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
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Salim Abdullah	Ifakara Health Research and Development Centre, United Republic of Tanzania
Audrey Albertini	Foundation for Innovative New Diagnostics, Switzerland
Frederic Ariey	Institut Pasteur, Cambodia
John Barnwell	US Centers for Disease Control and Prevention/National Center for Global Health/Division of Malaria and Parasitic Diseases, United States of America
John Bligh	Hospital for Tropical Diseases, United Kingdom of Great Britain and Northern Ireland
David Bell	WHO – Global Malaria Programme, Switzerland
Sandra Buisson	Hospital for Tropical Diseases, United Kingdom of Great Britain and Northern Ireland
Deborah Casandra	US Centers for Disease Control and Prevention/National Center for Global Health/Division of Malaria and Parasitic Diseases, United States of America
Qin Cheng	Army Malaria Institute, Australia
Peter Chiodini	Hospital for Tropical Diseases, United Kingdom of Great Britain and Northern Ireland
Jane Cunningham	TDR, Special Programme for Research and Training in Tropical Diseases, Switzerland
Linda Dantes	WHO – Regional Office for the Western Pacific, The Philippines
Djibrine Djalle	Institut Pasteur Bangui, Central African Republic
Babacar Faye	Université Cheikh Anta DIOP, Senegal
Nahla Gadalla	Hospital for Tropical Diseases, United Kingdom of Great Britain and Northern Ireland
Dionicia Gamboa	Universidad Peruana Cayetano Heredia Instituto de Medicina Tropical, Peru
Michelle Gatton	Queensland Institute of Medical Research, Australia
Iveth Gonzalez	Foundation for Innovative New Diagnostics, Switzerland
Sandra Incardona	Consultant, Foundation for Innovative New Diagnostics, Switzerland
Sophie Jones	US Centers for Disease Control and Prevention/National Center for Global Health/Division of Malaria and Parasitic Diseases, United States of America
Myat Phone Kyaw	Department of Medical Research, Myanamar
Jennifer Luchavez	Research Institute of Tropical Medicine, The Philippines
Lorraine Mationg	Research Institute of Tropical Medicine, The Philippines
James McCarthy	Queensland Institute of Medical Research, University of Queensland, Australia
Didier Menard	Institut Pasteur de Madagascar, Madagascar; Institut Pasteur, Cambodia
Claribel Murillo	Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM), Colombia
Sina Nhem	Institut Pasteur / National Malaria Centre (CNM), Cambodia

Bernhards Ogutu	Kenya Medical Research Institute (KEMRI), Kenya
Pamela Onyor	Kenya Medical Research Institute (KEMRI), Kenya
Daniel Orozco	Médecins Sans Frontières, The Netherlands
Wellington Oyibo	University of Lagos, Nigeria
Anita Pelecanos	Queensland Institute of Medical Research, Australia
Mark Perkins	Foundation for Innovative New Diagnostics, Switzerland
Roxanne Rees-Channer	Hospital for Tropical Diseases, United Kingdom of Great Britain and Northern Ireland
Charuni Senanayake	WHO – Regional Office for the Western Pacific, The Philippines
Muth Sinuon	National Malaria Centre (CNM), Cambodia
Michael Valentine	US Centers for Disease Control and Prevention/National Center for Global Health/Division of Malaria and Parasitic Diseases, United States of America
Melissa Vega	TDR, Special Programme for Research and Training in Tropical Diseases, Switzerland
Julie Vercruysse	Foundation for Innovative New Diagnostics, Switzerland
Kristin Wall	US Centers for Disease Control and Prevention/National Center for Global Health/Division of Malaria and Parasitic Diseases, United States of America

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.

