to distribution to the field, to ensure that expected performance is maintained (Section 15.2).

ii. In clinical settings, patients show a wide variety of parasite densities, the range of which will depend on the local epidemiology of the disease. The magnitude of the parasite density in the population tested affects the clinical sensitivity of the test. PDS against the test panel of blood samples diluted to 200 parasites/µL are likely to underestimate the clinical sensitivity of an RDT in areas of high-transmission where symptomatic patients often have much higher parasite densities in their blood. Many tests that showed only moderate detection of the 200 parasites/µL panel may perform very well in such settings, as shown by the better PDS of most products against the panel set at 2000 parasites/µL.

    Importantly, when interpreting Figures S1, S2, 7-9, and the colour coding in Tables 3, 4, the small differences in panel detection scores found among the better-performing RDTs in this evaluation are unlikely to result in noticeable differences in clinical sensitivity, and other issues such as stability, cost, or ease of use and manufacturing capacity may be more important factors in test selection.

[1] In the report of WHO Product Testing: Round 1 the PDS was termed the 'Detection Rate'(3).

Taking into consideration the parasite density of the target populations and the likely field sensitivity of RDTs, it is important to note that, even in areas with high transmission and strong malaria immunity, populations may include individuals with low parasite densities but clinically significant infections (e.g. young children, pregnant women, those regularly using bed nets, immigrants, and others with reduced immunity). The ability to detect low parasite density infections reliably therefore remains important in these cases. As some countries move towards elimination, population immunity will decrease and it will become increasingly important to use diagnostic tests that detect low parasite densities (i.e. with high PDS against 200 parasites/µL samples).

iii. Performance of tests against the challenge panel may not directly relate to sensitivity in clinical testing as there is variability in the amino acid sequence of the HRP2 antigen of *P. falciparum* that may affect the ability of RDTs to detect it. In certain *P. falciparum* parasites the HRP2 antigen may not be detectable at all. Specifically, there is evidence that *P. falciparum* strains in some areas of South America do not express HRP2 antigens due to gene deletions *(17)*. If a significant proportion of parasites in a given area do not express HRP2, it is necessary to use tests detecting other target antigens (pLDH or aldolase). The distribution of such strains is currently being mapped.

iv. The methods used to transport and store tests can affect their field sensitivity. The tests used in this evaluation were shipped and stored under conditions intended to safeguard against degradation caused by high temperature or other extreme conditions. If similar precautions are not taken with purchased RDTs, loss of performance could result. Ambient temperatures of storage conditions vary widely in settings where these tests are commonly used, as do temperatures during transport, and requirements for heat stability of a product will therefore differ.

v. Diagnostic sensitivity and specificity are dependent on the quality of preparation and interpretation of the tests. Highly trained individuals performed all the testing in this product evaluation. In clinical settings, malaria RDTs will often be used by health workers with limited training and supervision. Simplicity of design and clearly-interpretable results will have an influence on ensuring that the technical proficiency of a product translates into accurate diagnosis in the field.

14.2. False-positive rate and specificity

False-positive rates are reported here against a panel of clean-negative samples taken from blood donated in low-transmission settings by people without malaria symptoms. In addition, false-positive rates were calculated against a smaller number of samples with specific characteristics that affect the likelihood of a false-positive result from an immuno-diagnostic test (e.g. rheumatoid factor, anti-nuclear antibody), or that may be of significance in a specific population in malaria-endemic areas (e.g. leishmaniasis, dengue). The importance of these results will vary with the intended area of use. High false-positive rates against samples of blood from dengue patients, for example, may not be a significant factor to consider in regions where dengue does not occur. In view of the small number of samples in each category in this evaluation, the results should be considered primarily as a guide to highlight potential cross-reactions that will require close monitoring if relevant to the target population.

In general, it is preferable to procure a product with a low rate of false-positive reactions. In the case of many diagnostic tests, a trade-off must be made between a preference for a high rate of antigen detection (sensitivity) and a low false-positive rate (specificity). The context in which the test will be used will guide the relative importance of these two factors in choosing one product over another. In this evaluation there was no correlation of lower PDS (loss of sensitivity) associated with low false-positive rates (high specificity). A number of products attained both a high PDS and a low false-positive rate.

14.3. Heat (thermal) stability

RDTs in this evaluation were held for two months at 35°C and 45°C and 75% humidity and then retested to evaluate stability at these temperatures. The importance of thermal stability will vary according to the ambient conditions under which a product is expected to be transported and stored. Thus, stability at high temperatures will be vital if an RDT is to be stored at clinic level in a country where ambient temperatures can reach 45°C in the hot season, but less critical in a high-altitude or cooler tropical environment where temperatures rarely rise above 35°C. Most commercially-available RDTs list 30°C as the maximal storage temperature. Higher temperatures were used for this evaluation because it is common for malaria-endemic countries to have maximum ambient temperatures of 35°C or above, although the use of cool storage methods will allow safe storage and use of products designed for storage below these temperatures. Where transport and storage of RDTs is likely to occur at high ambient temperatures, heat (thermal) stability should be seen as a significant factor in ensuring maintenance of sensitivity.

High humidity will accelerate the degradation of malaria RDTs and other lateral flow tests. All the products in this evaluation were packaged in individual envelopes that contain a desiccant and are designed to be moisture-proof. This allows the user to open the envelope of a specific test at the time of use, limiting exposure to high humidity. During the stability testing phase of this evaluation, RDTs were stored at 75% humidity. The packaging should, if in good condition, protect the contents from exposure to high humidity during storage. As such, the stability testing results presented here provide an assessment of both the stability of the RDT and the quality of its packaging.

Several products showed high stability at the temperatures and time periods used in this evaluation. In general, pan-specific lines (pLDH) were less stable than HRP2 test lines, but there was overlap between the stability of tests against these targets with one pLDH test line on a combination tests maintaining very good positivity rates after two months at 45°C.

Though temperature and humidity were held constant in this evaluation, temperatures in the field fluctuate with time of day and season. While two months' storage at a set temperature can not accurately predict long-term stability under field conditions, loss of parasite detection over this period indicates a likelihood that significant sensitivity will be lost when similar or higher storage temperatures comprise a significant amount of the storage time, and indicates likelihood of a higher susceptibility to degradation during short periods of exposure to much higher temperatures, such as during transport *(18, 19)*.

14.4. Ease of use description

The sensitivity and specificity of RDT results are dependent on the quality of preparation and interpretation of the test. In general, a simpler format with fewer steps or fewer required extraneous materials is likely to be prepared and interpreted more reliably. Thus, cassette-format RDTs are generally more reliably prepared and interpreted than products in dip-stick format *(20)*. The extra cost involved in such a format may be offset by the advantages of increased accuracy and, in some cases, less additional equipment required to perform them.

The method of blood transfer from the patient to the test is important for the safety of the user, and for the accuracy of volume of blood transferred. Devices for blood transfer are supplied with RDTs, and vary widely in design. The performance of blood transfer devices was not formally assessed in this evaluation, as blood was transferred from a tube by a micro-pipette to ensure the manufacturer-specified volume was used. Programmes procuring RDTs should consider the adequacy of the blood transfer device supplied, including previous experience of health workers and the costs and time required for re-training. It may often be appropriate to discuss with manufacturers the possibility of changing the blood transfer device from that normally supplied.

Clarity of results is important to test interpretation. A clearly visible (intense) test line is less likely to be overlooked than a line that is barely visible. While reading proficiency and adequate work places should always be ensured, health workers may sometimes have sub-optimal vision or work in conditions of inadequate lighting. The intensity of the line of the test band is closely associated with the PDS achieved by RDTs in this report (Tables A4.2, A4.3).

The importance of format and simplicity of test design will depend on the intended end-users. Trained laboratory technicians may handle a complicated procedure more reliably than village-level volunteers with limited supervision. In all cases, specific proficiency-based training and adequate supervision should be included in any RDT-based diagnostic programme, and clear instructions should be provided in a language and format appropriate for the end-user[1] *(20-22)*.

14.5. Inter-lot variability

This testing programme evaluated only two production lots of each product. Malaria RDTs are complex biological products made of components commonly supplied from multiple sources, and subject to various conditions during manufacture that may affect the quality of the final product. All manufacturers entered in this evaluation have current ISO 13485:2003 certification, a standard designed to give assurance of consistency of quality of final product, if correctly implemented. The results presented here indicate that inter-lot variability does occur, and WHO strongly recommends that a sample of RDTs from each production lot be tested prior to dissemination to the field to ensure it meets an appropriate standard. This can be facilitated by WHO (Section 15.2).

Since inter-test variability also occurs, this will be detected to some extent by routine lot testing. Ensuring manufacturers have good manufacturing standards should minimize the likelihood of inconsistencies due to poor practice in the manufacturing process. Culture-based panels[2] that are subsets of the Phase 1 panel of this evaluation are available as reference standards for manufacturers to set their own lot-release criteria against, and the development of panels based on recombinant antigens is a focus of work by FIND, TDR and WHO.

14.6. Target antigens and species

Malaria RDTs included in this evaluation detect one or more of three parasite antigens (HRP2, pLDH, and aldolase) in various combinations. HRP2 is present only in *P. falciparum*, whereas aldolase and pLDH are present in all four species and may be used as pan or all-species targets. Some tests use differences in pLDH sequences between species as a means to differentiate *P. falciparum* from *P. vivax* and other species. There is considerable overlap in the PDS of products targeting the different antigens in this evaluation. While the products with the highest PDS for *P. falciparum* targeted HRP2, a number of pLDH-detecting products demonstrated high PDS against *P. vivax*. The stability of tests targeting these different antigens also overlapped.

The choice of RDT should take target antigen into account: HRP2-detecting RDTs should not be used in areas where high rates of HRP2 non-expression occur (*17*). Tests detecting only HRP2 (without pLDH or aldolase lines) will have limited utility where non-*falciparum* malaria is common. pLDH (and possibly aldolase) RDTs may have further advantages where antigen persistence (common with HRP2) may result in a high false-positive rate in areas where early retesting in the weeks immediately after treatment is common.

[1] See http://www.wpro.who.int/sites/rdt/using_rdts/training/ and http://www.finddiagnostics.org/programs/malaria/ for generic and product-specific examples.

[2] To access these panels, contact mal_rdt@wpro.who.int, cunninghamj@who.int or info@finddiagnostics.org.

The required sensitivity of a test may also vary with species; a less sensitive test may be acceptable for detection of *P. vivax* compared to detection of *P. falciparum*, as severe outcomes due to missed diagnoses are less likely. Use of a sufficiently sensitive pan-specific test may be appropriate in areas where both *P. falciparum* and *P. vivax* occur, if all infections were to be managed initially as a *P. falciparum* infection with artemisinin-based combination therapy (ACT). Tests with high PDS for both *P. falciparum* and *P. vivax* were demonstrated in the first round of product testing *(3)*.

It should be noted that pan-species tests were not evaluated for detection of *P. ovale* or *P. malariae* in this evaluation due to lack of sources of suitable mono-species infections of these parasites.

15. USING THESE RESULTS TO ENSURE QUALITY OF DIAGNOSIS IN THE FIELD

This report provides data to guide malaria control and management programmes in selecting products likely to perform to a high standard in the particular contexts in which the programme operates. The final decision on product selection requires that this data be considered in a systematic way, taking into context the distribution of parasite densities of the target population among whom the tests will be used, and the experience and training of the intended users. Further information should be sought from the manufacturer and other sources. An algorithm to guide this process is given in Annex 5.[1]

While malaria RDTs can be applied in a number of settings, the greatest potential for impact on public health is in extension of access to accurate, parasite-based diagnosis of malaria to regions and populations where good quality microscopy-based analysis is impractical to maintain, making possible the implementation of recent WHO recommendations on universal parasite-based diagnosis prior to anti-malarial therapy *(2)*. This currently applies to most people at risk of malaria in endemic countries (*1*). In many settings where RDTs have been introduced, the true rate of parasitaemia has been found to be considerably lower than expected, allowing health systems to reduce wastage of anti-malarial medicines and to focus on the appropriate management of non-malarial causes of fever, including early pneumonia and sepsis. A successful RDT programme must therefore address not just malaria but also the management of other common and severe febrile illnesses that occur locally, in the differential diagnoses of malaria, if the potential full public health impact of an RDT programme is to be achieved.

15.1. Beyond procurement

Diagnostic tests normally represent the starting point in a health system intervention, and their use presumes that appropriate patient management, based on testing, will follow. Thus, successful introduction of RDTs requires careful planning beyond rational procurement to ensure consistent

[1] An interactive guide designed to help short-list test according to individual programme needs, based on the performance of tests in rounds 1 and 2 of the WHO-TDR-FIND Product Testing Programme can be found at http://www.finddiagnostics.org/programs/malaria/find_activities/product_testing/

supplies of all necessary materials (including gloves, sharps disposal containers, and supplies required for further case management), training of end-users, community sensitization, and monitoring of diagnostic quality and results. This extends beyond malaria management to management of other febrile diseases and health service delivery systems, and requires an integrated approach with other health programmes impacting on the management of febrile illness.

This report provides information to guide procurement of RDTs within this framework. A number of factors beyond performance characteristics reported here must influence procurement decisions. An example algorithm to guide these decisions is given in Annex 5.

Details of implementation will vary widely between programmes according to local capacity and needs. Further recommendations on budgeting, planning and implementation can be found in Annex 6.

15.2. Lot testing

Complementary to the product testing programme, WHO, TDR and FIND currently support laboratories that perform continual quality assurance of RDTs in the form of lot testing. This programme responds to requests from national malaria programmes, manufacturers, and procurement bodies to assess the quality of RDT lots prior to purchase or when they arrive in country, prior to dispersal to the field and clinical use. Testing is performed against parasite-positive and negative panels prepared and characterized in the same way as the panels used in this evaluation. A number of other national institutions have also developed this capacity. Lot-testing reassures countries that the product they have purchased is performing to a high standard before distribution, and helps to ensure that manufacturers produce consistently good lots and improve their products.

Countries and/or manufacturers ship between 125-175 RDTs to the regional lot testing centre where they are evaluated against a small panel of parasites at high and low parasite densities and negative samples (Figure 2). They are subsequently incubated at a temperature close to the manufacturer's specified storage temperature and retested every six months until their expiry date. Initial results are available after five days and then sent at regular intervals. Details of the protocol can be found in the published methods manual for lot testing *(15)*. National malaria programmes and procuring agencies are encouraged to participate in the lot testing programme.

To access lot testing through the WHO-FIND programme, contact: *mal-rdt@wpro.who.int* or *info@finddiagnostics.org* at least 2 weeks before RDTs are ready for shipment. Following a request, a form and instructions for shipment will be issued. After shipments of RDTs are received at the lot testing laboratory, initial results are returned within five working days. Further information is available at *www.wpro.who.int/sites/rdt/who_rdt_evaluation/lot_testing.htm*, or at through *www.finddiagnostics.org*

16. CONCLUSIONS

This study adds to the large data set on malaria RDT performance published in 2009 after the first round of evaluations *(3)*. The product testing programme is a landmark in the field of malaria RDT evaluations because of the number of products evaluated and its comprehensiveness including, samples with low and high parasite densities, detailed parasite characterization, multiple test lots and heat stability assessment. New laboratory methods were developed and validated to support parasite characterization and this work generated new findings regarding the variation in antigen content at similar parasite densities and the variation in the structure and expression of HRP proteins. The publication of the WHO Product Testing Round 1 results impacted on the procurement practices of countries and procurement agencies, and this Report of Round 2 will add considerably to the number of well-performing RDTs for which comprehensive performance data is now available .

