

Characteristics of evaluation panel used for Round 2 of WHO malaria RDT product testing at U.S. CDC, 2009 WHO-FIND malaria RDT evaluation programme

Introduction

The second round of product testing of malaria RDTs under the WHO-FIND malaria RDT evaluation programme was completed in November 2009 at the Malaria Branch, Division of Parasitic Diseases Centers for Disease Control and Prevention (U.S. CDC). The panel is a sub-set of the global specimen bank, maintained by WHO, FIND and U.S. CDC. This document summarizes the characterization reports of the evaluation panel. The report of the evaluation was released in April 2010¹.

Sample collection and preparation

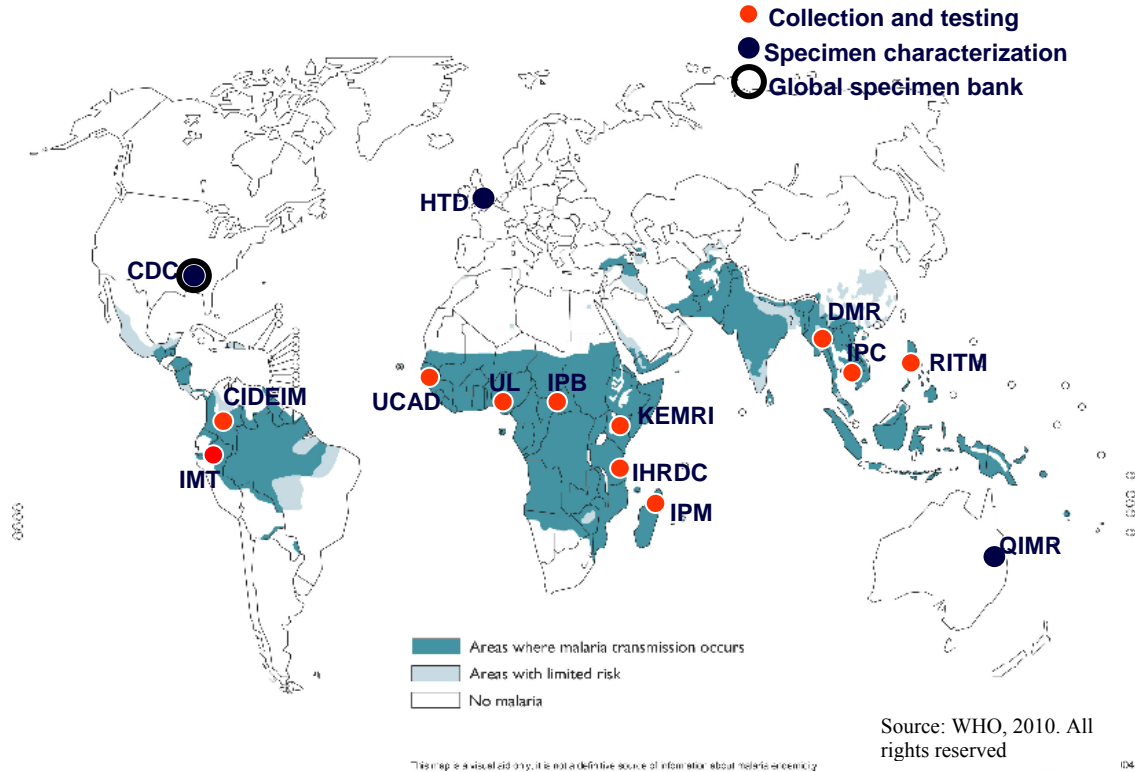
Evaluation panel samples consist of culture-derived *P. falciparum*, 'wild-type' *P. falciparum* or *P. vivax* derived from febrile malaria patients in malaria-endemic settings, or parasite-negative samples from people with or without differential diagnoses of malaria or other known blood characteristics that may influence test results. Panel samples are stored at below -70°C, and used once after thawing.

Samples were collected, prepared, and characterized at the following collection sites in partnership with the WHO and FIND.

Country	ISO Code	Institute	Address
Australia	AU	Army Malaria Institute	Enoggera, Brisbane
Cambodia	KH	Institut Pasteur Cambodge	Phnom Penh
Central African Republic	CF	Institut Pasteur Bangui	Bangui
Colombia	CO	CIDEIM	Cali
Kenya	KE	Kenya Medical Research Institute	Kisumu
Madagascar	MG	Institut Pasteur de Madagascar	Antananarivo
Myanmar	MM	Department of Medical Research	Yangon
Nigeria	NG	University of Lagos	Lagos
Peru	PE	Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia	Lima
Philippines	PH	Research Institute for Tropical Medicine	Muntinlupa City, Manila
Senegal	SN	Université Cheikh Anta DIOP	Dakar
Tanzania	TZ	Ifakara Health Research and Development Centre	Bagamoyo
United Kingdom	GB	Hospital for Tropical Disease	London
USA	US	Malaria Branch, Division of Parasitic Diseases Centers for Disease Control and Prevention	Atlanta

¹ WHO/TDR/FIND. *Malaria Rapid Diagnostic Test Performance - results of WHO product testing of malaria RDTs: Round 2(2009)*. Geneva, WHO, 2010.

