Global Problem of Malaria, Biology of Malaria Parasites and Implications for Transfusion-Transmitted Malaria and Detection Methods

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Malaria Workshop

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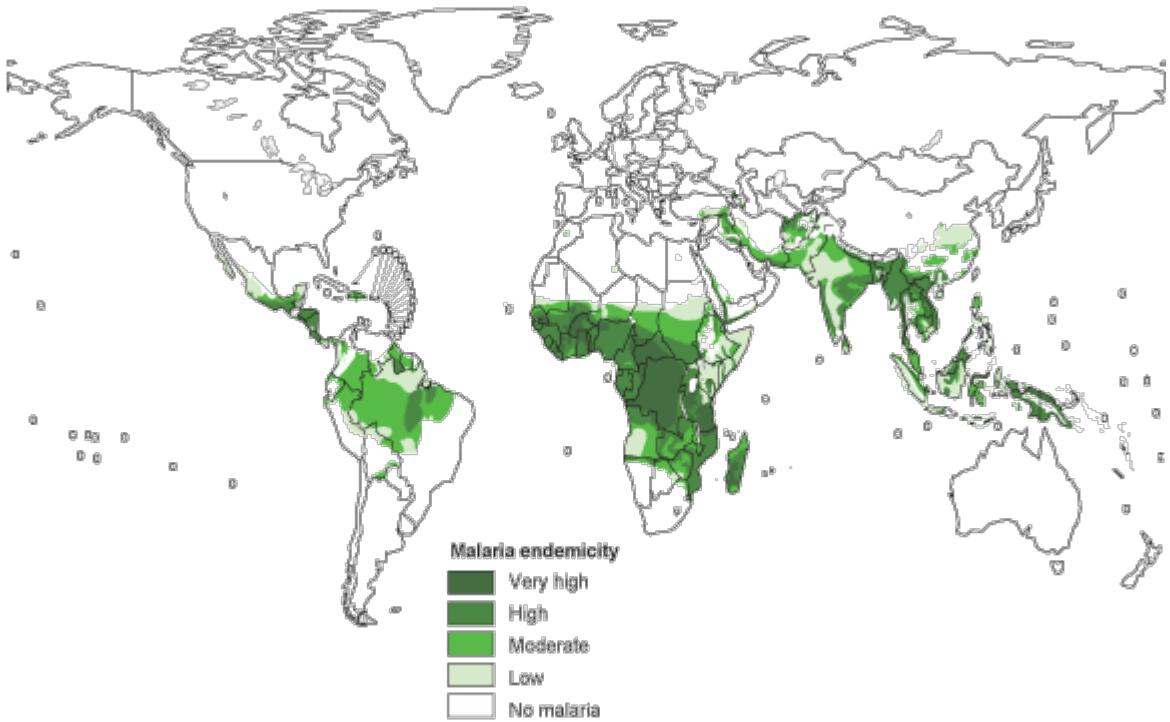
Epidemiology



- ***Plasmoidum falciparum, P. vivax, P. ovale, P. malariae***
- **Occurs in more than 100 countries throughout Africa, Asia, Latin America, and on certain Caribbean and Pacific Islands**
- **>3.2 billion inhabitants at risk**
- **300 – 500 million clinical cases**
- **~ 1 – 2 million per year**

World Malaria Report, WHO 2005

Representative Distributions of the Four Recognized Species of Human Malaria Parasites in the World Today

Distribution of species (%) in following areas (total no. of cases)					
Species	Sub-Saharan Africa		Asia (all) (863)	Central America and Caribbean (178,242)	South America (859,480)
	West and Central (858)	East and Southern (297)			
P. falciparum	88.2	78.8	4.2	12.9	29.2
P. vivax	1.2	9.8	95.6	87.1	70.6
P. malariae	2.2	3.0	0.0	0.0	0.2
P. ovale	8.4	8.4	0.2	0.0	0.0

Carter and Mendis, Clin Microbiol Rev 2002

Global Reach of Malaria

- In today's interconnected world, no country is immune from the hazards of malaria
- The problem of malaria is rising. There are more cases of malaria today than 30 years ago
- Major factors attributed to rise in malaria transmission
  - Environmental
  - Human activities
  - Drug resistance in malaria parasites
  - Vector populations