17. REFERENCES

1. *World Malaria Report 2009.* Geneva, World Health Organization, 2009.

2. *Guidelines for the Treatment of Malaria, Second Edition.* Geneva, World Health Organization, 2010.

3. *Malaria Rapid Diagnostic Test Performance : Results of WHO product testing of malaria RDTs: Round 1 (2008).* Geneva, World Health Organization, 2009. ISBN 978 92 4 1598071

4. *WHO Technical Consultation on Parasitological Confirmation of Malaria Diagnosis. Report.* Geneva, World Health Organization, 2010. (Unpublished)

5. Kolaczinski, J., et al., Comparison of the OptiMAL rapid antigen test with field microscopy for the detection of *Plasmodium vivax* and *P. falciparum*: considerations for the application of the rapid test in Afghanistan. *Ann Trop Med Parasitol*, 2004. 98(1): p. 15-20.

6. Richter, J., et al., Co-reactivity of plasmodial histidine-rich protein 2 and aldolase on a combined immuno-chromographic-malaria dipstick (ICT) as a potential semi-quantitative marker of high *Plasmodium falciparum parasitaemia. Parasitol Res*, 2004. 94(5): p. 384-5.

7. Huong, N.M., et al., Comparison of three antigen detection methods for diagnosis and therapeutic monitoring of malaria: a field study from southern Vietnam. *Trop Med Int Health*, 2002. 7(4): p. 304-8.

8. Mason, D.P., et al., A comparison of two rapid field immunochromatographic tests to expert microscopy in the diagnosis of malaria. *Acta Trop*, 2002. 82(1): p. 51-9.

9. Van den Broek, I., et al., Evaluation of three rapid tests for diagnosis of *P. falciparum* and *P. vivax* malaria in Colombia. *Am J Trop Med Hyg*, 2006. 75(6): p. 1209-15.

10. McMorrow, M.L., et al., Challenges in routine implementation and quality control of rapid diagnostic tests for malaria--Rufiji District, Tanzania. *Am J Trop Med Hyg*, 2008. 79(3): p. 385-90.

11. Wanji, S., et al., Performance and usefulness of the Hexagon rapid diagnostic test in children with asymptomatic malaria living in the Mount Cameroon region. *Malar J*, 2008. 7: p. 89.

12. Willcox, M.L., et al., Rapid diagnostic tests for the home-based management of malaria, in a high-transmission area. *Ann Trop Med Parasitol*, 2009. 103(1): p. 3-16.

13. Belizario, V.Y., et al., Field evaluation of malaria rapid diagnostic tests for the diagnosis of *P. falciparum* and non-*P. falciparum* infections. *Southeast Asian J Trop Med Public Health*, 2005. 36(3): p. 552-61.

14 WHO-TDR-FIND-CDC. *Methods manual for product testing of malaria rapid diagnostic tests (Version Two).* Geneva, World Health Organization, 2009.

15. WHO-TDR-FIND. M*ethods Manual for Laboratory Quality Control Testing of Malaria Rapid Diagnostic Tests, Version Six.* Geneva, World Health Organization, 2010.

16. *Methods for Field Trials of Malaria Rapid Diagnostic Tests.* Manila, World Health Organization Regional Office for the Western Pacific, 2009.

17. Gamboa, D., M. F. Ho, et al. *A large proportion of P. falciparum isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. PLoS One*, 2010: 5(1): e8091.

18. Jorgensen, P., et al., Malaria rapid diagnostic tests in tropical climates: The need for a cool chain. *American Journal of Tropical Medicine and Hygiene*, 2006. 74(5).

19. Chiodini, P.L., et al., The heat stability of *Plasmodium* lactate dehydrogenase-based and histidine-rich protein 2-based malaria rapid diagnostic tests. *Trans R Soc Trop Med Hyg*, 2007. 101(4): p. 331-7.

20. Rennie, W., et al., Minimising human error in malaria rapid diagnosis: clarity of written instructions and health worker performance. *Trans R Soc Trop Med Hyg*, 2007. 101(1): p. 9-18.

21. Harvey, S.A., et al., *Improving community health worker use of malaria rapid diagnostic tests in Zambia: package instructions, job aid and job aid-plus-training. Malar J,* 2008. 7(1): p. 160.

22. Tavrow, P., E Knebel, L Cogswell, *Using quality design to improve malaria rapid diagnostic tests in Malawi, in Operations Research Results 1(4).* 2000, Published for the United States Agency for International Development (USAID) by the Quality Assurance Project (QAP): Bethesda, Maryland.

ANNEXES

Annex 1: Characteristics of malaria rapid diagnostic tests in the evaluation

Manufacturer	Product name	Catalogue number	Plasmodium species targeted (*F = P. falciparum V = P. vivax O = P. ovale M = P. malariae* P = PAN; major Plasmodium species)	Target antigen[a](s)	Sequence and type of bound antibody[b]				Required volume (µl) of whole blood	Buffer volume (drops)	Minimum time to results[c] (mins)	Maximum reading time (mins)	Results Interpretation[d] (Type A–I)	Format type[e]
					C	T1	T2	T3						
Access Bio, Inc.	CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	F, V	pLDH (Pv); HRP2	√	pLDH (P.v.)	HRP2		5	2	20	20	E	A
	CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	F,VOM	pLDH (VOM); HRP2	√	pLDH (VOM)	HRP2		5	2	20	20	H	A
	CareStart™ Malaria HRP2/pLDH Pf test	G0181	F	HRP2; pLDH (Pf)	√ √	HRP2 pLDH (P.f.)			5	2	20	20	A	A
Amgenix International, Inc.	OnSight™ - Malaria Pf Test	511-25-DB	F	HRP2	√	HRP2			5	6	20	20	A	A
	OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	F,V	pLDH (Pv); HRP2	√	pLDH (P.v.)	HRP2		5	4	20	20	E	
	OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	P	pLDH (pan)	√	pLDH (pan)			5	4	20	20	B	A
Bhat Bio-Tech India (P) Ltd	Maleriscan® Malaria Pf/Pv	MAT-50	F,VOM	pLDH (VOM); HRP2	√	pLDH (VOM)	HRP2		5	3	20	20	H	
Blue Cross Bio-Medical (Beijing) Co., Ltd	One Step Malaria P.f. Test (cassette)	522352	F	HRP2	√	HRP2			5	2-3.	15	20	A	A
CTK Biotech, Inc.	OnSite Pf Ag Rapid Test	R0114C	F	HRP2	√	HRP2			5	3	30	30	A	
	Onsite Pf/Pan Ag rapid test	R0113C	F, P	HRP2; pLDH (pan)	√	HRP2	pLDH (pan)		5	3	20	20	D	A
	Onsite Pf/Pv Ag rapid test	R0112C	F,V	HRP2; pLDH (Pv)	√	HRP2	pLDH (P.v.)		5	3	20	20	F	
Guangzhou Wondfo Biotech Co. Ltd.	One Step Malaria P.f. test	W37-C	F	HRP2	√	HRP2			5	3	15	30	A	A
HBI Co., Ltd.	HiSens Malaria Ag P.f/P.v Card	HR2823	F,P	pLDH (pan); pLDH (Pf)	√	pLDH (pan)	pLDH (P.f.)		5	2	20	20	C	
	HiSens Malaria Ag P.f./ P.v. (HRP2/pLDH) Card	HR2923	F,P	pLDH (pan); HRP2	√	pLDH (pan)	HRP2		5	2	20	20	C	A
	HiSens Malaria Ag P.f. HRP2 Card	HR3023	F	HRP2	√	HRP2			5	2	20	20	A	
Premier Medical Corporation Ltd.	First Response® Malaria pLDH/HRP2 Combo Test	I16FRC30	F,P	pLDH (pan); HRP2	√	pLDH (pan)	HRP2		5	2	20	20	C	A
	First Response® Malaria Ag pLDH	I12FRC30	P	pLDH (pan)	√	pLDH (pan)			5	2	20	20	B	
Span Diagnostics Ltd	ParaHIT® total (dipstick)	55IC201-10	F,P	pLDH (pan); aldolase; HRPP2	√ √	pLDH (pan & aldolase)	HRP2		8	4 (into Test Tube)	15	30	C	D
	ParaHIT® Pan M (dipstick)	55IC301-10	P	pLDH (pan); aldolase	√ √	pLDH (pan & aldolase)			8	4 (into Test Tube)	15	30	B	D

Manufacturer	Product name	Catalogue number	Plasmodium species targeted (*F = P. falciparum* *V = P. vivax* *O = P. ovale* *M = P. malariae* P = PAN; major Plasmodium species)	Target antigen[a](s)	Sequence and type of bound antibody[b] C	T1	T2	T3	Required volume (µl) of whole blood	Buffer volume (drops)	Minimum time to results[c] (mins)	Maximum reading time (mins)	Results Interpretation[d] (Type A–I)	Format type[e]
SSA Diagnostics & Biotech Systems	diagnosticks- Malaria (Pf) Cassette	KMFC6001	F	HRP2	√	HRP2			5	6	20	20	A	
	diagnosticks- Malaria (Pf) Dipstick	KMFD6007	F	HRP2	√	HRP2			5	4 (into test tube)	20	20	A	D
	diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	F,V	pLDH (Pv); HRP2	√	pLDH (P.v.)	HRP2		5	4	20	20	E	A
Standard Diagnostics, Inc.	SD BIOLINE Malaria Ag Pv	05FK70	V	pLDH (Pv)	√	pLDH (P.v.)			5	4	15	30	I	
	SD BIOLINE Malaria Ag Pf/Pv	05FK80	F,V	pLDH (Pv); HRP2	√	pLDH (P.v.)	HRP2		5	4	15	30	E	A
Unimed International Inc.	FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	P	pLDH (pan)	√	pLDH (pan)			5	4	20	20	B	
	FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	F,P	pLDH (pan); HRP2	√	pLDH (pan)	HRP2		5	4	20	20	C	A
	FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	F,V,P	pLDH (pan); pLDH (Pv); HRP2	√	pLDH (pan)	pLDH (P.v.)	HRP2	5	4	20	20	G	
Zephyr Biomedicals	Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	F,V	pLDH (Pv); HRP2	√	pLDH (P.v.)	HRP2		5	4	20	20	E	A
	Paramax-3 Rapid Test for Malaria Pan/ Pv/Pf (device)	50320025	F,V,P	pLDH(pan); pLDH (Pv); HRP2	√	pLDH (pan)	pLDH (P.v.)	HRP2	5	4	20	20	G	

a pLDH = plasmodium lactate dehydrogenase ; HRP = histidine rich protein

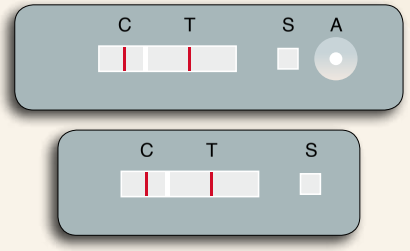
b Sequence when test held In a horizontal position and the sample well at far right and control line far left

c From placement of buffer, or from 'intermediate step' if this is present

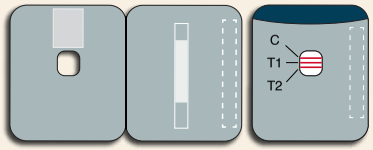
e see Annex 2

d Formats include: cassette (A), card (B), cassette hybrid (C), dipstick (D) or other. Each product should ideally be accompanied by all required materials (lancet, pipette etc.) particularly when used at the village health worker level; however, this is often not the case and the contents depend on the request of the procuring agent.

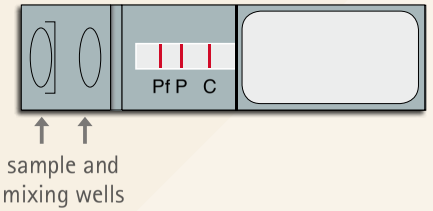
A Cassette



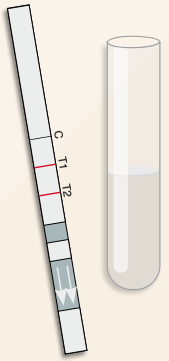
B Card



C Cassette hybrid



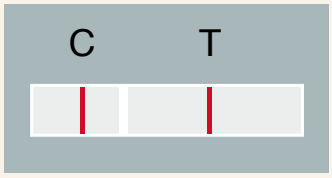
D Dipstick



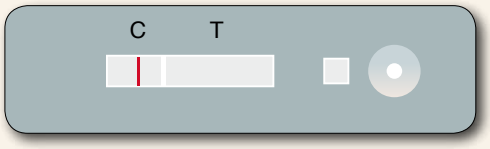
Annex 2: malaria RDT guide to results interpretation

Type A: Malaria Generic Pf RDT Results Guide

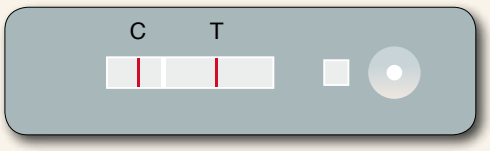
Results Window: C=control line; T=test line with bound HRP-2 or Pf-specific pLDH antibody.



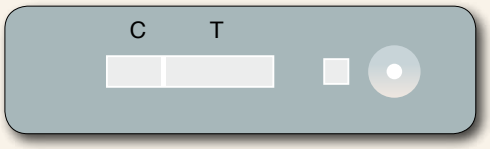
Negative Results: One line 'C' appears in the results window.

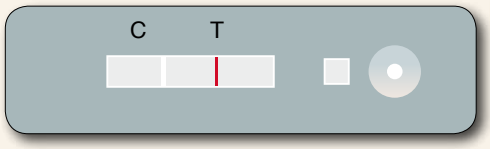


Positive Results: *P. falciparum* infection. Two lines 'C' and 'T' appear in the results window. Test is positive even if the test line is faint.



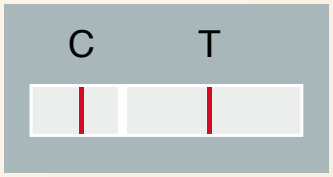
Invalid Results: No 'C' line appears in the results window. Repeat the test using a new RDT if no control line appears.



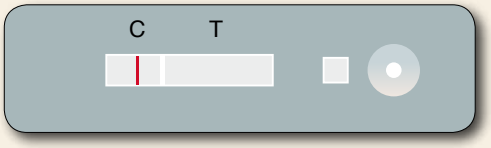


Type B: Malaria Generic Major Plasmodium species (pan) RDT Results Guide

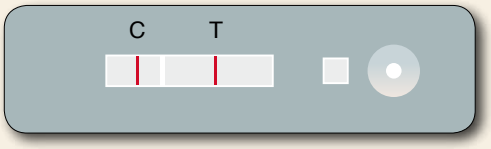
Results Window: C=control line; T=test line with bound pan-specific pLDH or aldolase antibody.



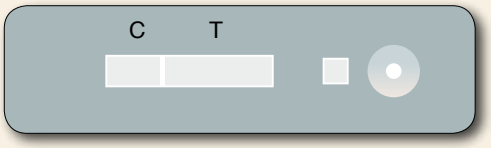
Negative Results: One line 'C' appears in the results window.

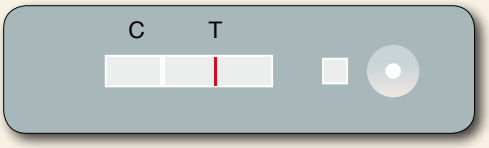


Positive Results: *Plasmodium* species (*P. falciparum*, *P. vivax*, *P.malariae*, *P.ovale*) infection. Two lines 'C' and 'T' appear in the results window. Test is positive even is the test line is faint.