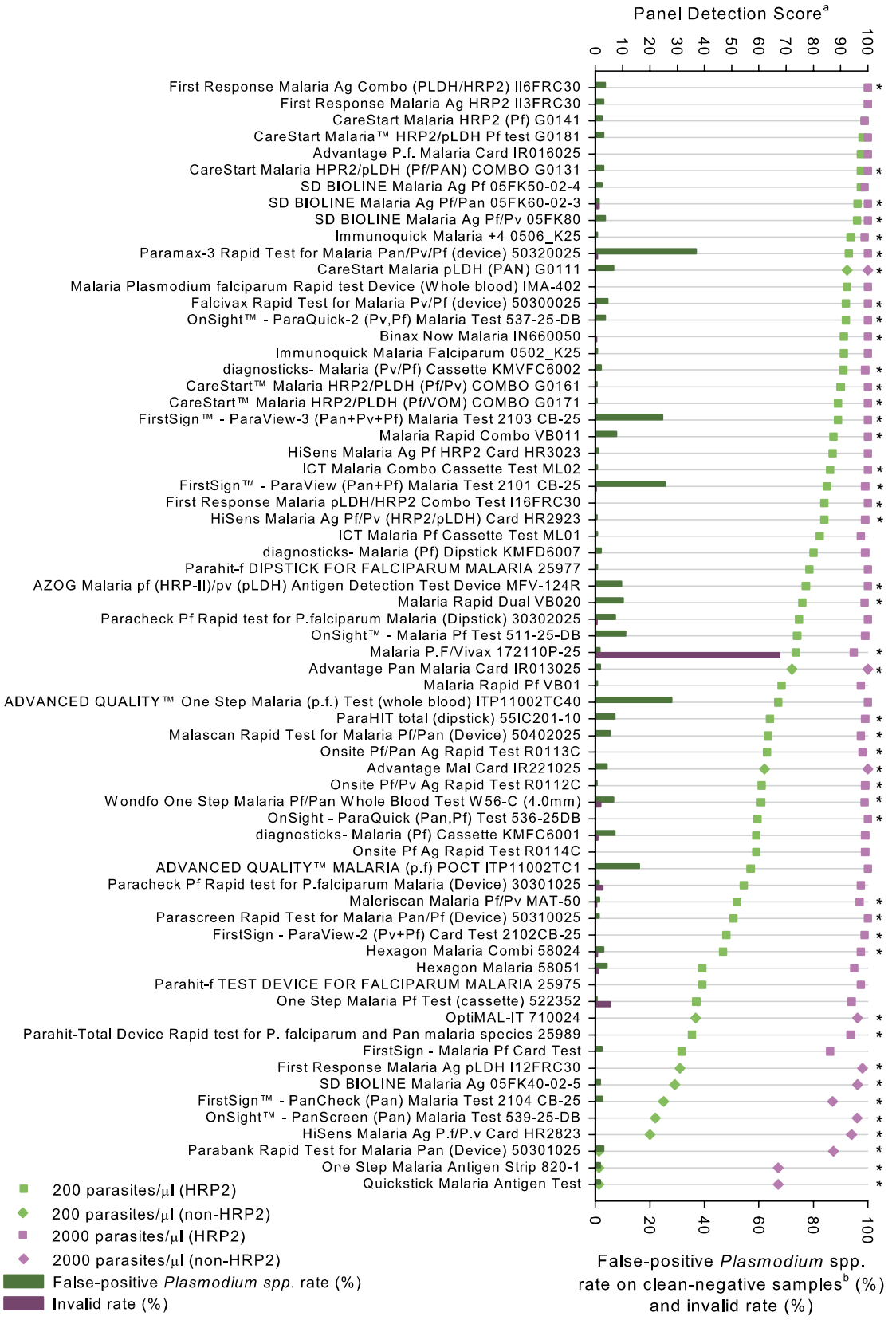
The results underscore the need for manufacturers to have adequate reference materials for product development and lot-release. The WHO-FIND malaria RDT evaluation programme, in collaboration with the CDC, offers quality standard panels to manufacturers to assist in this process.