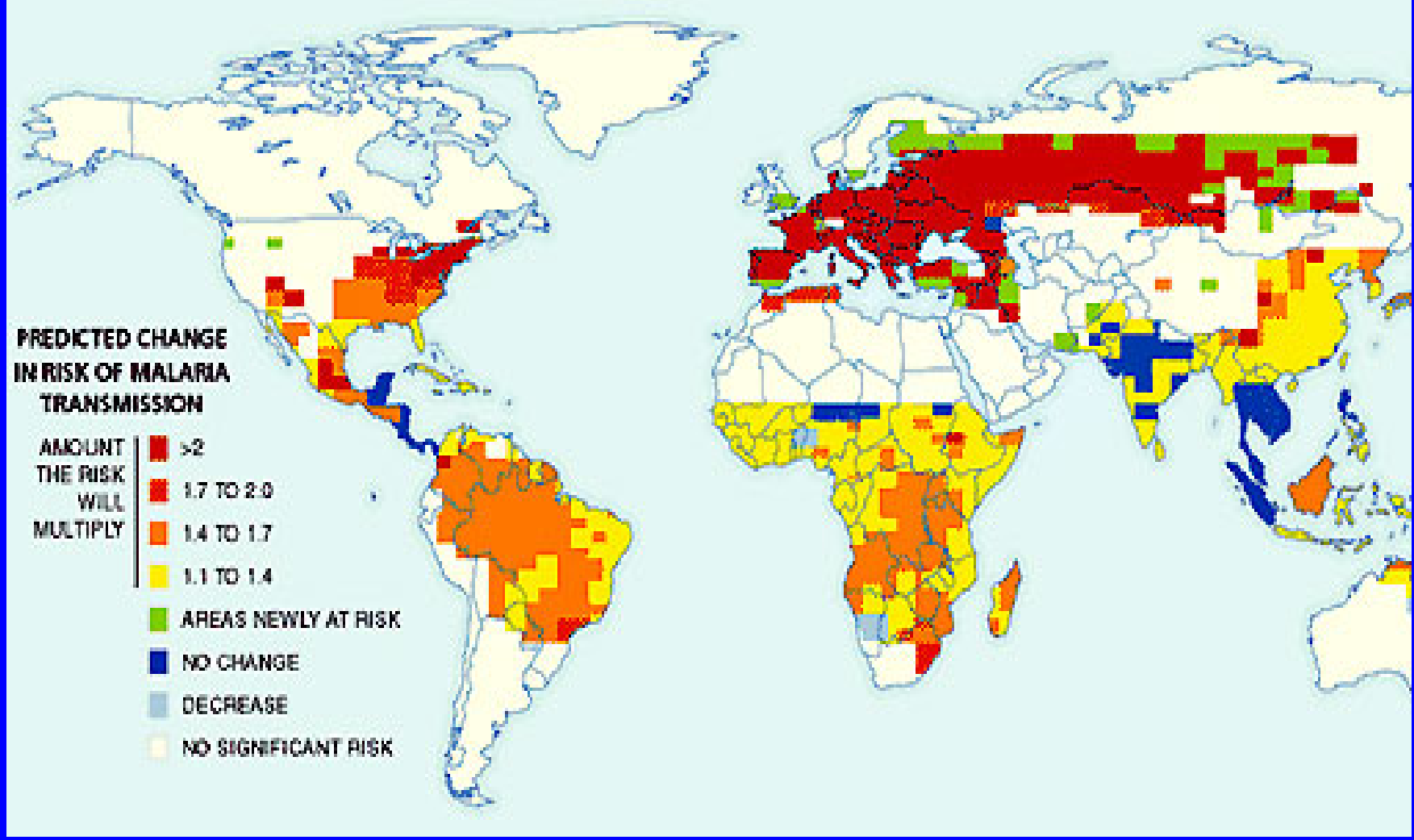
Malaria Mortality: Summary Statistics at the Beginning and End of the 20th Century

Region	Year	Total no. of deaths from malaria	% of all deaths due to malaria
Europe and North America	1900	80,000	0.8
	1997	20	0.0001
Caribbean, Central and South America	1900	42,000	2
	1997	4,000	0.05
Asia, China and Western Pacific	1900	2,800,000	9
	1997	65,000	0.1
Sub-Saharan Africa	1900	210,000	6
	1997	990,000	9
World minus Sub-Saharan Africa	1900	2,900,000	8
	1997	69,000	0.08
Total World	1900	3,132,000	
	1997	1,059,020	
Total World Annual Deaths/10,000	1900	19.4	
	1997	1.84	