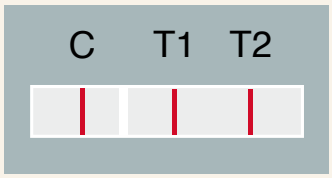
Invalid Results: No 'C' line appears in the results window. Repeat the test using a new RDT if no control line appears.



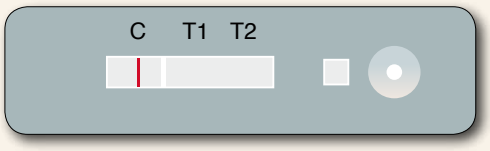


Type C: Malaria Generic Pan-Pf RDT Results Guide

Results Window: C=control line; T1=test line with bound pLDH or aldolase antibody; T2=test line with bound HRP2 or Pf specific pLDH antibody.

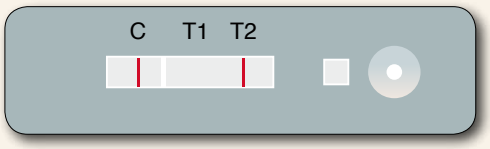


Negative Results: Only one line 'C' appears in the results window.

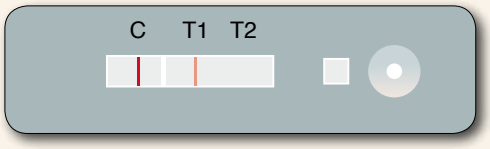


Positive Results:

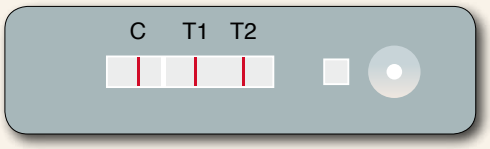
P. falciparum: Two lines 'C' and 'T2" appear in the results window.



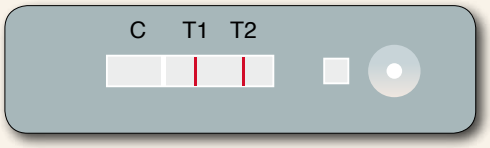
Non-falciparum infection (*P. vivax, P.ovale, P.malariae*) or mixed infection of these:
Two lines 'C' and 'T1" appear in the results window.

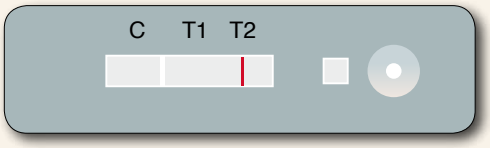


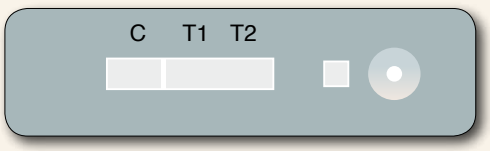
P. falciparum or mixed infection. Three lines 'C', 'T1' and 'T2' appear in the results window.

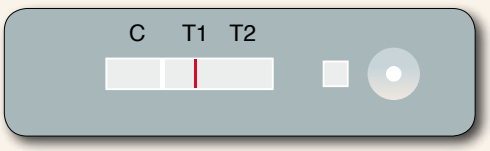


Invalid Results: No 'C' line appears in the results window. Repeat the test using a new RDT if no control line appears.



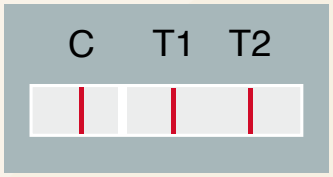




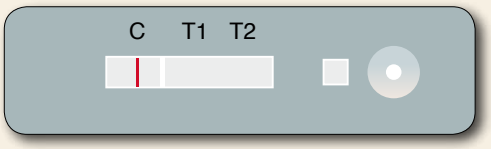


Type D: Malaria Generic Pf-Pan RDT Results Guide

Results Window: C=control line; T1=test line with bound HRP2 or Pf specific LDH antibody;
T2=test line with bound pLDH or aldolase antibody.

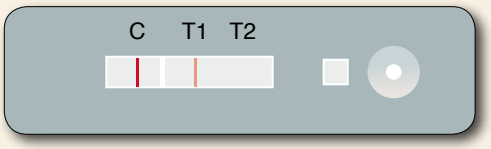


Negative Results: Only one line 'C' appears in the results window.

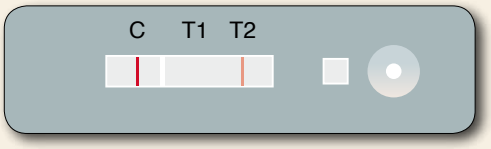


Positive Results:

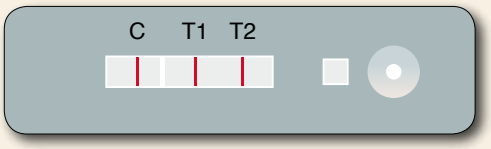
P. falciparum infection. Two lines 'C' and 'T1' appear in the results window.



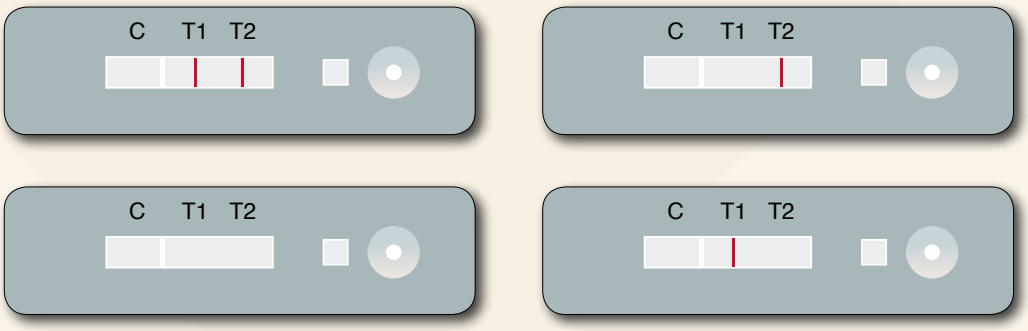
Non-falciparum infection (*P. vivax, P.ovale, P.malariae*) or mixed infection of these.
Two lines 'C' and 'T2' appear in the results window.



P. falciparum or mixed infection. Three lines 'C', 'T1' and 'T2' appear in the results window.

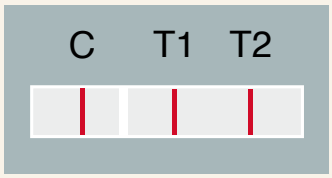


Invalid Results: No 'C' line appears in the results window. Repeat the test using a new RDT if no control line appears.

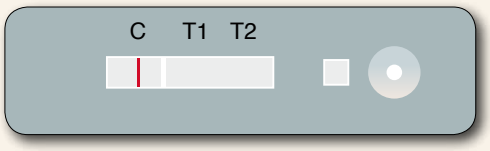


Type E: Malaria Generic Pv-Pf RDT Results Guide

Results Window: C=control line; T1=test line with bound *P. vivax* specific pLDH;
T2=test line with bound HRP2 or Pf-specific pLDH antibody.

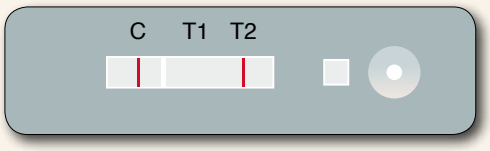


Negative Results: Only one line 'C' appears in the results window.

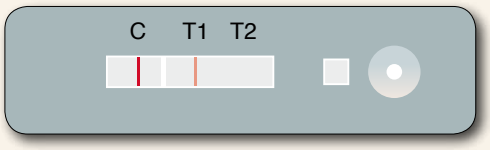


Positive Results:

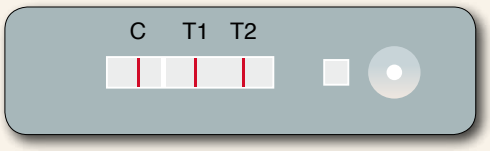
P. falciparum infection. Two lines 'C' and 'T2' appear in the results window.



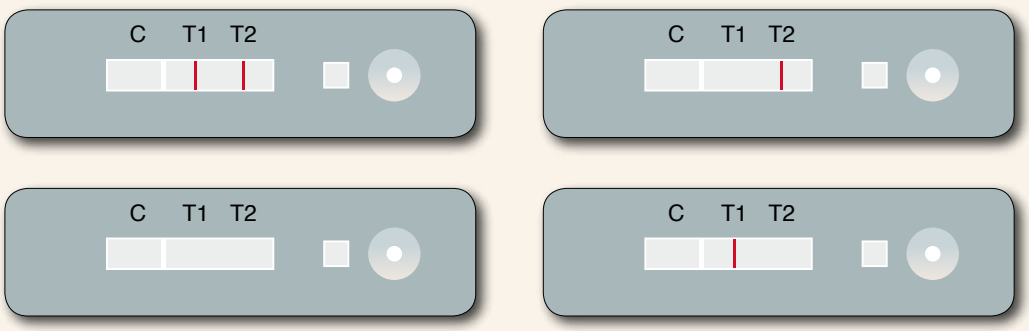
P. vivax infection. Two lines 'C' and 'T1' appear in the results window.



P. falciparum and *P. vivax* mixed infection. Three lines 'C', 'T1' and 'T2' appear in the results window.

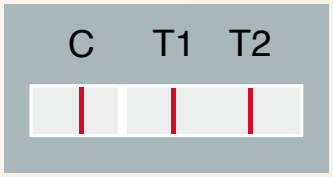


Invalid Results: No 'C' line appears in the results window. Repeat the test using a new RDT if no control line appears.

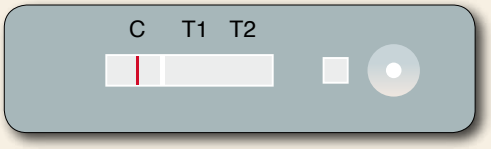


Type F: Malaria Generic Pf-Pv RDT Results Guide

Results Window: C=control line; T1= test line with bound HRP2 or Pf-specific pLDH antibody;
T2=test line with bound *P. vivax* specific pLDH.

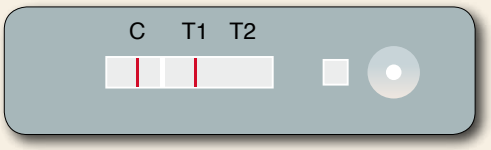


Negative Results: Only one line 'C' appears in the results window.

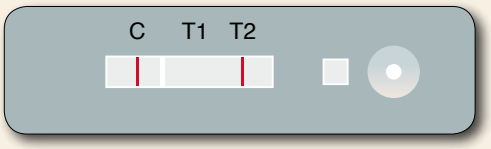


Positive Results:

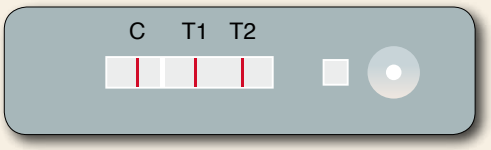
P. falciparum infection. Two lines 'C' and 'T1' appear in the results window.



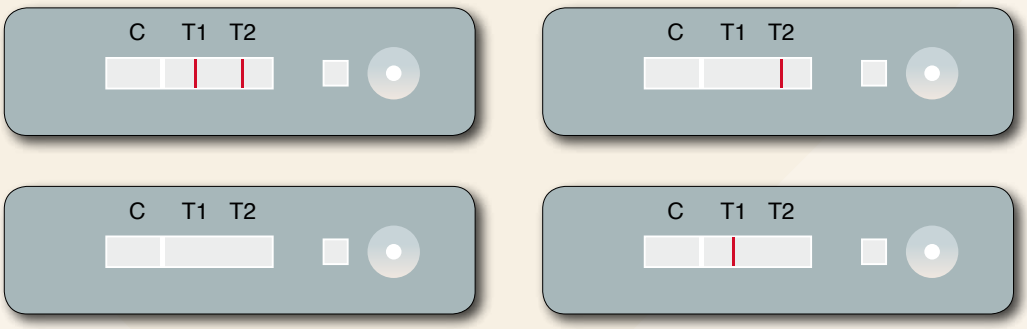
P. vivax infection. Two lines 'C' and 'T2' appear in the results window.



P. falciparum and *P. vivax* mixed infection. Three lines 'C', 'T1' and 'T2' appear in the results window.

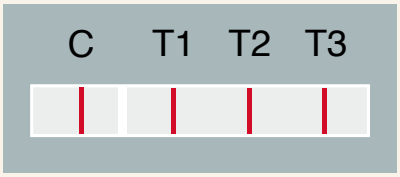


Invalid Results: No 'C' line appears in the results window. Repeat the test using a new RDT if no control line appears.

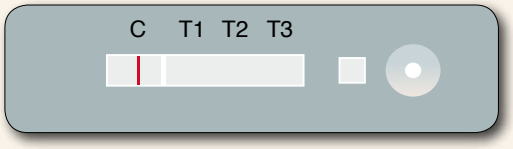


Type G: Malaria Generic Pan-Pv-Pf RDT Results Guide

Results Window: C=control line; T1=test line bound with pLDH or aldolase antibody; T2=test line with bound *P. vivax* specific pLDH; T3=test line with bound HRP2 or Pf-specific pLDH antibody

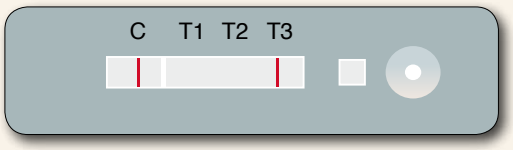


Negative Results: Only one line 'C' appears in the results window.

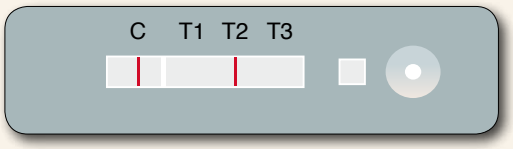


Positive Results:

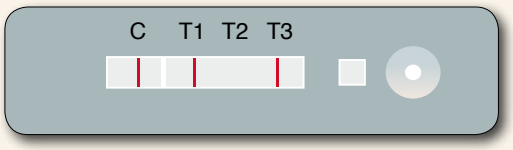
P. falciparum infection. Two lines 'C' and 'T3' appear in the results window.



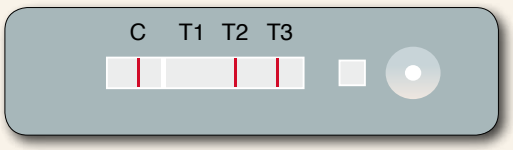
P. vivax infection. Two lines 'C' and 'T2' appear in the results window.



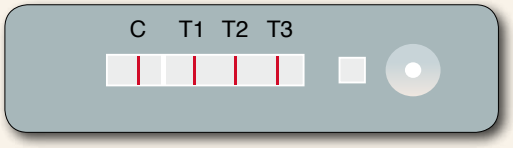
P. falciparum with or without mixed infection with *P. ovale* or *P. malariae*. Three lines 'C', 'T1' and 'T3' appear in the results window.



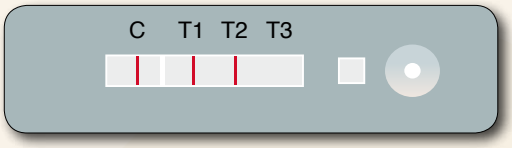
P. falciparum and *P. vivax* mixed infection. Three lines 'C', 'T2' and 'T3' appear in the results window.