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

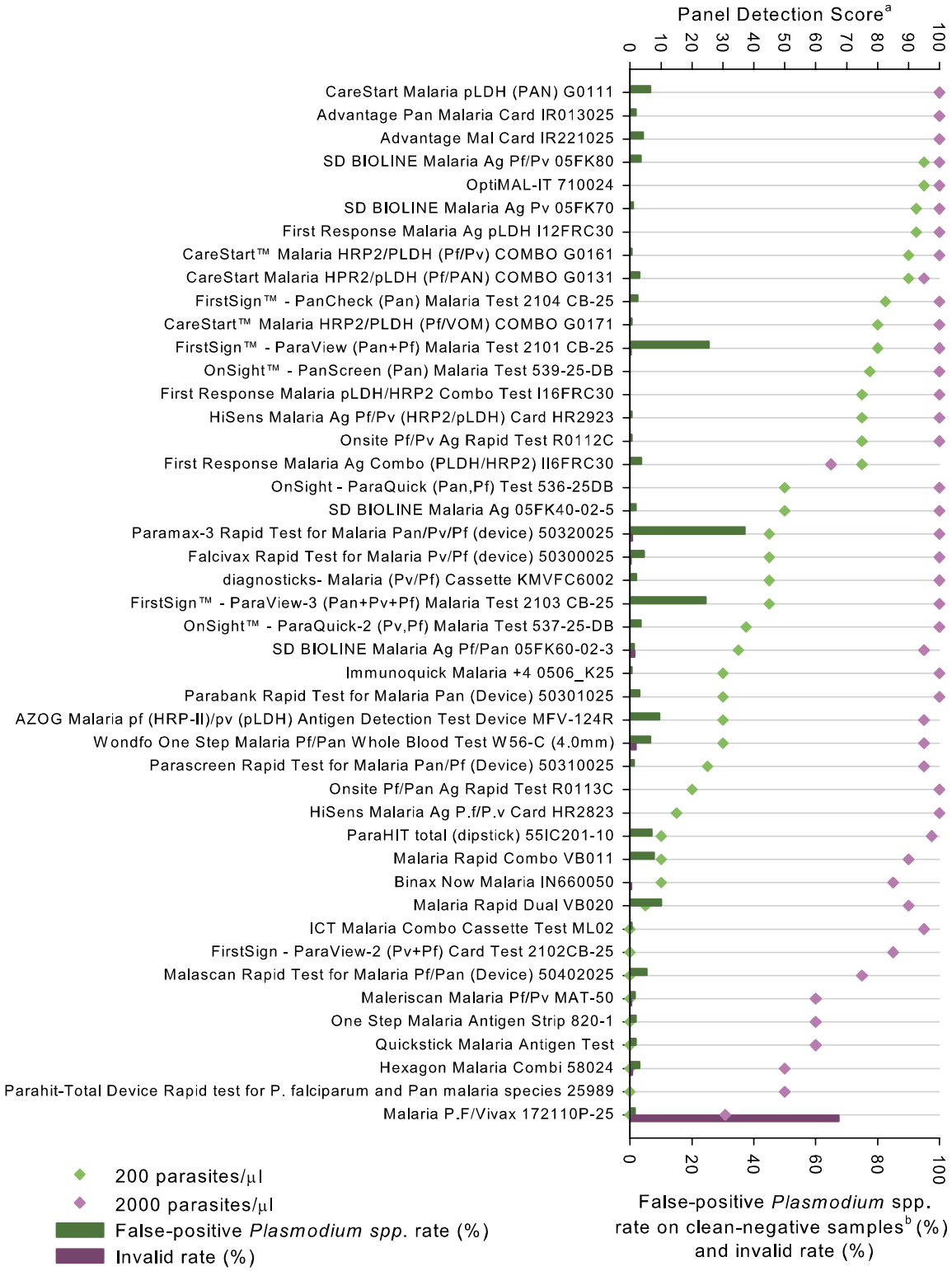


[a] panel detection score - A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive.;

[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.