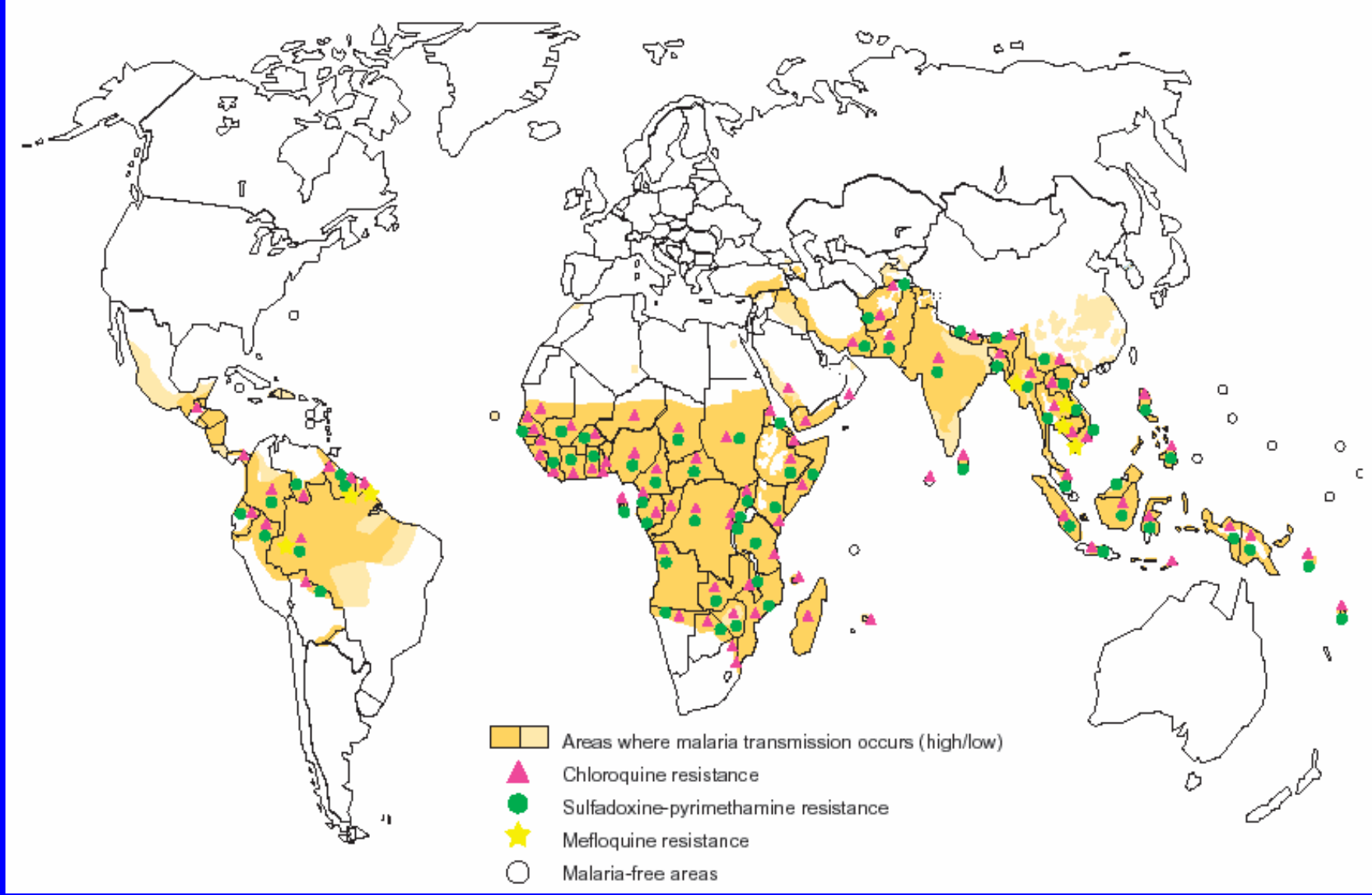
Carter and Mendis, Clin Microbiol Rev 2002