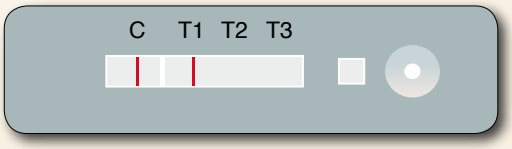
P. falciparum and *P. vivax* mixed infection with or without *P. ovale* and/or *P. malariae* infection. Four lines 'C', 'T1', 'T2' and 'T3' appear in the results window.



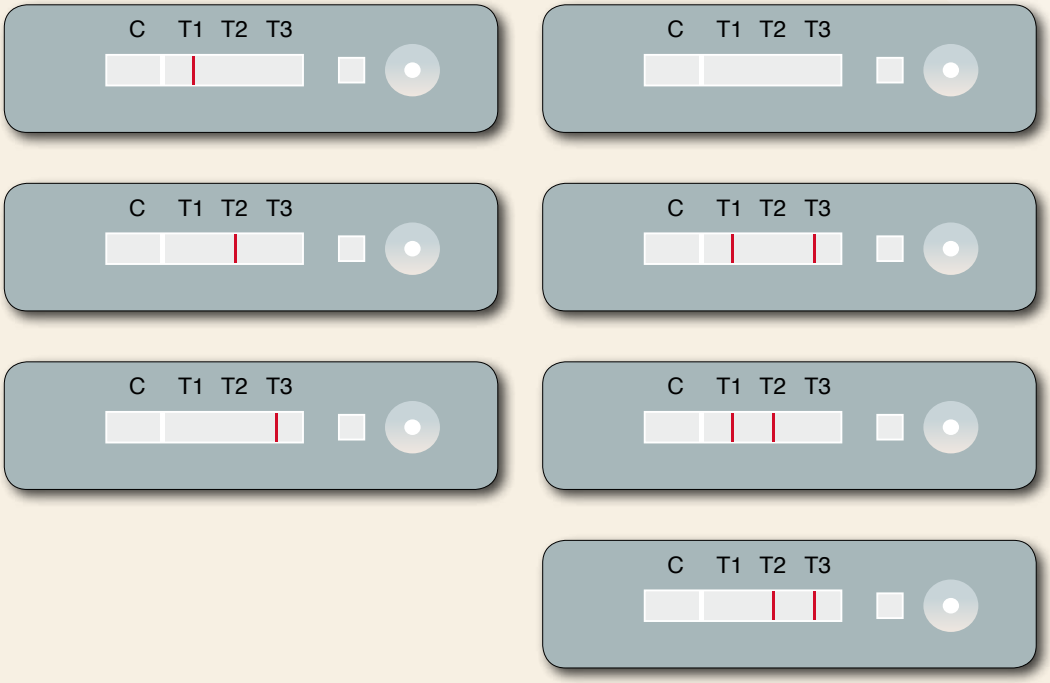
P. vivax with or without *P. ovale* and/or *P. malariae* infection. Three lines 'C', 'T1' and 'T2' appear in the results window.



P. malariae and/or *P. ovale P. vivax* infection. Two lines 'C' and 'T1' appear in the results window.

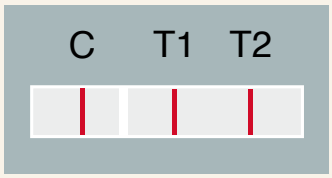


Invalid Results: No 'C' line appears in the results window. Repeat the test using a new RDT if no control line appears.

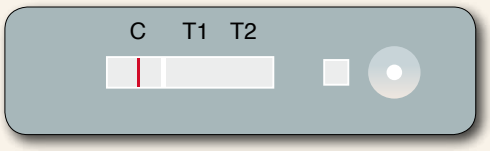


Type H: Malaria Generic VOM[1]-Pf RDT Results Guide

Results Window: C=control line; T1= test line bound with pLDH specific for non. *P. falciparum* (*P. vivax, P. ovale* and *P. malariae*); T2=test line with bound HRP2 or Pf-specific pLDH antibody

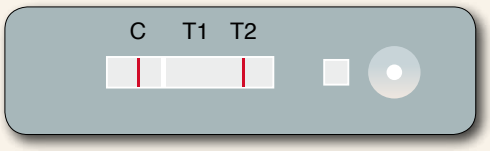


Negative Results: Only one line 'C' appears in the results window.

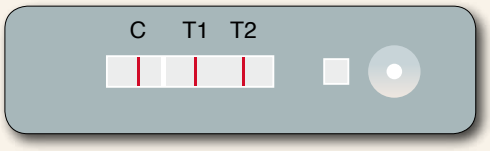


Positive Results:

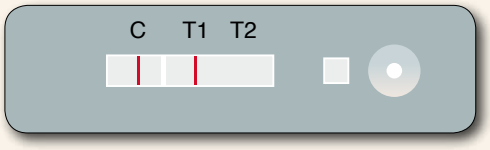
P. falciparum infection. Two lines 'C' and 'T2' appear in the results window.



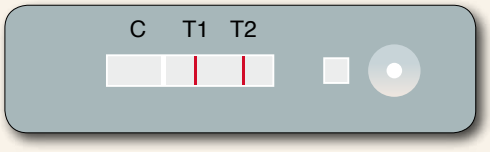
P. falciparum mixed infection (with anyone or more of *P. vivax, P. ovale* and *P. malariae*). Three lines 'C', 'T1' and 'T2' appear in the results window.

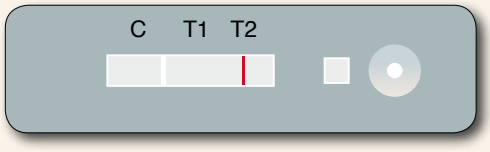


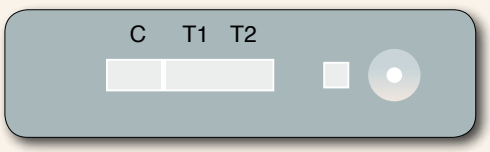
Non-*P. falciparum* infection (*P. vivax, P. ovale* and *P. malariae*) or mixed infection of these. Two lines 'C' and 'T1' appear in the results window.

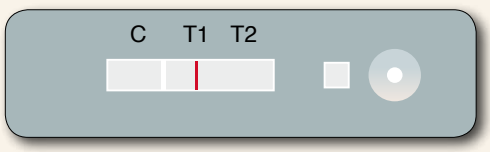


Invalid Results: No 'C' line appears in the results window. Repeat the test using a new RDT if no control line appears.





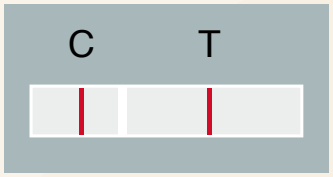




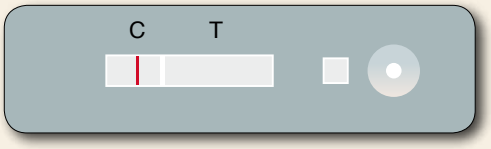
[1] VOM -*P. vivax, P. ovale, P. malariae*

Type I: Malaria Generic Pv RDT Results Guide

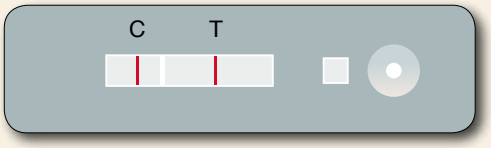
Results Window: C=control line; T=test line bound with *P. vivax* specific pLDH.



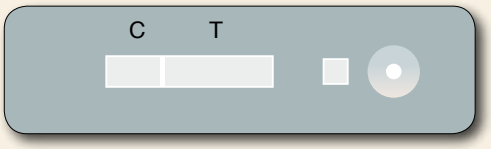
Negative Results: Only one line 'C' appears in the results window.

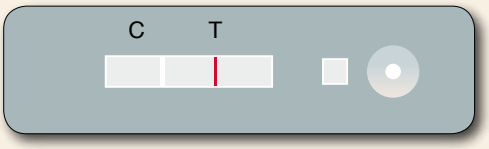


Positive Results: *P. vivax* infection. Two lines 'C' and 'T' appear in the results window.



Invalid Results: No 'C' line appears in the results window. Repeat the test using a new RDT if no control line appears.





Annex 3: Phase 1 results

TableA3.1: Lot variability in positive results[a] against *P. falciparum* culture samples at low (200) and high (2000) parasite density (parasites/µl)

Product	Catalogue number	Manufacturer	*P. falciparum* samples (n=20) Total positive results returned							
			200 parasites/µl						2000 parasites/µl	
			Lot 1			Lot 2			Lot 1	Lot 2
			Test 1	Test 2	No. positive agreements[b] (max=20)	Test 1	Test 2	No. positive agreements[b] (max=20)	Test 1	Test 2
Pf only										
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	20	20	20	20	20	20	20	20
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	14 (19)	16	14 (19)	14	9	9	20	20
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	18	17	17	17	16	16	20	20
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	20	20	20	20	20	20	20	20
One Step Malaria P.f. Test	W37-C	Guangzhou Wondfo Biotech Co. Ltd.	1 (8)	3 (9)	1 (3)	4 (17)	7 (17)	7 (17)	8	13
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	8 (17)	9 (16)	6 (14)	3 (19)	3 (18)	3 (18)	12	15
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	17	16	15	16	15	15	20	20
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	8	6	6	9	10	10	20	20
Pf and Pan										
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	20	19	19	19	18	18	20	20
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	19	15	15	19	18	18	20	20
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0	1	0	7	7	7	20	20
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	20	20	20	20	20	20	20	20
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	7	7	6	10	11	11	19	20
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	15	13	13	16	15	15	20	20
Pf and Pv										
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	18	19	17	18	17	17	20	20
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	17	20	17	16	17	17	20	20
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	20	19	19	18	19	19	20	20
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	19	20	19	19	18	18	20	20
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	15	14	13	12	16	16	19	20
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	19 (19)	20	19 (19)	19	19	19	20	20
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	5	7	5	7	9	9	20	20
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	20	20	20	19	18	18	20	20
Pf, Pv and Pan										
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	18	18 (19)	16 (19)	18	18	18	20	20
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	20	17 (19)	17 (19)	19	19	19	20	20
Pan only										
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	11	13	7	8	7	7	20	20
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	3	1	0	1	2	2	20	20
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	20	18	18	2	3	3	20	19
ParaHIT Pan M (dipstick)	55IC301-10	Span Diagnostics Ltd	3	3	2	3	5	5	11	16
Pv only										
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] Results are based on the first readers interpretation according to manufacturers instructions.

[b] Number of samples that returned a positive result for both tests. Where one test was invalid and the other positive, positive agreement was recorded.

Table A3.2: Distribution of test band intensity (0-4) scores against Phase 1 *P. falciparum* cultured parasites at low (200) and high (2000) parasite densities (parasites/µl)

Product	Catalogue number	Manufacturer	200 parasites/µl					2000 parasites/µl					200 parasites/µl					2000 parasites/µl				
			Percentage distribution of Pf test band intensity (n=80)					Percentage distribution of Pf test band intensity (n=40)					Percentage distribution of Pan test band intensity (n=80)					Percentage distribution of Pan test band intensity (n=40)				
			0[a]	1	2	3	4	0[a]	1	2	3	4	0[a]	1	2	3	4	0[a]	1	2	3	4
Pf only																						
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	0.0	22.5	45.0	27.5	5.0	0.0	2.5	5.0	20.0	72.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	33.8	36.3	18.8	7.5	3.8	0.0	5.0	7.5	30.0	57.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	15.0	37.5	35.0	11.3	1.3	0.0	2.5	17.5	45.0	35.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	0.0	13.8	43.8	31.3	11.3	0.0	0.0	5.0	17.5	77.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
One Step Malaria P.f. Test	W37-C	Guangzhou Wondfo Biotech Co. Ltd.	81.3	15.0	3.8	0.0	0.0	47.5	10.0	25.0	12.5	5.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
One Step Malaria Pf Test (cassette)	522352	BlueCrossBio-Medical(Beijing)Co.,Ltd	71.3	20.0	3.8	3.8	1.3	32.5	7.5	17.5	17.5	25.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	20.0	16.3	36.3	20.0	7.5	0.0	2.5	5.0	7.5	85.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	58.8	31.3	10.0	0.0	0.0	0.0	10.0	35.0	40.0	15.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pf and Pan																						
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	5.0	25.0	45.0	25.0	0.0	0.0	0.0	5.0	35.0	60.0	22.5	73.8	3.8	0.0	0.0	0.0	5.0	67.5	27.5	0.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	11.3	23.8	37.5	20.0	7.5	0.0	0.0	12.5	12.5	75.0	47.5	52.5	0.0	0.0	0.0	0.0	65.0	32.5	2.5	0.0
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	81.3	18.8	0.0	0.0	0.0	0.0	40.0	55.0	5.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	0.0	12.5	42.5	38.8	6.3	0.0	0.0	5.0	17.5	77.5	0.0	67.5	32.5	0.0	0.0	0.0	2.5	22.5	62.5	12.5
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	56.3	35.0	8.8	0.0	0.0	2.5	10.0	35.0	40.0	12.5	100.0	0.0	0.0	0.0	0.0	97.5	2.5	0.0	0.0	0.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	26.3	50.0	21.3	2.5	0.0	0.0	7.5	22.5	32.5	37.5	77.5	21.3	1.3	0.0	0.0	15.0	77.5	7.5	0.0	0.0
Pf and Pv																						
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	10.0	38.8	37.5	11.3	2.5	0.0	0.0	15.0	42.5	42.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	12.5	33.8	41.3	12.5	0.0	0.0	0.0	10.0	27.5	62.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	5.0	17.5	28.8	42.5	6.3	0.0	0.0	2.5	20.0	77.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	5.0	21.3	30.0	35.0	8.8	0.0	0.0	5.0	20.0	75.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	28.8	45.0	20.0	6.3	0.0	2.5	7.5	15.0	47.5	27.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	3.8	12.5	15.0	58.8	10.0	0.0	0.0	0.0	15.0	85.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	65.0	23.8	11.3	0.0	0.0	0.0	12.5	45.0	20.0	22.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	3.8	21.3	38.8	27.5	8.8	0.0	0.0	2.5	25.0	72.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pf, Pv and Pan																						
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	10.0	26.3	42.5	16.3	5.0	0.0	0.0	2.5	37.5	60.0	56.3	43.8	0.0	0.0	0.0	12.5	72.5	15.0	0.0	0.0
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025		6.3	21.3	21.3	38.8	12.5	0.0	2.5	0.0	15.0	82.5	53.8	43.8	2.5	0.0	0.0	5.0	52.5	35.0	7.5	0.0
Pan only																						
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	51.3	47.5	1.3	0.0	0.0	0.0	7.5	77.5	15.0	0.0
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	91.3	8.8	0.0	0.0	0.0	0.0	40.0	47.5	12.5	0.0
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	46.3	13.8	17.5	15.0	7.5	0.0	2.5	22.5	30.0	45.0
ParaHIT Pan M (dipstick)	55IC301-10	Span Diagnostics Ltd	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	82.5	17.5	0.0	0.0	0.0	32.5	55.0	10.0	0.0	2.5
Pv only																						
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species* - N/A: not applicable

a Denotes no band visible

Annex 4: Phase 2 results

TableA4.1: Lot variability in positive results against Pase 2 wild type *P. falciparum* and *P. vivax* samples at low (200) and high (2000) parasite density (parasites/µl)