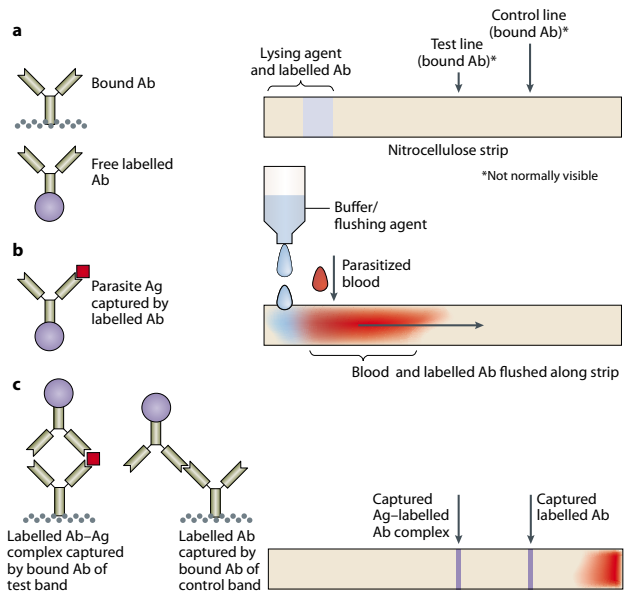
Despite WHO recommendations for laboratory-confirmed diagnosis of malaria infections prior to treatment in all cases (*2*), diagnosis is often made on clinical grounds (*4*). However, in most endemic areas malaria makes up a minority of 'malaria-like' febrile illness. Microscopy has been the cornerstone 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



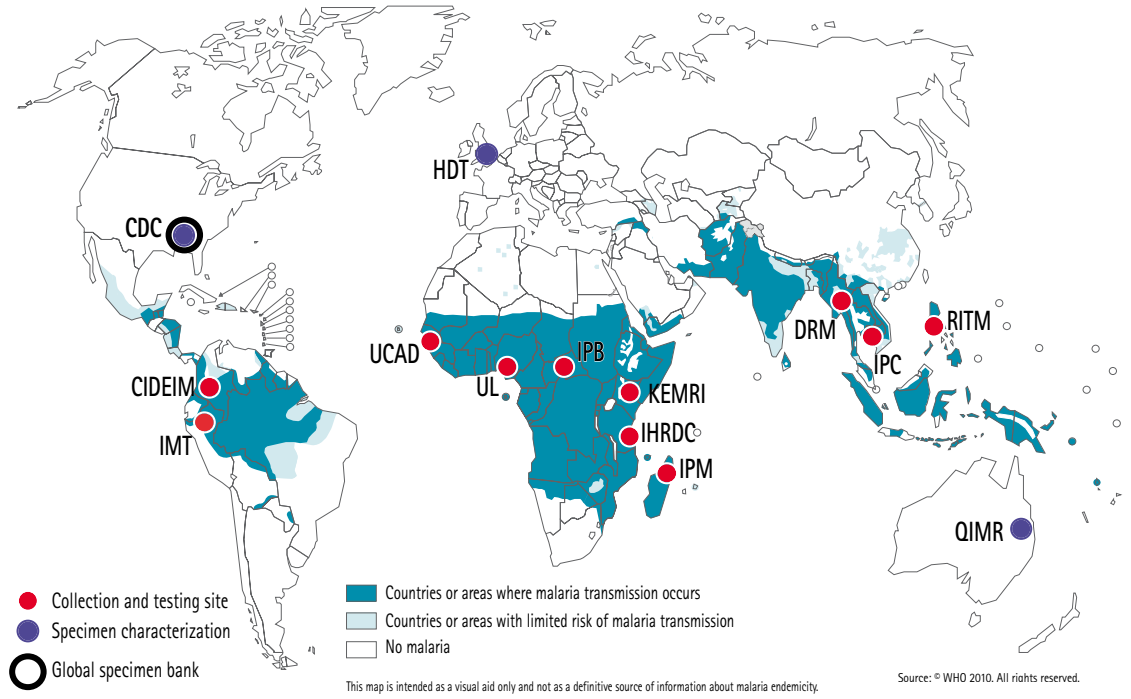
Mode of action of common malaria RDT format:

(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

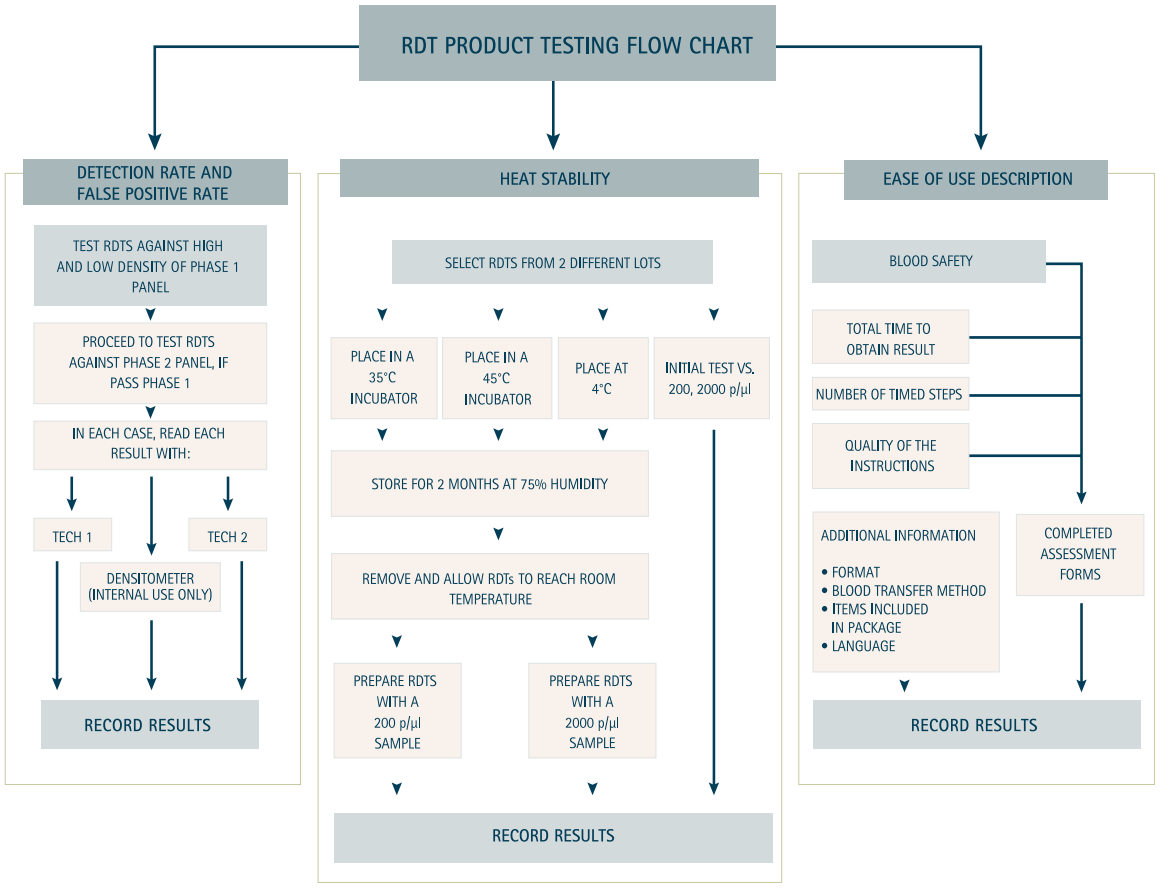
iv) Antigen concentration, determined by quantitative ELISA for HRP2, pLDH, aldolase

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)

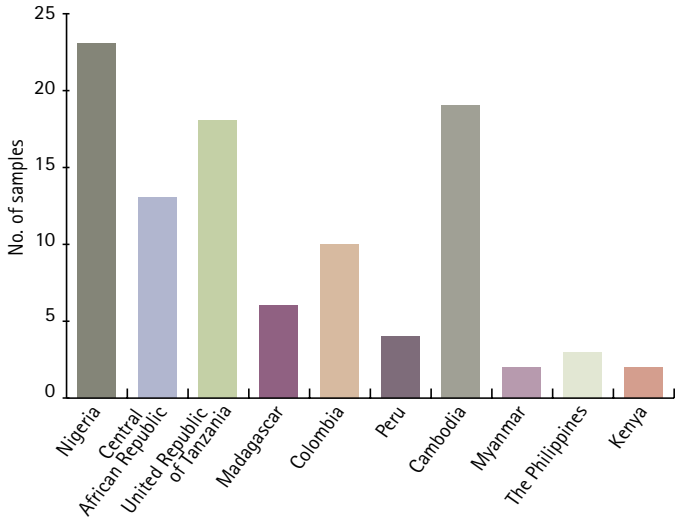
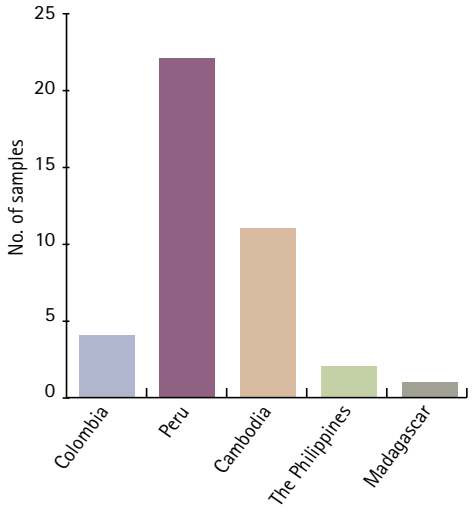