Malaria Incidence in India

Source: Tom Wellems, NIH

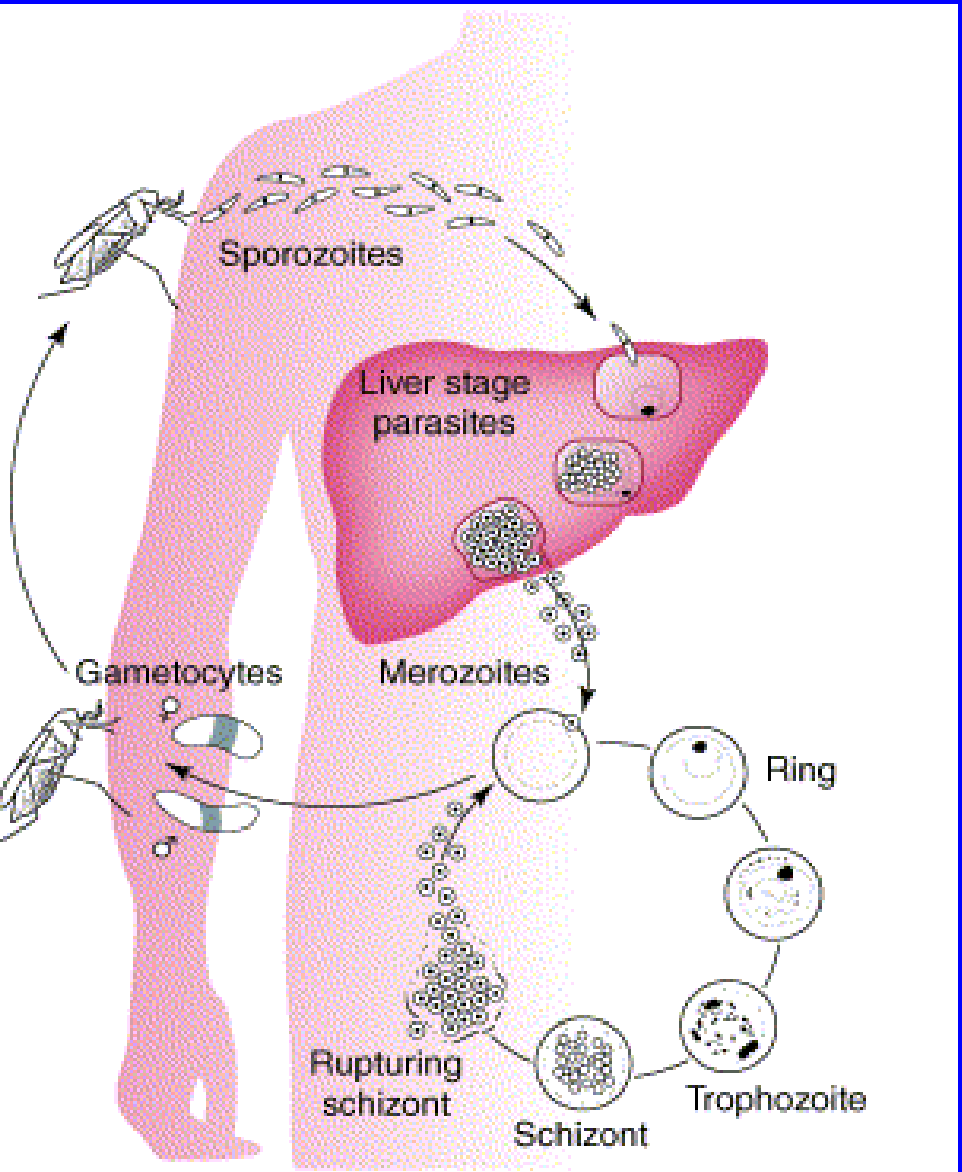


Projected Risk of Malaria Transmission in the year 2020 based on a global temperature increase of 2°F and no human efforts to contain the spread of malaria. *Source:* Pim Martens (http://www.exploratorium.edu/climate/global-effects/data3.html)



Drug resistance to *P. falciparum* from studies in sentinel sites, up to 2004. World Malaria Report, WHO 2005.

Malaria Life Cycle and Biology



- Liver stage: 6 – 14 days
- Blood stage: 48 – 72 hrs
- Incubation period: 21 days
- Primary infections: Clinical disease of varying manifestations
- Adults from endemic areas develop clinical immunity but carry low-grade parasitemia

Malaria Parasite Biology, Clinical Disease, Immunity, and implications for TTM

- **Incubation period: Time between infection to first appearance of blood form parasites (varies between species). *P. vivax* and *P. ovale* have dormant liver form stage causing relapse infection (months to a year or more)**
- **Chronicity of infection: *P. malariae* can be present in a host for up to 40 years**
- **Asymptomatic carriers: Multiple exposures in individuals born in endemic countries or expatriates with prolonged residence develop partial immunity while carrying low-grade parasite burden**
- **Parasite burden in asymptomatic carriers is not known**
- **Infectious dose of blood form malaria parasites is very low**

Donor populations that cause TTM and Implication for a Donor Screening Test

- **Travelers**
  - **No prior immunity**
  - **Infection can be acquired shortly before departure**
  - **Infection with a strain of Plasmodium with prolonged latency**

- **Residents**
  - **Born in an endemic country or had a prolonged residence**
  - **Asymptomatic carriers**
  - **Parasite burden in asymptomatic carriers is not known**

- **History of clinical malaria**
  - **Inadequate treatment**
  - **Relapse from liver form parasites**

Considerations for laboratory tests to detect malaria parasites in blood donors

- **Direct parasite demonstration (microscopy or DNA detection) is most suitable for all donor groups**
  - **Window period of exposure in travelers before testing should be allowed and low-parasite burden in asymptomatic carriers**
- **A surrogate of exposure such as the presence of anti-malaria antibodies can be indicative of a current infection or a previous exposure**
  - **Time lapse between parasite exposure and first appearance of antibodies in travelers and assay sensitivity in donors with primary infections and asymptomatic carriers**
  - **A few reports suggest that seroconversion occurs within a few weeks after appearance of blood form parasites**
- **A screening test should be able to detect all Plasmodium species that frequently cause TTM**

Methods to Detect Malaria Parasites

- Direct parasite demonstration
  - Microscopy
    - Thick blood film
    - QBC method
- Antigen detection
  - HRP, LDH etc. based dip sticks
  - Nucleic acid based methods
    - PCR test, TaqMan assay, Real-time PCR and Microarray
- Indirect demonstration of parasite exposure
  - Antibody based methods: IFAT, ELISA

THE LANCET.

A Journal of British and Foreign Medicine, Surgery, Obstetrics, Physiology, Chemistry, Pharmacology, Public Health, and News.

IN TWO VOLUMES ANNUALLY.