Product	Catalogue number	Manufacturer	*P. falciparum* samples (n=100) Total positive results[a] returned								*P. vivax* samples (n=40) Total positive results[a] returned							
			200 parasites/µl						2000[b] parasites/µl		200 parasites/µl						2000[b] parasites/µl	
			Lot 1			Lot 2			Lot 1	Lot 2	Lot 1			Lot 2			Lot 1	Lot 2
			Test 1	Test 2	No. positive agreements[c] (max=100)	Test 1	Test 2	No. positive agreements[c] (max=100)	Test 1	Test 2	Test 1	Test 2	No. positive agreements[c] (max=100)	Test 1	Test 2	No. positive agreements[c] (max=100)	Test 1	Test 2
Pf only																		
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	99	99	98	99	100	99	100	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	71 (99)	70 (99)	62 (98)	79	76	70	97 (98)	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	90	86	84	92	87	84	99	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	95	94	92	92	95	91	100	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	50 (96)	52 (97)	43 (93)	45 (97)	49 (94)	40 (92)	93 (96)	83 (88)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	83	82	77	81	86	79	99	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	70	67	62	73	68	64	100	99	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pf and Pan																		
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	89	91	86	94	92	90	100	100	34	35	34	32	36	31	40	40
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	95	93	92	93	91	88	99 (99)	99	34	38	34	36 (39)	35	35 (39)	40	40
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	38	37	28	37	40	28	96	96	12	11	7	20	15	13	40	40
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	90	90	88	91	90	87	100	99	34	34	30	39	38	38	40	40
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	72	75	68	74	75	70	100	98	18	16	11	17	16	13	40	40
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	71	68	66	80	78	75	99	100	14	16	11	12	9	4	39	40
Pf and Pv																		
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	93	91	90	98	92	92	100	100	38	34	33	38	39	38	40	40
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	97	92	91	98	93	93	100	100	38	38	36	39	39	39	40	40
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	96	94 (99)	92 (99)	96	96	95	99	100	29	30	26	23	24	21	40	40
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	97	98	95	96	96	94	100	100	27	24	21	29	28	27	40	39 (39)
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	69	70	59	78	69 (99)	66 (99)	99	98	1	0	0	0	0	0	28	29 (39)
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	98	99	97	95	95	93	100	100	25	28	23	23	21	17	40	40
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	73	71	66	74	71	66	100	99	35	34	31	39	38	37	40	40
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	98	99	97	98	97	97	100	99 (99)	39	39	39	37 (39)	39	37 (39)	40	40
Pf, Pv and Pan																		
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	95	95	93	96	94 (99)	92 (99)	100	100	29	27	24	27	29	24	40	40
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	96	96 (99)	94 (99)	97	92 (97)	91 (97)	100	99 (99)	28	27	22	27 (39)	31	24 (39)	40	40
Pan only																		
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	59	51	41	55	53	40	99	98	40	39	39	38	39	38	40	40
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	36	36 (98)	29 (98)	33	35	28	97	97	36	38	36	36	38	35	40	40
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	41 (99)	45	34 (99)	37	33	24	96	97	36	34	34	37	34	33	40	40
Pv only																		
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	39	38	38	40	38	38	40	40

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] Results are based on the first readers interpretation according to manufacturers instructions.

[b] 6 (6%) of the 100 *P. falciparum* dilution samples sets were 200 and 5000 parasites/µl and 2 (5%) of the 40 *P. vivax* dilution sample sets were 200 and 5000 parasites/µl

[c] Number of samples that returned a positive result for both tests. Where one test was invalid and the other positive, positive agreement was recorded.

Table A4.2: Distribution of test band intensity (0-4) scores against Phase 2 wild type *P. falciparum* samples (n=100) at low (200) and high (2000) parasite densities (parasites/µl)

Product	Catalogue number	Manufacturer	200 parasites/µl Percentage distribution of Pf test band intensity (n=400)					2000[b] parasites/µl Percentage distribution of Pf test band intensity (n=200)					200 parasites/µl Percentage distribution of pan test band intensity (n=400)					2000[b] parasites/µl Percentage distribution of pan test band intensity (n=200)				
			0[a]	1	2	3	4	0[a]	1	2	3	4	0[a]	1	2	3	4	0[a]	1	2	3	4
Pf only																						
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	0.8	6.8	33.0	33.3	26.3	0.0	0.0	2.0	13.0	85.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	25.8	16.5	25.0	18.5	14.3	1.0	3.0	10.0	20.0	66.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	11.3	18.3	28.3	23.0	19.3	0.5	0.0	4.5	16.5	78.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	6.0	13.3	39.0	27.0	14.8	0.0	0.5	4.5	22.5	72.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	51.0	17.3	18.0	10.0	3.8	10.0	4.0	18.5	17.0	50.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	17.0	12.5	26.0	20.0	24.5	0.5	0.0	13.0	12.0	74.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	30.5	21.5	31.0	12.5	4.5	0.5	2.0	16.5	27.0	54.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pf and Pan																						
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	8.5	16.3	33.5	29.0	12.8	0.0	1.0	5.0	20.5	73.5	25.0	44.8	28.3	1.8	0.3	0.5	3.5	41.0	36.0	19.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	7.0	12.0	20.3	30.5	30.3	0.5	0.5	4.5	10.5	84.0	55.3	32.0	11.8	0.8	0.3	4.0	10.5	36.0	34.5	15.0
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	62.0	29.3	8.5	0.3	0.0	4.0	18.0	41.0	21.0	16.0	99.8	0.3	0.0	0.0	0.0	81.5	14.5	4.0	0.0	0.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	9.8	18.8	32.0	27.3	12.3	0.5	1.5	5.5	18.5	74.0	18.5	44.3	33.8	3.5	0.0	1.0	2.5	27.0	42.0	27.5
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	26.0	24.0	28.5	15.8	5.8	1.0	2.5	13.5	30.0	53.0	97.3	1.8	1.0	0.0	0.0	37.0	28.5	28.5	6.0	0.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	25.8	24.8	21.8	14.3	13.5	0.5	3.0	8.0	20.5	68.0	88.5	10.3	1.3	0.0	0.0	35.0	30.5	22.0	12.0	0.5
Pf and Pv																						
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	6.5	15.5	41.3	24.3	12.5	0.0	0.5	6.0	19.5	74.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	5.0	17.0	36.0	26.0	16.0	0.0	0.5	5.0	19.0	75.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	4.5	11.3	30.8	29.0	24.5	0.5	0.5	3.0	24.5	71.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	3.3	6.0	34.8	31.8	24.3	0.0	0.5	3.0	16.0	80.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	28.5	24.0	37.3	7.8	2.5	1.5	3.5	33.5	32.5	29.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	3.3	9.0	23.5	24.0	40.3	0.0	0.0	3.0	12.5	84.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	27.8	19.8	31.5	15.3	5.8	0.5	2.0	16.0	28.5	53.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	2.0	9.8	33.5	29.0	25.8	0.5	0.0	2.5	12.0	85.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pf, Pv and Pan																						
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	5.0	9.3	25.3	31.3	29.3	0.0	1.0	3.5	12.5	83.0	56.0	31.3	11.8	0.8	0.3	6.5	15.0	45.0	26.0	7.5
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	4.5	7.0	38.3	29.3	21.0	0.5	1.0	2.5	17.0	79.0	43.0	40.3	16.0	0.8	0.0	7.5	8.5	60.0	20.0	4.0
Pan only																						
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	45.5	36.5	15.8	2.3	0.0	1.5	7.5	33.0	35.0	23.0
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	65.0	20.3	12.5	1.3	1.0	3.0	7.0	24.0	35.0	31.0
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	61.0	22.8	13.5	2.5	0.3	3.5	6.0	26.0	32.5	32.0
Pv only																						
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] Denotes no visible band

[b] 6 (6%) of the 100 *P. falciparum* dilution samples sets were 200 and 5000 parasites/µl

Table A4.3: Distribution of Pan/Pv test band intensity (0–4) scores for Phase 2 wild type *P. vivax* samples (n=40) at low (200) and high (2000) parasite densities (parasites/µl)

Product	Catalogue number	Manufacturer	200 parasites/µl Percentage distribution of pan or Pv test band intensity (n=160)					2000[b] parasites/µl Percentage distribution of pan or Pv test band intensity (n=80)				
			0[a]	1	2	3	4	0[a]	1	2	3	4
Pf only												
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pf and Pan												
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	14.4	42.5	41.9	1.3	0.0	0.0	1.3	13.8	42.5	42.5
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	10.0	26.3	48.1	11.9	3.8	0.0	1.3	3.8	13.8	81.3
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	63.8	27.5	8.1	0.6	0.0	0.0	6.3	36.3	30.0	27.5
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	9.4	35.6	52.5	2.5	0.0	0.0	0.0	13.8	38.8	47.5
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	58.1	35.6	5.0	1.3	0.0	0.0	2.5	40.0	41.3	16.3
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	68.1	26.3	5.6	0.0	0.0	1.3	5.0	36.3	37.5	20.0
Pf and Pv												
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	6.9	17.5	65.0	8.8	1.9	0.0	0.0	1.3	20.0	78.8
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	3.8	28.8	59.4	6.9	1.3	0.0	0.0	3.8	28.8	67.5
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	33.8	36.9	26.9	2.5	0.0	0.0	1.3	8.8	32.5	57.5
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	32.5	23.8	41.9	1.9	0.0	0.0	2.5	12.5	37.5	47.5
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	99.4	0.6	0.0	0.0	0.0	28.8	40.0	31.3	0.0	0.0
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	39.4	30.6	27.5	2.5	0.0	0.0	1.3	7.5	36.3	55.0
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	8.8	34.4	48.8	8.1	0.0	0.0	0.0	7.5	38.8	53.8
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	3.8	11.9	56.3	25.6	2.5	0.0	0.0	0.0	16.3	83.8
Pf, Pv and Pan												
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test[c]	2103 CB-25	Unimed International Inc.	30.0	47.5	22.5	0.0	0.0	1.3	6.3	50.0	35.0	7.5
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test[d]	2103 CB-25	Unimed International Inc.	30.0	37.5	30.6	1.9	0.0	0.0	1.3	8.8	42.5	47.5
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)[c]	50320025	Zephyr Biomedicals	27.5	31.3	41.3	0.0	0.0	1.3	2.5	72.5	20.0	3.8
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)[d]	50320025	Zephyr Biomedicals	29.4	28.1	40.0	2.5	0.0	0.0	0.0	17.5	55.0	27.5
Pan only												
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	2.5	19.4	56.3	21.9	0.0	0.0	0.0	2.5	30.0	67.5
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	7.5	25.6	44.4	18.1	4.4	0.0	0.0	1.3	17.5	81.3
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	11.9	26.3	45.0	15.0	1.9	0.0	0.0	5.0	8.8	86.3
Pv only++												
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	3.1	11.3	54.4	27.5	3.8	0.0	0.0	0.0	16.3	83.8

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] Denotes no visible band

[b] 2 (5%) of the 40 *P. vivax* dilution sample sets were 200 and 5000 parasites/µl

[c] Pan test line

[d] *P.vivax* test line

TableA4.4: Panel detection score of Phase 2 wild type *P. falciparum* at low (200) and high (2000) parasite densities (parasites/µl) by continent

Product	Catalogue number	Manufacturer	200 parasites/µl Panel detection score[a] by continent of sample origin			2000[b] parasites/µl Panel detection score[a] by continent of sample origin		
			Africa (n=62)	Asia (n=24)	South America (n=14)	Africa (n=62)	Asia (n=24)	South America (n=14)
Pf only								
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	96.8	100.0	100.0	100.0	100.0	100.0
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	62.9	54.2	50.0	100.0	100.0	92.9
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	77.4	87.5	78.6	100.0	100.0	92.9
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	82.3	95.8	92.9	100.0	100.0	100.0
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	41.9	29.2	28.6	93.6	95.8	92.9
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	75.8	75.0	64.3	98.4	100.0	100.0
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	59.7	54.2	64.3	98.4	100.0	100.0
Pf and Pan								
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	77.4	95.8	92.9	100.0	100.0	100.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	80.7	95.8	85.7	100.0	100.0	92.9
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	27.4	8.3	7.1	91.9	100.0	92.9
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	80.7	95.8	78.6	100.0	100.0	92.9
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	59.7	66.7	71.4	96.8	100.0	100.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	61.3	75.0	57.1	98.4	100.0	100.0
Pf and Pv								
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	83.9	100.0	92.9	100.0	100.0	100.0
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	85.5	100.0	92.9	100.0	100.0	100.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	87.1	95.8	100.0	100.0	95.8	100.0
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	88.7	100.0	92.9	100.0	100.0	100.0
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	58.1	45.8	35.7	98.4	100.0	85.7
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	88.7	95.8	100.0	100.0	100.0	100.0
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	61.3	58.3	64.3	98.4	100.0	100.0
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	95.2	100.0	92.9	100.0	100.0	100.0
Pf, Pv and Pan								
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	85.5	95.8	92.9	100.0	100.0	100.0
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	91.9	95.8	92.9	100.0	100.0	100.0
Pan only								
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	41.9	12.5	14.3	98.4	100.0	92.9
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	35.5	12.5	0.0	96.8	100.0	92.9
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	32.3	8.3	0.0	95.2	100.0	92.9
Pv only								
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A

Africa - United Republic of Tanzania, Central African Republic, Madagascar, Nigeria, Kenya

Asia - Myanmar, The Philippines, Cambodia

South America - Peru, Colombia

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive

[b] 6 (6%) of the 100 *P. falciparum* dilution samples sets were 200 and 5000 parasites/µl

Table A4.5: ***P. falciparum*** **test line false positive rates for Phase 2** ***P. vivax*** **samples (n=40) at low (200) and high (2000) parasite densities (parasites/µl)**

Product	Catalogue number	Manufacturer	*P. vivax* samples (n=40)					
			200 parasites/µl False positive Pf infection[b] (%)			2000[a] parasites/µl False positive Pf infection[b] (%)		
			Lot 1 (n=80)	Lot 2 (n=80)	Overall (n=160)	Lot 1 (n=40)	Lot 2 (n=40)	Overall (n=80)
Pf only								
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	0.0	1.3	0.6	2.5	0.0	1.3
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	2.5	1.3	1.9	5.4 (37)	0.0	2.6 (77)
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	5.0	0.0	2.5	5.0	2.5	3.8
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	2.5 (79)	0.0 (74)	1.31 (153)	0.0 (39)	0.0 (38)	0.0 (77)
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	12.5	3.8	8.1	2.5	2.5	2.5
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	0.0
Pf and Pan								
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	0.0	0.0	0.0	0.0	0.0	0.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	0.0	1.3 (79)	0.6 (159)	0.0	0.0	0.0
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	0.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	0.0	0.0	0.0	0.0	0.0	0.0
Pf and Pv								
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	0.0	0.0	0.0	0.0	0.0	0.0
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	1.3	0.0	0.6	0.0	0.0	0.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	1.3	0.0	0.6	0.0	0.0	0.0
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	2.5	0.0	1.3	0.0	0.0 (39)	0.0 (79)
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	3.8	1.3	2.5	2.5	2.5 (39)	2.5 (79)
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	2.5	1.3	1.9	0.0	0.0	0.0
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	0.0
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	0.0	0.0 (79)	0.0 (159)	0.0	0.0	0.0
Pf, Pv and Pan								
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	3.8	1.3	2.5	0.0	0.0	0.0
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	0.0	0.0 (79)	0.0 (159)	0.0	0.0	0.0
Pan only								
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	N/A	N/A	N/A	N/A	N/A	N/A
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	N/A	N/A	N/A	N/A	N/A	N/A
Pv only								
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] 2 (5%) of the 40 *P. vivax* dilution sample sets were 200 and 5000 parasites/µl