Map of sample collection, preparation and characterization institutions.



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).

Details of specimen collection, preparation, characterization and use of panel samples are found in the Methods Manual for Laboratory Quality Control Testing of Malaria Rapid Diagnostic Tests (1), and the Methods Manual for Product Testing of Malaria Rapid Diagnostic Tests (2).

Collection and characterization of parasite-positive samples is as follows:

- Parasites are derived from monoclonal culture lines, or volunteering patients with fever and evidence of malaria parasitaemia.

- Parasite-positive samples have parasite density determined by two pre-qualified microscopists performing blinded readings, with a required concordance on parasite density within 20%. Dilution to standardized parasite densities is then based on a mean of these two results.
- All wild-type parasite samples are screened for HIV, hepatitis B and hepatitis C viruses, and virus-positive (HIV, HepB, HepC) samples are excluded.
- Species is confirmed by PCR for *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*, and multi-species infections are excluded.
- The concentration of the three target antigens, HRP2 (*falciparum* only); pLDH; and aldolase, which is determined by quantitative ELISA.
- HRP2 primary sequence is determined.

Collection and characterization of parasite-negative samples is as follows:

- Parasite-negative samples are derived from volunteers with specific blood types or diseases considered differential diagnoses of malaria, or from stored blood bank blood of other volunteers.
- Malaria is excluded by PCR for *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*.

Note on HRP2 sequence classification:

The primary structure of HRP2 is classified according to a value calculated using the number of the common Type 2 and Type 7 amino acid repeat sequences in the protein, as identified by Baker *et al.* (3): A (≥ 100 repeats), Type B (50 to 99 repeats), and Type C (≤ 50 repeats). The primary structure is determined as it could theoretically affect RDT sensitivity, but its effect may be limited (5,6).

Samples used in Round 2 evaluation panel

RDTs in Round 2 were evaluated against three panels, specifically:

- i) *P. falciparum* culture lines (includes a subset, 'manufacturer's panel') at low (200 parasites/ μ l) and high parasite densities (2000 or 5000 parasites/ μ l), one sample (US01FNigeriaXII) was also used as the standard for heat stability testing.
- ii) Wild-type *Plasmodium* samples (*P. falciparum*, *P. vivax*) from naturally infected humans and parasite-negative samples at low (200 parasites/ μ l) and high densities (2000 or 5000 parasites/ μ l).
- iii) Parasite-negative panel ('clean' samples and disease-specific or blood factor-specific samples).

A summary of outcomes of characterization of samples, including summary statistics for antigen concentrations at 200 parasites/ μ L, are given below.

Phase 1 (culture-derived) panel

HRP2 primary structure classification of Phase 1 panel

Culture line	HRP2 structure
US01F7G8	A
US01FBeninI	A
US01FBorneol	C
US01FECP	B
US01FFC27/A3	B
US01FFCH4	B
US01FGhanallI	B
US01FGhanaV	B
US01FGuineal	B
US01FHB3	B
US01FKE4	B
US01FKI28	B
US01FMalawil	B
US01FNigeriaXII	B
US01FPeru01064	B
US01FPH1	C
US01FPNG144	B
US01FSabahIII	B
US01FSantaLucia	B
US01FUgandall	A

Antigen concentration (ng/mL) of Phase 1 panel at 200 parasites/ μ L

	pLDH	HRP2	Aldolase
Mean	5.20	9.40	0.99
Median	3.72	4.56	0.96
Standard deviation	5.21	17.46	0.52
Maximum	18.57	79.90	2.03
Minimum	0.00	0.04	0.21

Results based on 20 samples (HRP2, pLDH) and 12 samples (aldolase)

Phase 2 patient-derived *P. falciparum* parasite panel

Geographical origin of Phase 2 *P. falciparum* -positive panel

Region	Country	N
South America	Colombia	10
	Peru	4
Africa	Central African Republic	13
	Kenya	2
	Madagascar	6
	Nigeria	23
	Tanzania	18
Asia	Cambodia	19
	Myanmar	2
	Philippines	3
Total		100

Antigen concentration (ng/mL) of Phase 2 (patient derived) *P. falciparum* -positive panel at 200 parasites/ μ L

	pLDH	HRP2	Aldolase
Mean	13.77	12.70	1.80
Median	10.41	6.76	1.50
Standard deviation	10.94	15.75	1.65
Maximum	47.15	73.70	9.90
Minimum	0.19	0.62	0.00

Concentrations of 1 or 2 antigens excluded from 11 samples where insufficient correctly-labeled sample were available for analysis