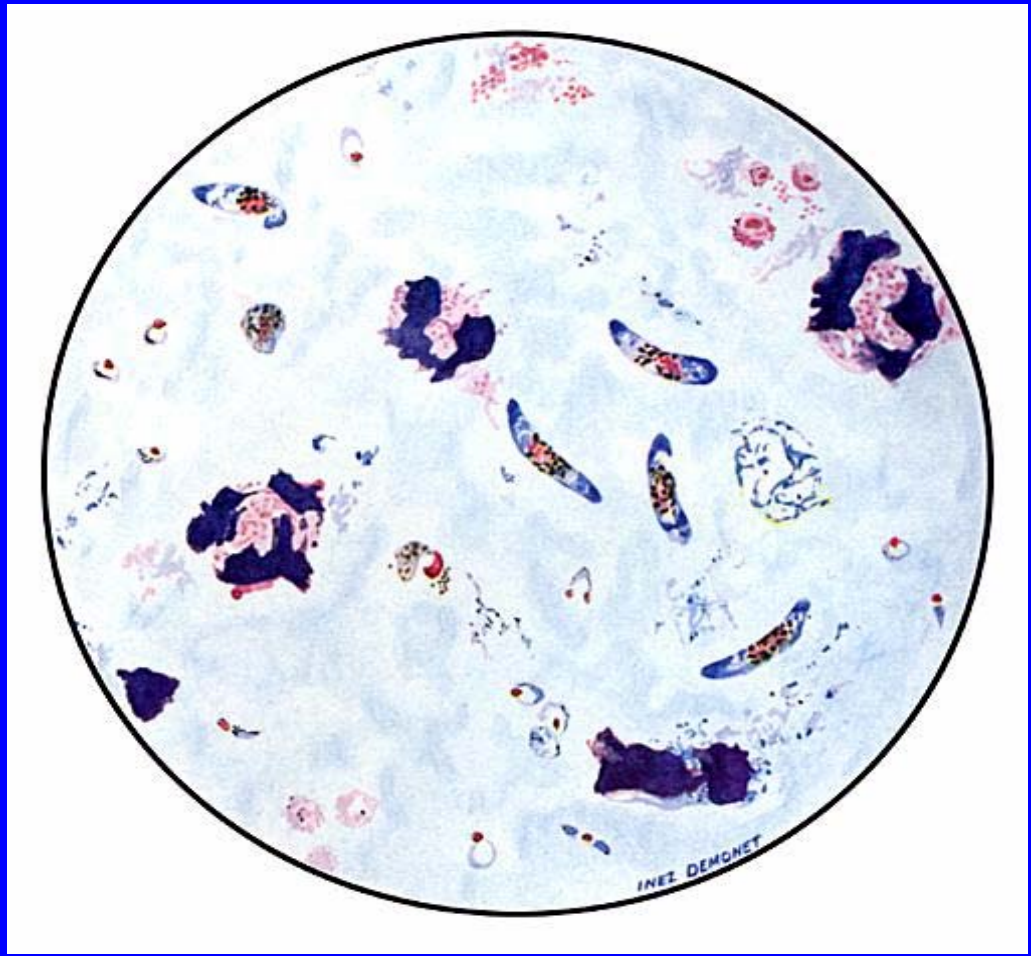
VOL I. FOR 1903.

AN IMPROVED METHOD FOR THE MICROSCOPICAL DIAGNOSIS OF INTERMITTENT FEVER.

BY RONALD ROSS, C.B., F.R.S., F.R.C.S. ENG., D.P.H.,

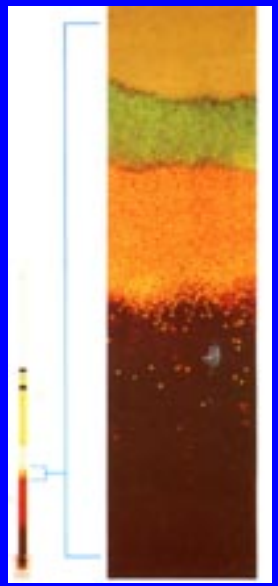
LECTURER ON TROPICAL MEDICINE AT UNIVERSITY COLLEGE, LIVERPOOL; WALTER MYERS LECTURER AT THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE; LATE MAJOR, I.M.S.

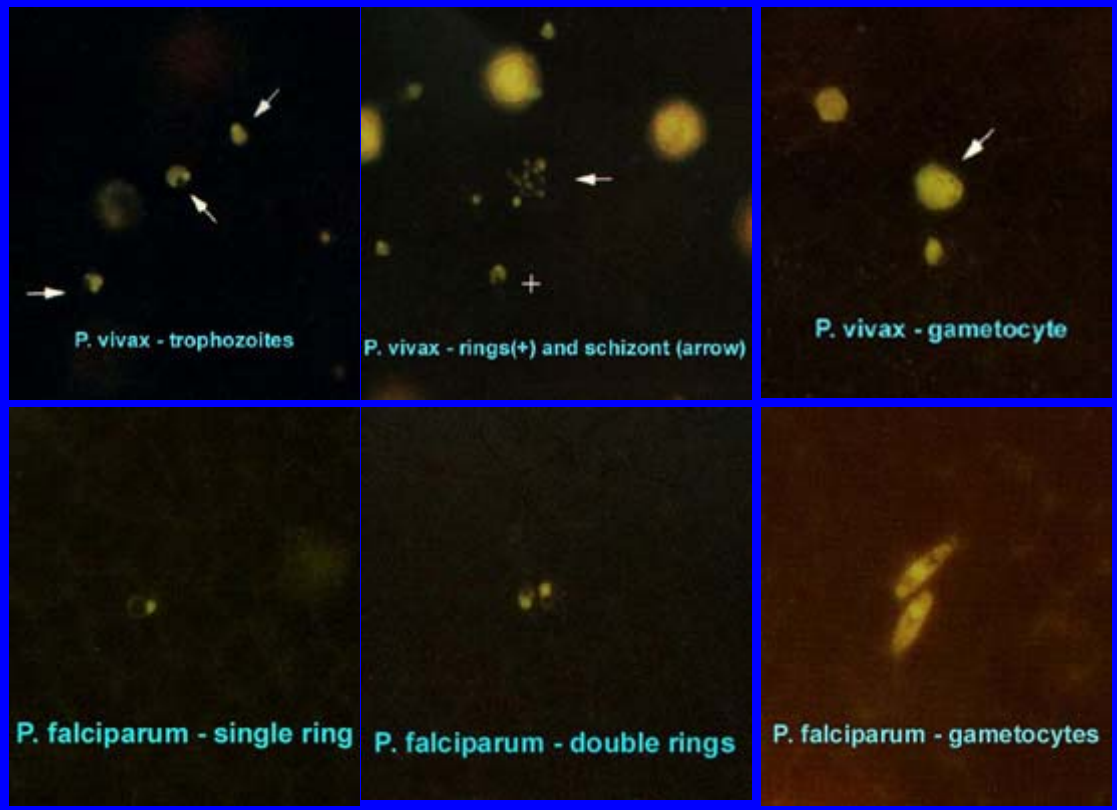
Plasmodium falciparum: Blood Stage Parasites Thick Blood Smears