[b] Pf line positive indicates a false positive *P. falciparum* infection

Table A4.6: Pan (or Pv) test line false positive rate for non-Pf infection on Phase 2 *P. falciparum* samples (n=100) at low (200) and high (2000) parasite densities (parasites/µl)

Product	Catalogue number	Manufacturer	*P. falciparum* samples (n=100)					
			200 parasites/µl False positive non-Pf infection (%)			2000[a] parasites/µl False positive non-Pf infection (%)		
			Lot 1 (n=200)	Lot 2 (n=200)	Overall (n=400)	Lot 1 (n=100)	Lot 2 (n=100)	Overall (n=200)
Pf only								
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	N/A	N/A	N/A	N/A	N/A	N/A
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	N/A	N/A	N/A	N/A	N/A	N/A
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	N/A	N/A	N/A	N/A	N/A	N/A
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	N/A	N/A	N/A	N/A	N/A	N/A
Pf and Pan								
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	0.0	0.0	0.0	0.0	0.0	0.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	0.0	0.0	0.0	0.0 (99)	1.0	0.5 (199)
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	0.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	0.0	0.0	0.0	0.0	0.0	0.0
Pf and Pv								
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	1.0	1.5	1.3	0.0	1.0	0.5
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	0.5	0.0	0.3	0.0	0.0	0.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	0.5 (199)	0.0	0.25 (399)	0.0	0.0	0.0
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	0.0	0.0	0.0	0.0	0.0	0.0
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	3.0	0.5 (199)	1.75 (399)	31.0	34.0	32.5
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	1.0	0.0	0.5	0.0	0.0	0.0
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	0.0	0.5	0.3	0.0	1.0	0.5
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	0.0	0.0	0.0	0.0	0.0 (99)	0.0 (199)
Pf, Pv and Pan								
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	0.0	0.0 (199)	0.0 (399)	0.0	0.0	0.0
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	0.0 (199)	0.0 (197)	0.0 (396)	0.0	0.0 (99)	0.0 (199)
Pan only								
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	N/A	N/A	N/A	N/A	N/A	N/A
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	N/A	N/A	N/A	N/A	N/A	N/A
Pv only								
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	0.5	0.0	0.3	1.0	1.0	1.0

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] 6 (6%) of the 100 *P. falciparum* dilution samples sets were 200 and 5000 parasites/µl

Table A4.7: Phase 2 false positive rate for *P. falciparum* test line results on all malaria-negative samples

Product	Catalogue number	Manufacturer	Percentage of false positive Pf test lines on "clean[a]" negative samples			Percentage of false positive Pf test lines on samples containing non-*Plasmodium* spp. infectious agents[b]			Percentage of false positive Pf test lines on samples containing immunological factors[c]		
			Lot 1 (n=100)	Lot 2 (n=100)	Overall (n=200)	Lot 1 (n=42)	Lot 2 (n=42)	Overall (n=84)	Lot 1 (n=58)	Lot 2 (n=58)	Overall (n=116)
Pf only											
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	3.0	3.0	3.0	0.0	0.0	0.0	1.7	1.7	1.7
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	5.0	9.0	7.0	0.0 (40)	4.8	2.44 (82)	8.93 (56)	1.7	5.26 (114)
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	2.0	2.0	2.0	4.8	0.0	2.4	5.2	0.0	2.6
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	2.0	0.0	1.0	0.0 (41)	0.0	0.0 (83)	0.0	0.0	0.0
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	0.0 (98)	1.1 (88)	0.5 (186)	2.4(41)	0.0 (40)	1.2 (81)	3.5 (57)	3.9 (51)	3.7 (108)
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	14.0	8.0	11.0	9.5	9.5	9.5	6.9	3.5	5.2
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.9
Pf and Pan											
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	3.5	3.5	3.5
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	3.0	0.0	1.5	0.0 (41)	0.0	0.0 (83)	1.7	0.0	0.9
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	3.5	3.5	3.5
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	2.0	0.0	1.0	0.0	0.0	0.0	3.5	1.7	2.6
Pf and Pv											
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	2.0	1.0	1.5	2.4	0.0	1.2	0.0	0.0	0.0
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	5.0	3.0	4.0	2.4	0.0	1.2	0.0	0.0 (56)	0.0 (114)
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	0.0 (99)	3.0	1.5 (199)	0.0 (40)	0.0	0.0 (82)	1.7	3.5	2.6
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	2.0	1.0	1.5	4.9 (41)	0.0	2.4 (83)	1.7	0.0	0.9
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	3.5	3.5	3.5
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	5.0	2.0	3.5	0.0	0.0	0.0	6.9	8.6	7.8
Pf, Pv and Pan											
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	3.0	2.0	2.5	4.8	0.0	2.4	1.7	0.0	0.9
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	7.0	4.1 (98)	5.5 (198)	2.4	0.0	1.2	3.5 (57)	0.0	1.7 (115)
Pan only											
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pv only											
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] Blood samples from healthy volunteers with no known current illness or blood abnormality

[b] See Table A4.8 for details

[c] See Table A4.9 for details

Table A4.8: Phase 2 false positive rate for *P. falciparum* in samples containing specific non-malarial infectious pathogens

Product	Catalogue number	Manufacturer	Percentage of false positive for *Plasmodium* spp. by infectious pathogen							
			Dengue		Schistosomiasis		Leishmaniasis		Chagas	
			Lot 1 (n=8)	Lot 2 (n=8)	Lot 1 (n=20)	Lot 2 (n=20)	Lot 1 (n=10)	Lot 2 (n=10)	Lot 1 (n=4)	Lot 2 (n=4)
Pf only										
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	0.0 (7)	0.0	0.0	0.0	0.0 (9)	10.0	0.0	25.0
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	12.5	0.0	0.0	0.0	10.0	0.0	0.0	0.0
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0 (9)	0.0	0.0	0.0
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	0.0	0.0 (6)	0.0	0.0	11.1 (9)	0.0	0.0	0.0
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	0.0	12.5	20.0	10.0	0.0	10.0	0.0	0.0
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pf and Pan										
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	0.0 (7)	0.0	0.0	0.0	20.0	50.0	0.0	25.0
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Pf and Pv										
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	0.0	0.0	0.0 (18)	0.0	0.0	0.0	0.0	0.0
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	0.0 (7)	0.0	5.0	0.0	10.0	0.0	0.0	0.0
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0
Pf, Pv and Pan										
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	0.0	0.0	25.0	0.0	70.0	50.0	25.0	50.0
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	0.0	0.0	10.0	10.0	40.0	50.0	50.0	75.0
Pan only										
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	0.0	0.0	10.0	5.0	0.0	0.0	0.0	0.0
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	12.5	0.0	0.0	0.0 (19)	0.0	0.0	0.0	50.0
Pv only										
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

Table A4.9: Phase 2 false positive rate for *P. falciparum* in samples containing potentially cross-reacting blood immunological factors

Product	Catalogue number	Manufacturer	Percentage of false positive for *Plasmodium* spp. by blood immunological factor							
			Rheumatoid factor		Anti-nuclear antibodies		Anti-mouse antibodies		Rapid plasma reagin (RPR) positive	
			Lot 1 (n=8)	Lot 2 (n=8)	Lot 1 (n=26)	Lot 2 (n=26)	Lot 1 (n=6)	Lot 2 (n=6)	Lot 1 (n=18)	Lot 2 (n=18)
Pf only										
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	0.0	0.0	0.0	0.0	16.7	16.7	0.0	0.0
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	12.5	0.0	12.0 (25)	0.0	0.0	0.0	5.9 (17)	5.6
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	0.0	0.0	3.9	0.0	0.0	0.0	11.1	0.0
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	28.6 (7)	25.0	0.0	0.0 (23)	0.0	0.0	0.0	0.0 (14)
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	0.0	0.0	3.9	3.9	0.0	16.7	16.7	0.0
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	16.7	0.0	0.0
Pf and Pan										
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	25.0	25.0	0.0	3.9	33.3	33.3	0.0	0.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	37.5	12.5	15.4	19.2	50.0	33.3	44.4	33.3
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	25.0	25.0	0.0	3.9	100.0	100.0	0.0	0.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	25.0	25.0	11.5	0.0	33.3	33.3	5.6	5.6
Pf and Pv										
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	0.0	12.5	0.0	0.0	0.0	0.0	0.0	5.6
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	0.0	0.0	0.0	0.0	0.0	0.0 (5)	0.0	0.0 (17)
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	25.0	25.0	11.5	0.0	16.7	33.3	5.6	0.0
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	0.0	0.0	0.0	0.0	16.7	0.0	0.0	0.0
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	25.0	25.0	0.0	11.5	66.7	66.7	0.0	0.0
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	25.0	37.5	0.0	0.0	33.3	33.3	0.0	0.0
Pf, Pv and Pan										
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	50.0	25.0	42.3	7.7	33.3	33.3	50.0	50.0
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	50.0	50.0	44.0 (25)	19.2	66.7	66.7	33.3	44.4
Pan only										
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	0.0	12.5	3.9	0.0	0.0	0.0	5.6	0.0
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	25.0	0.0	0.0	3.9	0.0	0.0	11.1	0.0
Pv only										
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

Table A4.10: Phase 2 false positive rate of pan/*P. vivax* test line results on all malaria-negative samples

Product	Catalogue number	Manufacturer	Percentage of false positive pan or Pv test lines on "clean[a]" negative samples			Percentage of false positive pan or Pv test lines on samples containing non-*Plasmodium* spp. infectious agents[b]			Percentage of false positive pan or Pv test lines on samples containing immunological factors[c]		
			Lot 1 (n=100)	Lot 2 (n=100)	Overall (n=200)	Lot 1 (n=42)	Lot 2 (n=42)	Overall (n=84)	Lot 1 (n=58)	Lot 2 (n=58)	Overall (n=116)
Pf only											
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pf and Pan											
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	6.9	6.9	6.9
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	24.0	24.0	24.0	4.9 (41)	14.3	9.6 (83)	29.3	24.1	26.7
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	0.0	0.0	0.0	0.0	0.0	0.0	12.1	12.1	12.1
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	9.0	5.0	7.0	2.4	0.0	1.2	10.3	8.6	9.5
Pf and Pv											
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	1.0	0.0	0.5	0.0	0.0	0.0	0.0	3.5	1.7
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	1.0	0.0	0.5	0.0	0.0	0.0	0.0	1.7	0.9
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	2.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0 (56)	0.0 (114)
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	0.0 (99)	0.0	0.0 (199)	0.0 (40)	0.0	0.0 (82)	10.3	3.5	6.9
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	3.0	1.0	2.0	0.0 (41)	0.0	0.0 (83)	0.0	0.0	0.0
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	0.0	1.0	0.5	0.0	4.8	2.4	6.9	12.1	9.5
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	0.0	0.0	0.0	2.4	0.0	1.2	0.0	0.0	0.0
Pf, Pv and Pan											
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test[d]	2103 CB-25	Unimed International Inc.	30.0	17.0	23.5	28.6	16.7	22.6	43.1	25.9	34.5
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test[e]	2103 CB-25	Unimed International Inc.	1.0	0.0	0.5	9.5	0.0	4.8	8.6	0.0	4.3
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)[d]	50320025	Zephyr Biomedicals	34.0	37.8 (98)	35.9 (198)	14.3	21.4	17.9	43.9 (57)	36.2	40.0 (115)
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)[e]	50320025	Zephyr Biomedicals	4.0	5.1 (98)	4.5 (198)	4.8	2.4	3.6	8.8 (57)	5.2	7.0 (115)
Pan only[d]											
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	4.0	1.0	2.5	4.8	2.4	3.6	3.5	1.7	2.6
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	3.0	2.0	2.5	2.4	4.9 (41)	3.6 (83)	6.9	1.7	4.3
Pv only											
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	0.0	2.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

[a] Blood samples from healthy volunteers with no known current illness or blood abnormality

[b] See Table A4.8 for details

[c] See Table A4.9 for details

[d] Pan test line

[e] *P.vivax* test line

Table A4.11: Heat stability testing results for *P. falciparum* (or pan[a]) test line on a *P. falciparum* samples at low parasite density (200 parasites/µl). Positivity rate at baseline, and after 60 days incubation at 4°C, 35°C and 45°C

Product	Catalogue number	Manufacturer	Baseline testing						35°C						45°C						4°C					
			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)		
			No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity
Pf only																										
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	10.0	0.0	3.0	10.0	0.0	3.0	10.0	0.0	2.9	10.0	0.0	2.9	10.0	0.0	3.0	10.0	0.0	2.9	10.0	0.0	2.9	10.0	0.0	3.0
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	9.0	0.0	2.0	10.0	0.0	1.0	4.0	1.0	1.3	10.0	0.0	1.9	3.0	1.0	1.0	8.0	1.0	1.6	9.0	0.0	1.4	8.0	0.0	1.6
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	10.0	0.0	1.9	10.0	0.0	1.9	10.0	0.0	1.8	10.0	0.0	1.8	10.0	0.0	2.0	10.0	0.0	1.8	10.0	0.0	2.0	10.0	0.0	2.0
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	10.0	0.0	2.3	10.0	0.0	3.0	10.0	0.0	2.0	10.0	0.0	2.8	10.0	0.0	1.8	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.0
One Step Malaria Pf Test (cassette)	522352	BlueCross Bio-Medical (Beijing) Co., Ltd	6.0	0.0	1.0	0.0	0.0	N/A	3.0	0.0	1.0	0.0	0.0	N/A	2.0	2.0	1.0	0.0	2.0	N/A	4.0	3.0	1.0	0.0	0.0	N/A
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	10.0	0.0	1.8	10.0	0.0	2.7	10.0	0.0	2.0	9.0	0.0	1.8	10.0	0.0	2.0	8.0	1.0	1.8	10.0	0.0	1.9	10.0	0.0	2.0
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	10.0	0.0	1.0	10.0	0.0	1.0	10.0	0.0	1.0	8.0	0.0	1.0	5.0	0.0	1.0	8.0	0.0	1.8	8.0	0.0	1.0	10.0	0.0	1.2
Pf and Pan																										
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	10.0	0.0	2.1	10.0	0.0	1.9	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	1.8	10.0	0.0	1.5	10.0	0.0	2.1	10.0	0.0	2.1
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	10.0	0.0	2.7	10.0	0.0	2.0	10.0	0.0	2.5	10.0	0.0	2.0	10.0	0.0	1.6	10.0	0.0	2.3	10.0	0.0	2.0	10.0	0.0	3.0
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	1.0	0.0	1.0	6.0	0.0	1.0	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	1.0	0.0	1.0	0.0	0.0	N/A	1.0	0.0	1.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	10.0	0.0	2.0	10.0	0.0	3.0	10.0	0.0	2.0	10.0	0.0	1.7	10.0	0.0	1.0	10.0	0.0	1.8	10.0	0.0	2.0	10.0	0.0	2.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	10.0	0.0	1.1	10.0	0.0	1.0	8.0	0.0	1.0	10.0	0.0	1.0	2.0	0.0	1.0	6.0	0.0	1.0	10.0	0.0	1.0	10.0	0.0	1.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	7.0	0.0	1.0	4.0	0.0	1.0	8.0	0.0	1.0	9.0	0.0	1.0	4.0	0.0	1.0	7.0	0.0	1.0	3.0	0.0	1.0	9.0	0.0	1.0
Pf and Pv																										
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	10.0	0.0	2.0	10.0	0.0	2.3	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	3.0	10.0	0.0	2.0
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	10.0	0.0	3.0	10.0	0.0	2.5	10.0	0.0	2.0	10.0	0.0	2.0	9.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.8	10.0	0.0	2.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	10.0	0.0	2.0	10.0	0.0	3.0	9.0	1.0	2.0	10.0	0.0	2.0	9.0	0.0	2.0	10.0	0.0	2.5	10.0	0.0	2.0	10.0	0.0	3.0
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	10.0	0.0	3.0	10.0	0.0	3.0	10.0	0.0	3.0	10.0	0.0	2.0	10.0	0.0	2.2	10.0	0.0	2.1	10.0	0.0	3.0	10.0	0.0	2.9
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	10.0	0.0	1.0	10.0	0.0	1.0	7.0	0.0	1.0	5.0	0.0	1.2	1.0	0.0	1.0	5.0	0.0	1.0	10.0	0.0	1.0	9.0	0.0	1.0
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	10.0	0.0	2.0	10.0	0.0	2.9	10.0	0.0	3.0	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.8	10.0	0.0	2.7	10.0	0.0	2.0
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	10.0	0.0	1.0	10.0	0.0	1.6	9.0	0.0	1.0	10.0	0.0	1.0	7.0	0.0	1.0	2.0	0.0	1.0	10.0	0.0	1.0	ND	ND	ND
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	1.8	10.0	0.0	2.0	10.0	0.0	2.0
Pf, Pv and Pan																										
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	10.0	0.0	2.2	10.0	0.0	2.6	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.3	10.0	0.0	2.8	10.0	0.0	2.9
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	10.0	0.0	2.6	10.0	0.0	3.0	10.0	0.0	2.7	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.9	9.0	1.0	3.0
Pan only																										
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	10.0	0.0	1.1	0.0	0.0	N/A	10.0	0.0	1.0	6.0	0.0	1.2	7.0	0.0	1.0	4.0	1.0	1.0	7.0	0.0	1.0	8.0	0.0	1.0
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	0.0	0.0	N/A	5.0	0.0	1.0	0.0	0.0	N/A	1.0	1.0	1.0	0.0	0.0	N/A	2.0	0.0	1.0	1.0	1.0	1.0	2.0	0.0	1.0
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	0.0	0.0	N/A	1.0	0.0	1.0	7.0	0.0	1.0	0.0	0.0	N/A	3.0	0.0	1.0	0.0	0.0	N/A	9.0	0.0	1.1	0.0	0.0	N/A
Pv only																										
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species* - ND: not done

