

Characteristics of evaluation panel used for Round 1 of WHO malaria RDT product testing at U.S. CDC, 2008

WHO-FIND malaria RDT evaluation programme

Introduction

The first round of product testing of malaria RDTs under the WHO-FIND malaria RDT evaluation programme was completed in November 2008 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 2009¹.

Sample collection and preparation

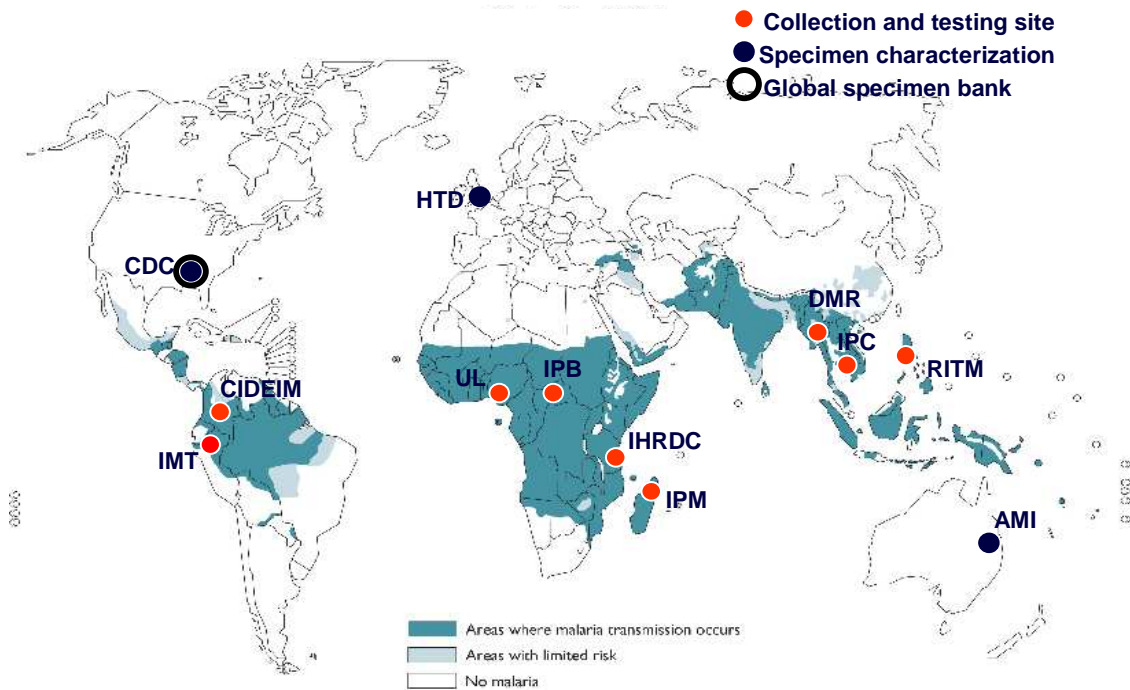
Evaluation panel samples consist of culture-derived *P. falciparum*, 'wild-type' *P. falciparum* or *P. vivax* derived from febrile malaria patients in malaria-endemic settings, or parasite-negative samples from people with or without differential diagnoses of malaria or other known blood characteristics 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 World Health Organization (WHO) and FIND (Foundation for Innovative New Diagnostics).

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

¹ WHO/TDR/FIND. *Malaria Rapid Diagnostic Test Performance - results of WHO product testing of malaria RDTs: Round 1 (2008)*. Geneva, WHO, 2009.

Map of sample collection, preparation and characterization institutions



Abbreviations: AMI Army Malaria Institute (Queensland, Australia); CDC Centers for Disease Control and Prevention (Atlanta, United States of America); CIDEIM Centro Internacional de Entrenamiento e 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, 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); RITM Research Institute of Tropical Medicine (Manila, Philippines); 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 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 IP (*falciparum* only); pLDH; and aldolase, which is determined by quantitative ELISA.
- HRP2 primary sequence is sequenced

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

- Parasite-negative samples are derived from people 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*, and multi-species infections are excluded.

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). This value has been previously shown to be to some extent predictive of the lower detection limit of certain HRP2-detecting RDTs which may reflect the affinity of anti-HRP2 antibodies to bind the protein (3,4). Aldolase and pLDH have been shown to have minimal variation in primary structure within species (5,6).