Sensitivity limit: 5 – 500 parasites per µL of blood. Source: CDC
http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Malaria_il.htm.

The Quantitative Buffy Coat (QBC) Test

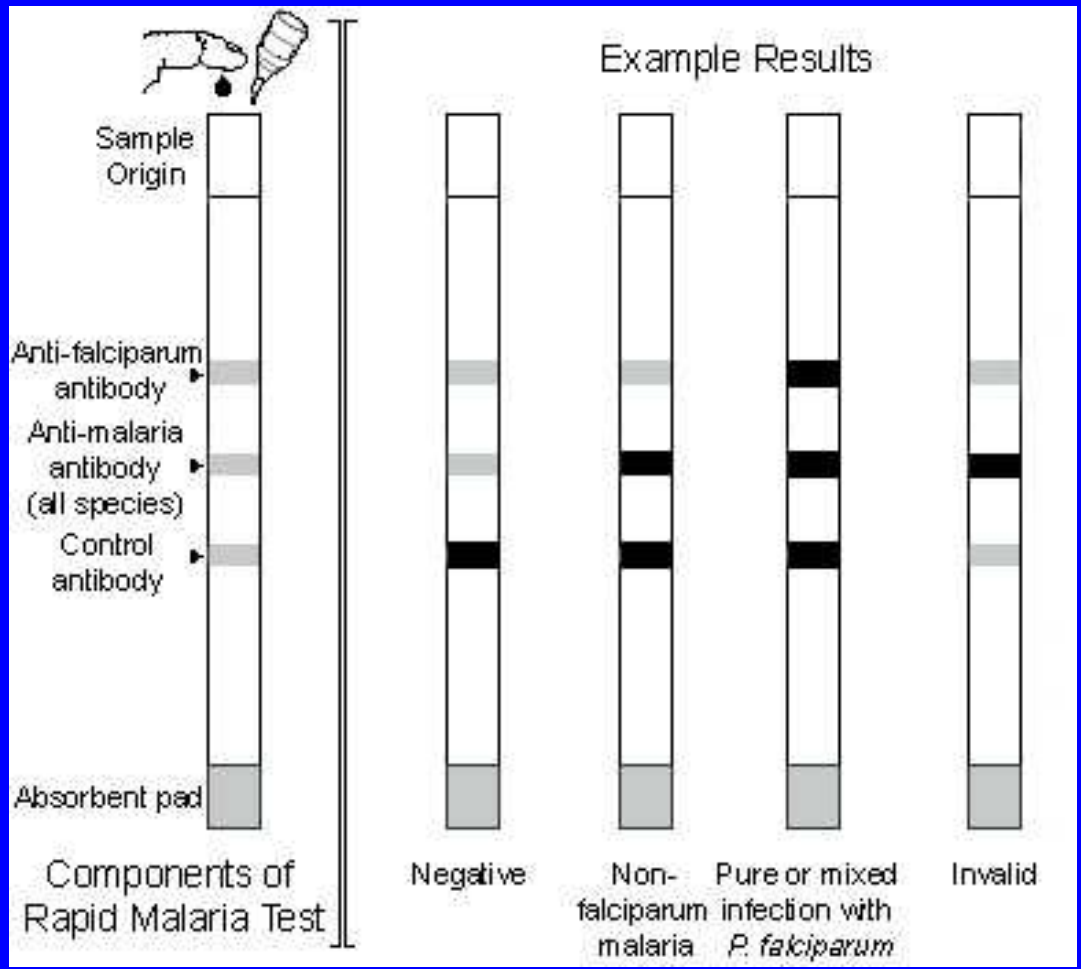




- A high precision glass hematocrit tube, pre-coated with acridine orange is filled with 55-65 µl of blood
- Centrifugation at 12, 000 rpm separates cells based on their densities
- Sensitivity is claimed to be as good as a thick smear

Source: http://www.malariasite.com/malaria/QBC.htm.