[a] For pan-only tests

Table A4.11a: Heat stability testing results for pan test line of combination RDTs on a *P. falciparum* sample at low parasite density (200 parasites/µl). Positivity rate at baseline, and after 60 days incubation at 4°C, 35°C and 45°C

Product	Catalogue number	Manufacturer	Baseline testing						35°C						45°C						4°C					
			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)		
			No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity
Pf and Pan																										
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	10.0	0.0	1.0	7.0	0.0	1.0	10.0	0.0	1.0	1.0	0.0	1.0	6.0	0.0	1.0	5.0	0.0	1.0	10.0	0.0	1.0	8.0	0.0	1.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	9.0	0.0	1.0	10.0	0.0	1.0	0.0	0.0	N/A	8.0	0.0	1.0	6.0	0.0	1.0	2.0	0.0	1.0	10.0	0.0	1.0	6.0	0.0	1.3
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	10.0	0.0	1.0	10.0	0.0	2.0	10.0	0.0	1.0	10.0	0.0	1.0	10.0	0.0	1.0	9.0	0.0	1.4	10.0	0.0	1.0	9.0	0.0	1.4
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	0.0	0.0	N/A	1.0	0.0	1.0	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	2.0	0.0	1.0	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A
Pf, Pv and Pan																										
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	9.0	0.0	1.0	3.0	0.0	1.0	10.0	0.0	1.0	0.0	0.0	N/A	3.0	0.0	1.0	0.0	0.0	N/A	10.0	0.0	1.0	5.0	0.0	1.0
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	10.0	0.0	1.3	10.0	0.0	2.0	3.0	0.0	1.0	2.0	0.0	1.0	0.0	0.0	N/A	6.0	0.0	1.0	8.0	0.0	1.0	8.0	1.0	1.6

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

Table A4.12: Heat stability testing results for Pf (or pan[a]) test line on a *P. falciparum* sample at high parasite density (2000 parasites/µl). Positivity rate at baseline, and after 60 days incubation at 4°C, 35°C and 45°C

Product	Catalogue number	Manufacturer	Baseline testing						35°C						45°C						4°C					
			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)		
			No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity
Pf only																										
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	9.0	1.0	4.0	10.0	0.0	4.0	10.0	0.0	2.2	9.0	1.0	3.9	9.0	1.0	2.0	10.0	0.0	4.0	10.0	0.0	1.9	9.0	0.0	4.0
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	10.0	0.0	3.9	10.0	0.0	4.0	10.0	0.0	3.0	10.0	0.0	4.0	10.0	0.0	2.0	10.0	0.0	3.9	10.0	0.0	2.9	10.0	0.0	4.0
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	3.9
One Step Malaria Pf Test (cassette)	522352	BlueCross Bio-Medical (Beijing) Co., Ltd	7.0	3.0	4.0	9.0	1.0	2.9	9.0	1.0	4.0	9.0	1.0	2.1	9.0	1.0	4.0	7.0	3.0	3.7	9.0	1.0	4.0	9.0	1.0	1.9
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	10.0	0.0	3.4	10.0	0.0	4.0	10.0	0.0	3.0	10.0	0.0	3.8	8.0	2.0	2.0	5.0	5.0	4.0	10.0	0.0	2.7	10.0	0.0	4.0
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	10.0	0.0	4.0	10.0	0.0	3.0	10.0	0.0	3.9	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	3.1	10.0	0.0	4.0	10.0	0.0	4.0
Pf and Pan																										
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	3.9	10.0	0.0	4.0	10.0	0.0	4.0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	10.0	0.0	4.0	10.0	0.0	3.9	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	10.0	0.0	2.6	10.0	0.0	2.5	10.0	0.0	2.0	10.0	0.0	2.4	10.0	0.0	1.5	10.0	0.0	1.8	9.0	0.0	2.0	10.0	0.0	2.0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	3.8	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	10.0	0.0	4.0	10.0	0.0	3.0	10.0	0.0	3.9	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	2.9	10.0	0.0	4.0	10.0	0.0	3.5
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	2.9	10.0	0.0	4.0	10.0	0.0	2.8	9.0	0.0	4.0	10.0	0.0	3.0	10.0	0.0	4.0
Pf and Pv																										
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	3.8	10.0	0.0	4.0	9.0	1.0	3.0	10.0	0.0	4.0	10.0	0.0	3.0	10.0	0.0	4.0
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	3.8	10.0	0.0	4.0	10.0	0.0	4.0
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	10.0	0.0	3.1	10.0	0.0	3.3	10.0	0.0	2.0	8.0	0.0	2.9	10.0	0.0	2.6	9.0	0.0	2.8	10.0	0.0	3.0	10.0	0.0	3.1
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	7.0	3.0	3.9	10.0	0.0	4.0	9.0	1.0	3.4	10.0	0.0	4.0
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	10.0	0.0	4.0	10.0	0.0	3.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	3.1	10.0	0.0	3.0	10.0	0.0	3.9	ND	ND	ND
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	10.0	0.0	3.8	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	9.0	1.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0
Pf, Pv and Pan																										
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	3.7	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0	10.0	0.0	4.0
Pan only																										
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	10.0	0.0	2.2	10.0	0.0	2.8	10.0	0.0	2.8	10.0	0.0	2.8	10.0	0.0	2.8	10.0	0.0	2.2	10.0	0.0	2.9	10.0	0.0	3.0
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	10.0	0.0	2.6	10.0	0.0	3.0	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.9	10.0	0.0	2.9	10.0	0.0	3.7	10.0	0.0	3.5
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	10.0	0.0	2.4	10.0	0.0	3.5	10.0	0.0	2.0	10.0	0.0	2.2	10.0	0.0	2.0	10.0	0.0	2.2	9.0	0.0	2.2	10.0	0.0	2.0
Pv only																										
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species* - ND: not done

[a] For pan-only tests

Table A4.12a: Heat stability testing results for pan test line of combination RDTs on a *P. falciparum* sample at high parasite density (2000 parasites/µl). Positivity rate at baseline, and after 60 days incubation at 4°C, 35°C and 45°C

Product	Catalogue number	Manufacturer	Baseline testing						35°C						45°C						4°C					
			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)			Lot 1 (n=10)			Lot 2 (n=10)		
			No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity	No. positive	No. invalid	Mean band intensity
Pf and Pan																										
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	10.0	0.0	2.1	10.0	0.0	3.0	10.0	0.0	3.0	10.0	0.0	2.9	10.0	0.0	3.0	10.0	0.0	3.0	10.0	0.0	3.0	10.0	0.0	3.2
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	10.0	0.0	2.4	10.0	0.0	1.9	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.3	10.0	0.0	2.0	10.0	0.0	2.0	10.0	0.0	2.9
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0.0	0.0	N/A	7.0	0.0	1.0	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A	0.0	0.0	N/A
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	10.0	0.0	3.0	10.0	0.0	3.0	10.0	0.0	3.0	10.0	0.0	3.8	10.0	0.0	3.0	10.0	0.0	3.0	10.0	0.0	3.0	10.0	0.0	3.0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	10.0	0.0	1.1	10.0	0.0	1.3	8.0	0.0	1.0	6.0	0.0	1.0	2.0	0.0	1.0	7.0	0.0	1.6	10.0	0.0	1.0	2.0	0.0	1.0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	10.0	0.0	1.7	0.0	0.0	N/A	2.0	0.0	1.0	7.0	0.0	1.0	8.0	0.0	1.0	6.0	0.0	1.7	10.0	0.0	1.0	9.0	0.0	1.0
Pf, Pv and Pan																										
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	10.0	0.0	2.0	10.0	0.0	1.9	10.0	0.0	2.0	8.0	0.0	1.1	10.0	0.0	1.2	10.0	0.0	1.7	10.0	0.0	1.9	10.0	0.0	1.6
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	10.0	0.0	2.1	10.0	0.0	2.0	10.0	0.0	2.0	9.0	0.0	1.9	10.0	0.0	1.3	10.0	0.0	1.6	10.0	0.0	2.0	10.0	0.0	1.8

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

Table A4.13: Heat stability testing results for *P. falciparum* (or pan) test line on parasite-negative samples. Positivity rate at baseline, and after 60 days incubation at 4°C, 35°C and 45°C

Product	Catalogue number	Manufacturer	Baseline testing				35°C				45°C				4°C			
			Lot 1 (n=4)		Lot 2 (n=4)		Lot 1 (n=4)		Lot 2 (n=4)		Lot 1 (n=4)		Lot 2 (n=4)		Lot 1 (n=4)		Lot 2 (n=4)	
			No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid
Pf only																		
CareStart™ Malaria HRP2/pLDH Pf test	G0181	Access Bio, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
diagnosticks- Malaria (Pf) Cassette	KMFC6001	SSA Diagnostics & Biotech Systems	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
diagnosticks- Malaria (Pf) Dipstick	KMFD6007	SSA Diagnostics & Biotech Systems	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HiSens Malaria Ag Pf HRP2 Card	HR3023	HBI Co., Ltd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
One Step Malaria Pf Test (cassette)	522352	Blue Cross Bio-Medical (Beijing) Co., Ltd	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0
OnSight™ - Malaria Pf Test	511-25-DB	Amgenix International, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Onsite Pf Ag Rapid Test	R0114C	CTK Biotech, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pf and Pan																		
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pf and Pv																		
CareStart™ Malaria HRP2/PLDH (Pf/VOM) COMBO	G0171	Access Bio, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CareStart™ Malaria HRP2/PLDH (Pf/Pv) COMBO	G0161	Access Bio, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
diagnosticks- Malaria (Pv/Pf) Cassette	KMVFC6002	SSA Diagnostics & Biotech Systems	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Falcivax Rapid Test for Malaria Pv/Pf (device)	50300025	Zephyr Biomedicals	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Maleriscan Malaria Pf/Pv	MAT-50	Bhat Bio-Tech India (P) Ltd	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OnSight™ - ParaQuick-2 (Pv,Pf) Malaria Test	537-25-DB	Amgenix International, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Onsite Pf/Pv Ag Rapid Test	R0112C	CTK Biotech, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
SD BIOLINE Malaria Ag Pf/Pv	05FK80	Standard Diagnostics, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pf, Pv and Pan																		
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pan only																		
First Response Malaria Ag pLDH	I12FRC30	Premier Medical Corporation Ltd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FirstSign™ - PanCheck (Pan) Malaria Test	2104 CB-25	Unimed International Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OnSight™ - PanScreen (Pan) Malaria Test	539-25-DB	Amgenix International, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pv only																		
SD BIOLINE Malaria Ag Pv	05FK70	Standard Diagnostics, Inc.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

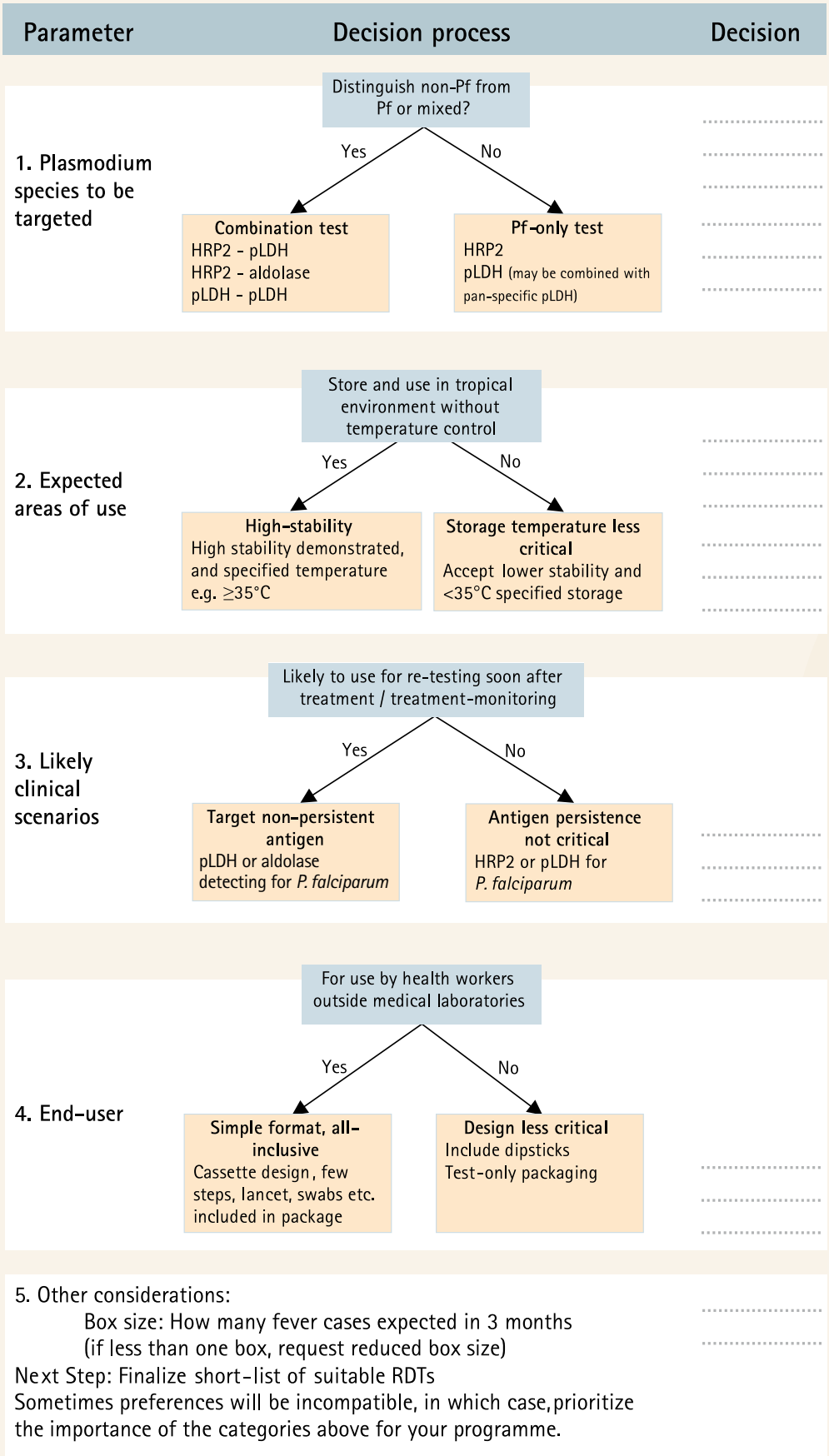
Table A4.13a: Heat stability testing results for pan test line of combination RDTs on parasite-negative samples. Positivity rate at baseline, and after 60 days incubation at 4°C, 35°C and 45°C