Samples used in Round 1 evaluation panel

RDTs in Round 1 were then 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).
- ii) Wild-type *Plasmodium* species (*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
US01FECP-200	B
US01FMalawil-200	B
US01FPeru1064-200	B
US01FGuineal-200	B
US01FSabahIII-200	B
US01FBorneol-200	C
US01FUgandall-200	A
US01FGhanall-200	B
US01FGhanaV-200	B
US01FHB3-200	B
US01F7G8-200	A
US01FKI28-200	B
US02FBeninI-200	A
US02FFCH4-200	B
US02FNigeriaXII-200	B
US02FPH1-200	C
US01FSantaLucia-200	B
US02FFC27/A3-200	B
US01FKE4-200	B
US01FPNG144-200	B

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

	pLDH	HRP2	Aldolase
Mean	2.7	9.1	0.9
Median	2.6	4.8	0.7
Standard deviation	2.2	18.5	0.5
Minimum	0.0	0.0	0.2
Maximum	8.3	79.9	2.0

Results based on first 17 samples (HRP2, pLDH) and samples 2-17 (aldolase)

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

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

Region	Country	N
South America	Colombia	7
	Peru	1
Africa	Central African Republic	13
	Madagascar	6
	Nigeria	12
	Tanzania	18
Asia	Cambodia	14
	Myanmar	5
	Philippines	3
Total		79

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

	pLDH	HRP2	Aldolase
Mean	15.3	15.3	2.0
Median	12.0	9.6	1.6
Standard deviation	11.3	17.0	1.7
Maximum	47.2	73.7	9.9
Minimum	0.7	0.8	0.1

Concentrations of 1 or 2 antigens excluded from 5 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	KH03F01	MC	MM01F01	B	PH08F09	C
CF01F02	MC	KH03F03	B	MM02F01	B	TZ01F01	MC
CF01F04	B	KH03F04	B	MM04F118	Awaited	TZ01F02	MC
CF01F05	A	KH03F05	B	MM06F01	B	TZ01F07	C
CF01F06	C	KH03F06	C	MM09F01	B	TZ01F11	B
CF01F07	B	KH03F07	C	NG01F01	B	TZ01F12	B
CF01F09	C	KH03F08	B	NG01F02	B	TZ01F13	Awaited
CF01F13	B	KH03F09	B	NG01F05	B	TZ01F14	A
CF01F15	B	KH04F01	C	NG01F06	B	TZ01F15	B
CF01F18	MC	KH04F02	C	NG01F08	A	TZ01F16	Awaited
CF01F19	Awaited	KH04F03	C	NG01F16	B	TZ01F17	MC
CF01F22	Awaited	KH04F04	B	NG01F17	B	TZ01F18	B
CF01F23	Awaited	KH04F05	C	NG01F20	MC	TZ01F20	B
CO01F02	B	KH04F06	B	NG01F22	B	TZ01F23	B
CO01F04	B	MG01F01	A	NG01F23	B	TZ01F24	B
CO01F05	B	MG01F02	B	NG01F24	B	TZ01F25	B
CO01F08	B	MG01F14	B	NG01F25	A	TZ01F26	MC
CO01F09	B	MG01F19	B	PE01F04	Awaited	TZ01F27	A
CO01F11	B	MG01F20	B	PH07F04	A	TZ01F28	A
CO01F12	B	MG01F22	B	PH08F07	A		

1. MC: Probable multi-clone *P. falciparum* infection

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

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

Region	Country	N
South America	Colombia	3
	Peru	10
Africa	Madagascar	1
Asia	Cambodia	4
	Philippines	2
Total		20

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

	pLDH	Aldolase
Mean	17.4	6.7
Median	12.7	6.3
Standard deviation	11.6	2.9
Minimum	5.1	3.2
Maximum	44.4	13.2

Phase 2 parasite-negative panel

Geographical origin of 'clean' parasite-negative panel

Country	N
Madagascar	1
Nigeria	3
Philippines	20
USA	18
Total	42

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	1
Total	48

References

1. WHO, *Rapid Diagnostic Tests for Malaria: Methods Manual for Laboratory Quality Control Testing. Version 5a*. 2008, WHO - Regional Office for the Western Pacific.
2. WHO/FIND/CDC, *Methods manual for product testing of malaria rapid diagnostic tests (version 2)*. 2009: Geneva.
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.