Antigen Detection Tests for Malaria

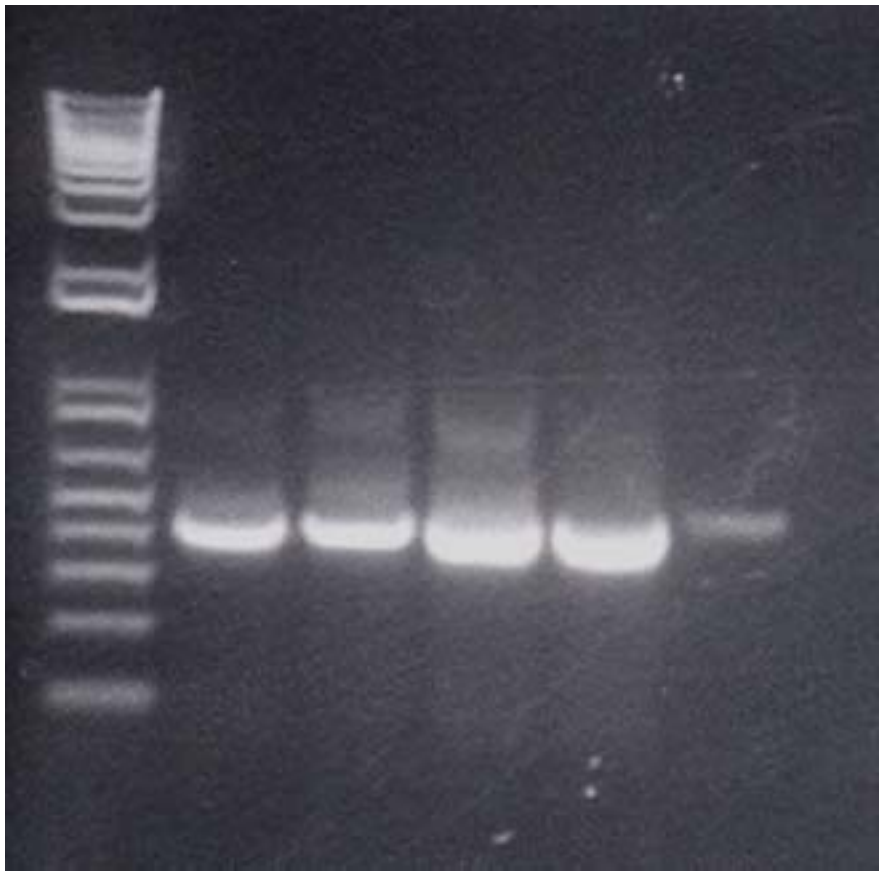


- Antigen capture by mAb or polyclonal antibody
- HRP-2, specific to Pf
- Aldolase and pLDH: pan-Plasmodium recognization
- Sensitivity: >83%
- Less sensitive in non-immune travelers
- Reactivity with auto-antibodies

Source: http://www.malariasite.com/malaria/rdts.htm#parasight

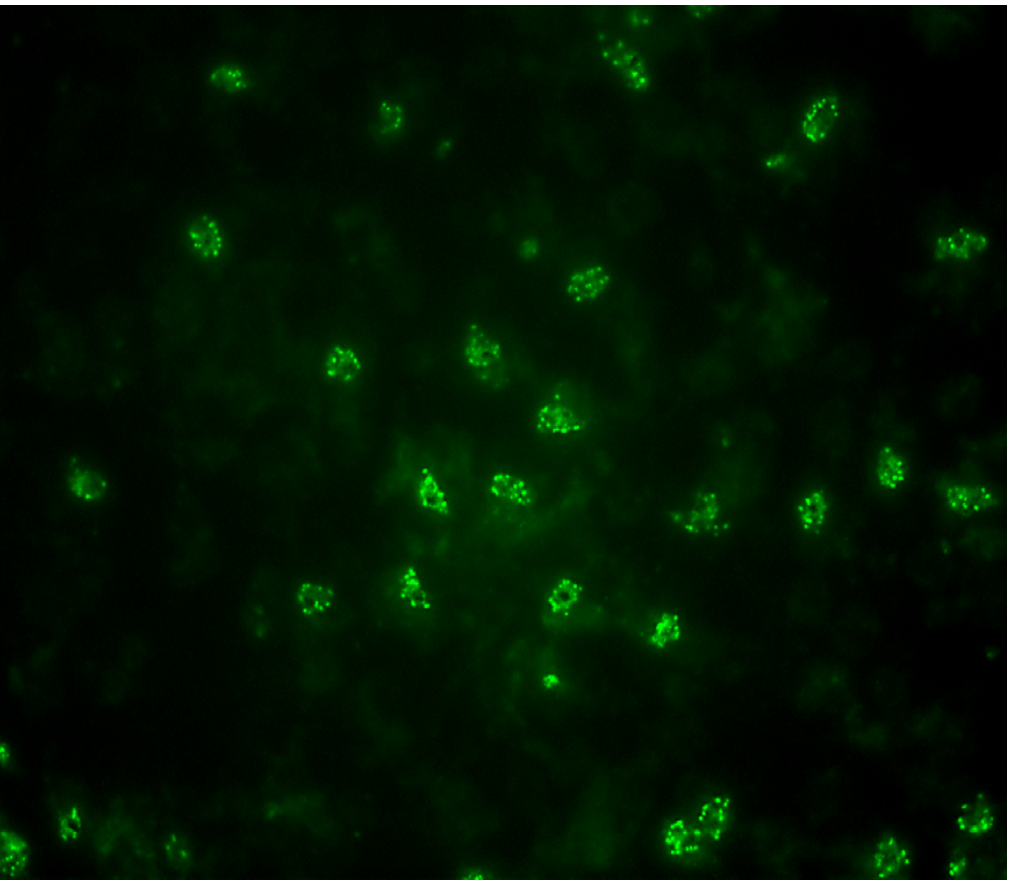
Nested PCR Amplification of 18 S rRNA Gene Fragment from *P. falciparum* Spiked in 1 µL of Human Blood