Product	Catalogue number	Manufacturer	Baseline testing				35°C				45°C				4°C			
			Lot 1 (n=4)		Lot 2 (n=4)		Lot 1 (n=4)		Lot 2 (n=4)		Lot 1 (n=10)		Lot 2 (n=10)		Lot 1 (n=10)		Lot 2 (n=10)	
			No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid	No. positive	No. invalid
Pf and Pan																		
First Response Malaria pLDH/HRP2 Combo Test	I16FRC30	Premier Medical Corporation Ltd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FirstSign™ - ParaView (Pan+Pf) Malaria Test	2101 CB-25	Unimed International Inc.	0	0	4	0	0	0	1	0	0	0	1	0	0	0	2	0
HiSens Malaria Ag P.f/P.v Card	HR2823	HBI Co., Ltd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HiSens Malaria Ag Pf/Pv (HRP2/pLDH) Card	HR2923	HBI Co., Ltd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Onsite Pf/Pan Ag Rapid Test	R0113C	CTK Biotech, Inc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ParaHIT total (dipstick)	55IC201-10	Span Diagnostics Ltd	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pf, Pv and Pan																		
FirstSign™ - ParaView-3 (Pan+Pv+Pf) Malaria Test	2103 CB-25	Unimed International Inc.	2	0	0	0	4	0	0	0	0	0	1	0	0	0	1	0
Paramax-3 Rapid Test for Malaria Pan/Pv/Pf (device)	50320025	Zephyr Biomedicals	0	0	4	0	2	0	0	0	0	0	3	0	2	0	1	0

Pf: *Plasmodium falciparum* - Pv: *Plasmodium vivax* - pan: *Plasmodium species*

Annex 5: Example algorithm for selecting a malaria RDT

Using Table S1/Figures S1, S2 or the interactive guide[a], select a RDT with sufficiently high detection rate, low false-positive rate and invalid rate and then follow the algorithm below.



[a] An interactive guide designed to help short-list test according to individual programme needs, based on the performance of the tests in rounds 1 and 2 of the WHO Product Testing Programme can be found at *http://www.finddiagnostics.org/programs/malaria/find_activities/product_testing/*

CHOOSING SPECIFIC PRODUCT FROM RDT SHORT-LIST

1. Contact manufacturers and request:
   a. Quoted price (include necessary accessories, delivery to country, and staggered delivery in 2-3 batches over 12 months)
   b. Request heat stability data as evidence of manufacturer's stated storage temperature and shelf-life
   c. Request sample of product to assess format, ease of use, compatibility of other materials with health system requirements

2. Assess experience of others:
   If possible, obtain written assessments of field experience from other countries / programmes that have experience in using the product

Make preliminary procurement decision, then consider...

3. Options to improve implementation:
   a. Negotiate replacement of product if supplied product fails lot-testing after delivery by a method approved by manufacturer / WHO-coordinated laboratory
   b. Develop appropriately-formatted instructions in appropriate language and consider their inclusion of these in kits at the manufacturing site
   c. Negotiate delivery dates for staggered delivery to reduce in-country storage times, ensure long (e.g. 18 months) shelf-life after delivery

4. Organize lot-testing prior to dispersal to the field

Annex 6: Introducing RDT-based malaria diagnosis into national programmes

As parasite-based diagnosis is introduced at smaller clinics and village level for case management, a large number of challenges arise not only in logistical administration but also in managing the health-seeking and health-providing behaviour of patients and health workers. These can be addressed by a systematic approach to planning, implementation, monitoring and evaluation of the diagnostic programme; a process that must commence well before RDTs are procured. The following information is derived from existing WHO documents addressing this area.[1] More information may be obtained at www.wpro.who.int/sites/rdt .

Many health workers and communities will have been taught that "fever equals malaria unless proven otherwise". Introducing RDTs will demonstrate that this is not the case. To have an impact on anti-malarial diagnosis and treatment, RDTs must be seen to provide an accurate diagnosis by both health workers and patients alike, that is, they must be as good or better than those relied on previously. A health worker will also need a good alternative to anti-malarial medicines for the management of parasite-negative febrile patients. To achieve and maintain confidence in RDT-based diagnosis, a good quality assurance system must be in place (detailed elsewhere on this website). There must be satisfactory education of health workers, and widespread community sensitization. Knowledge of other causes of fever will be necessary to develop appropriate management algorithms for parasite-negative cases.

At the national level, regulatory requirements may need to be developed to control the importation and use of malaria RDTs, and new procedures for storage, distribution and inventory management, such as those used for medicines, may need to be developed. If changing from a different product or mode of diagnosis, an adequate phase-out plan for this must also be developed.

This requires a clear strategic plan to be developed well in advance of RDT introduction, with a clear timeline to ensure that the various components of the RDT programme are in place at the right time. A focal person, or persons, will be needed to coordinate the overall implementation plan and ensure that the various agencies that may be involved understand the process and their particular roles. To achieve this, funding for the programme must include a significant component for planning and coordination, sensitization/IEC, training, quality assurance, monitoring and supervision, and logistics, in addition to procurement. Without this, much of the funds expended on RDTs may be wasted, and a loss of confidence in RDT-based diagnosis may hinder the process of strengthening appropriate malaria case management.

An example of a national implementation plan is shown below. This will need to be modified considerably for each programme, preferably through a collaborative process involving all the major agencies concerned in its implementation. Budgeting for all the components of the programme at the outset is vital. An example of components to be considered in an overall budget is shown in Figure A6.1.

[1] Developed by WHO Regional Office for the Western Pacific and the WHO Global Malaria Programme, with support from the Uganda Ministry of Health (National Malaria Control Programme), Management Sciences for Health (MSH), and other partners.

Summary of introduction plan (see following page)

Program planning and management

- Identify key stakeholders, and secure commitment for introduction of RDTs
- Establish working group and develop terms of reference
- Identify specific focal person(s) responsible for day to day oversight of the implementation plan
- Develop a timeline, scope, and budget for implementation
- Identify human and other resource needs, and a strategy for accessing them
- Review and update, if needed, case-management algorithms for malaria and other causes of febrile illness

Policy and regulatory issues

- Develop appropriate regulatory documents if required
- Register RDT products

Procurement of RDTs

- Develop product specifications and packaging requirements
- Develop product short-list
- Conduct quantification (estimation of needs)
- Procure RDTs
- Procure sharps boxes, gloves etc.

Logistics

- Develop distribution plan
- Train logistics and storage personnel in handling and distribution of RDTs
- Implement a system for data collection and information flows
- Arrange for appropriate transport and storage
- Review and strengthen inventory management, as needed
- Develop a plan for discontinuation and disposal of other diagnostic supplies, if appropriate

Quality Assurance

- Develop mechanisms for assessing samples at a national level (lot-testing), and regular (and random) testing at the level of use (e.g. microscopy-sentinel sites)
- Implement post-marketing surveillance

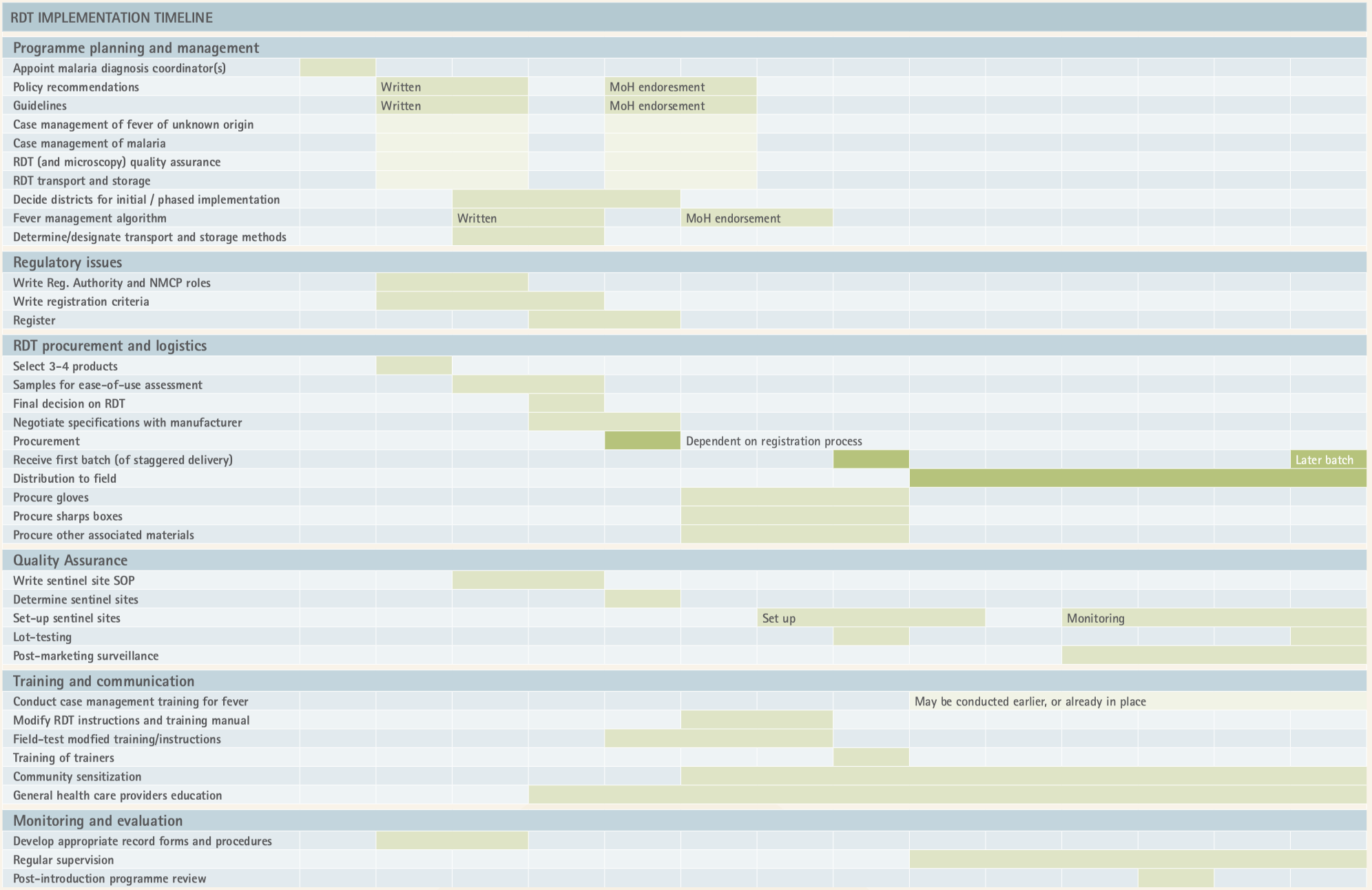
Training and communication

- Develop appropriate training and supervision materials
- Train health workers in case management and managing commodities
- Train in RDT use
- Develop and implement a program for community education/ sensitization

Monitoring and Evaluation

- Implement effective supervision and monitoring
- Strengthen recording and reporting procedures

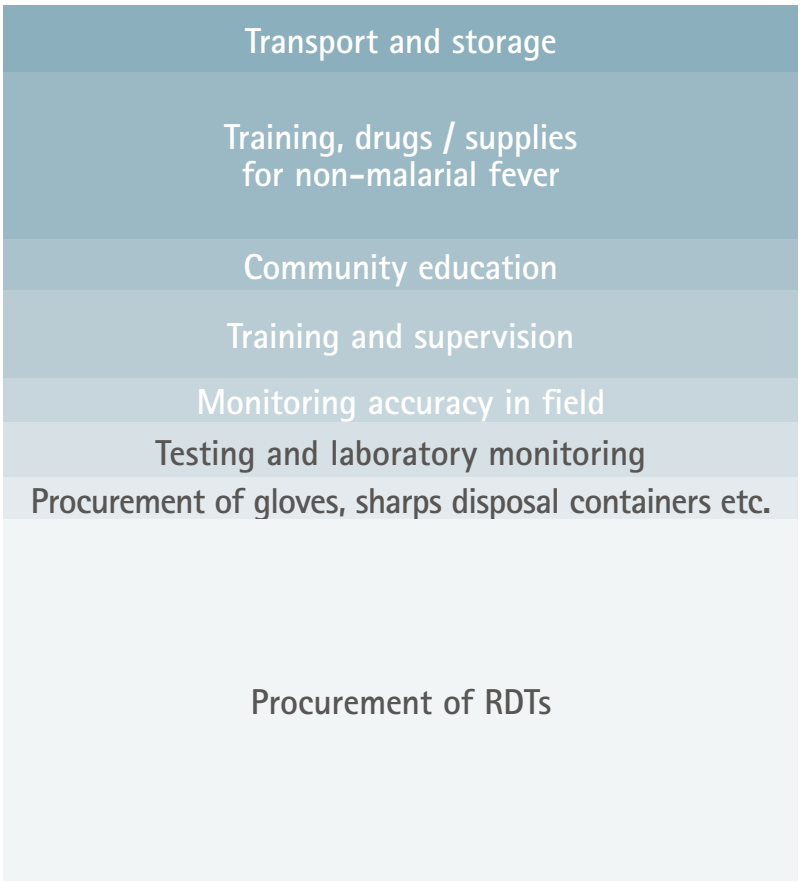
Recommended sequence of activities for implementation of RDT-based diagnosis in a national malaria programme and the relative time allotment.[a]



[a] time requ irements will vary between programmes

Figure A6.1 Example malaria RDT implementation budget

Below is an example of major components of a programme budget to be considered when introducing RDTs into a malaria programme. Without adequate provision for each of these factors, it is likely that an RDT-based diagnostics programme will fail to achieve its goals. These components should therefore be addressed in proposals for programme funding, or provisions should be made for them in collaborating programmes.



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