Figure 4b: Origin of Phase 2 *P. vivax* wild type (clinical) samples (n=40)



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.

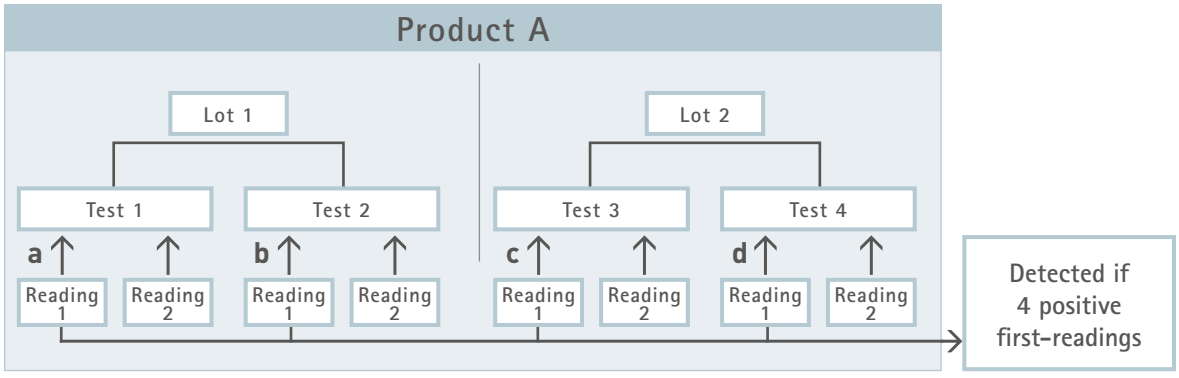
5.8. Interpretation of results

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.

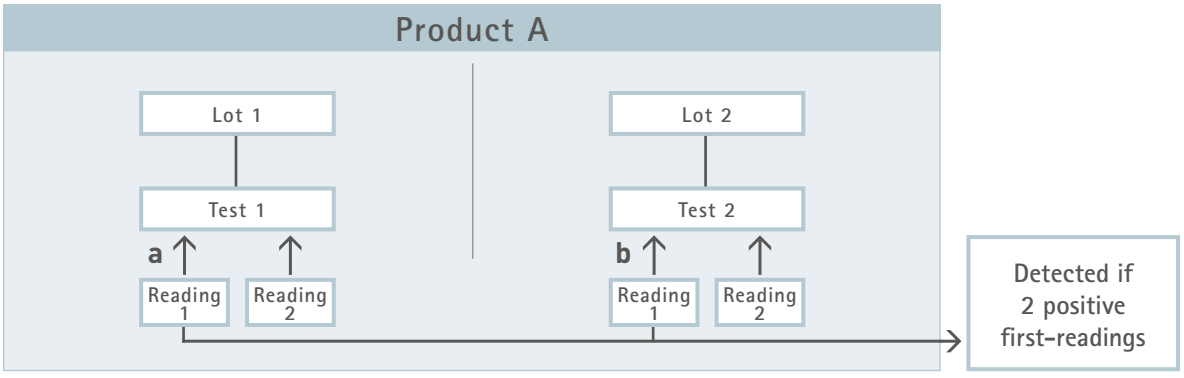
The results of the product panel testing and heat stability testing conducted at the CDC were recorded on report forms by each technician individually, as per the SOP. These results were double-data entered, and analysed for discrepancies.

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.