HRP2 primary structure classification of Phase 2 *P. falciparum*-positive panel

Sample ID	HRP2 type ¹	Sample ID	HRP2 type ¹	Sample ID	HRP2 type ¹	Sample ID	HRP2 type ¹
CF01F01	B	KH03F06	C	NG01F17	B	TZ01F13	MC
CF01F02	MC	KH03F07	C	NG01F20	MC	TZ01F14	A
CF01F04	B	KH03F08	B	NG01F22	B	TZ01F15	B
CF01F05	A	KH03F09	B	NG01F23	B	TZ01F16	MC
CF01F06	C	KH04F01	C	NG01F24	B	TZ01F17	MC
CF01F07	B	KH04F02	C	NG01F25	A	TZ01F18	B
CF01F09	C	KH04F03	C	NG02F01	A	TZ01F20	B
CF01F13	B	KH04F04	B	NG02F02	B	TZ01F23	B
CF01F15	B	KH04F05	C	NG02F08	B	TZ01F24	B
CF01F18	MC	KH04F06	B	NG02F13	A	TZ01F25	B
CF01F19	NA	KH05F01	A	NG02F16	B	TZ01F26	MC
CF01F22	NA	KH05F06	B	NG02F17	B	TZ01F27	A
CF01F23	NA	KH05F09	B	NG02F19	B	TZ01F28	A
CO01F02	B	KH05F10	MC	NG02F22	A		
CO01F04	B	KH05F11	B	NG02F25	B		
CO01F05	B	MG01F01	A	NG02F26	B		
CO01F08	B	MG01F02	B	NG02F33	B		
CO01F09	B	MG01F14	B	PE01F04	B		
CO01F11	B	MG01F19	B	PE02F01	B		
CO01F12	B	MG01F20	B	PE02F04	B		
CO01F13	B	MG01F22	B	PE02F29	B		
CO01F16	B	MM04F02	NA	PH7F04	A		
CO01F18	B	MM09F01	B	PH08F07	A		
KE01F05	NA	NG01F01	B	PH08F09	C		
KE01F14	NA	NG01F02	B	TZ01F01	MC		
KH03F01	MC	NG01F05	B	TZ01F02	MC		
KH03F03	B	NG01F06	B	TZ01F07	C		
KH03F04	B	NG01F08	A	TZ01F11	B		
KH03F05	B	NG01F16	B	TZ01F12	B		

1. MC: Probable multi-clone *P. falciparum* infection
2. NA: Not available

Phase 2 patient-derived *P. vivax* parasite panel

Geographical origin of Phase 2 *P. vivax*-positive panel

Region	Country	N
South America	Colombia	4
	Peru	22
Africa	Madagascar	1
Asia	Cambodia	11
	Philippines	2
Total		40

Antigen concentration (ng/mL) of Phase 2 *P. vivax*-positive panel at 200 parasites/ μ L

	pLDH	Aldolase
Mean	19.47	6.40
Median	16.80	6.03
Standard deviation	13.51	2.86
Maximum	47.90	13.40
Minimum	1.54	1.70

Concentrations of 1 or 2 antigens excluded from 5 samples where insufficient correctly-labeled sample were available for analysis

The distribution of antigen levels for Pf HRP2, Pf pLDH and Pv pLDH were compared between the Round 1 and Round 2 parasite panels to ensure consistency. No statistically significant differences in median antigen levels between the Round 1 and 2 panels were detected for any of the 3 antigens ($P > 0.2$, Mann-Whitney test).

Phase 2 parasite-negative panel

Geographical origin of 'clean' parasite-negative panel

Country	N
Madagascar	1
Nigeria	3
Philippines	20
Senegal	8
USA	18
Total	50

Parasite-negative panel with known disease or blood abnormality characteristics

Sample type	N
Dengue	4
RPR positive	9
ANA	13
Schistosomiasis	10
Rheumatoid Factor	4
Leishmaniasis	5
Chagas	2
Human anti-mouse antibody	3
Total	50

References

1. WHO/FIND/TDR, *Rapid Diagnostic Tests for Malaria: Methods Manual for Laboratory Quality Control Testing (Version 6)* 2010, WHO - Regional Office for the Western Pacific, TDR, FIND.
<http://www.wpro.who.int/sites/rdt/documents/list.htm>
2. WHO/FIND/CDC, *Methods manual for product testing of malaria rapid diagnostic tests (version 3)* 2010.
http://www.wpro.who.int/NR/rdonlyres/B9E0219D-6AA1-41AA-9404-110BC3D097D2/0/METHODSMANUALMaIRDTPPT_FINAL_Version3.pdf
3. Baker, J., et al., *Genetic diversity of Plasmodium falciparum histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests*. J Infect Dis, 2005. **192**(5): p. 870-7.
4. Lee, N., et al., *Effect of sequence variation in Plasmodium falciparum histidine-rich protein 2 on binding of specific monoclonal antibodies: Implications for rapid diagnostic tests for malaria*. J Clin Microbiol, 2006. **44**(8): p. 2773-8.
5. Lee, N., et al., *Assessing the genetic diversity of the aldolase genes of Plasmodium falciparum and Plasmodium vivax and its potential effect on performance of aldolase-detecting rapid diagnostic tests*. J Clin Microbiol, 2006. **44**(12): p. 4547-9.
6. Talman, A.M., et al., *Evaluation of the intra- and inter-specific genetic variability of Plasmodium lactate dehydrogenase*. Malar J, 2007. **6**: p. 140.