Direct Boiling Method



- 7-8 copies of 18 S rRNA
- Genus and species specific identification
- Potentially feasible for donor screening
- Sensitivity: 0.25 parasite/µl or 250 parasites/ml of blood
- 20-fold superior to thick blood film

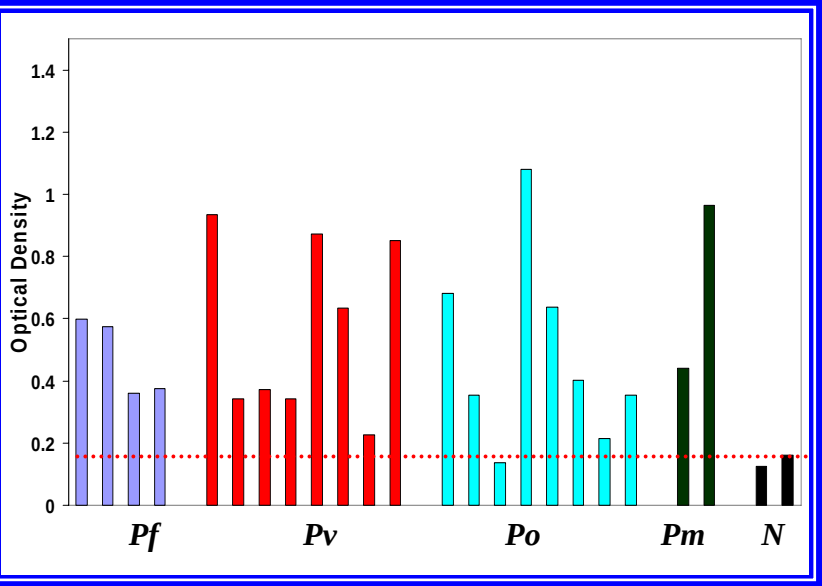
Indirect Fluorescent Antibody Test



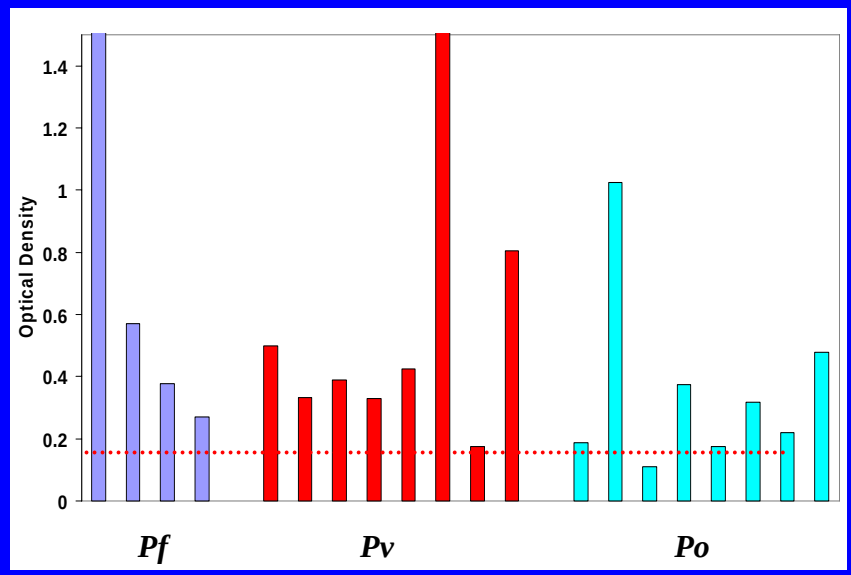
- **“Gold Standard” serology test**
- **Possible to detect all four Plasmodium species**
- **Highly effective in detecting antibodies in prior residents**
- **Cumbersome and difficult to develop an automated format**

Pan-Plasmodium reactivity of sera from malaria patients detected against individual & combination recombinant antigens in ELISA

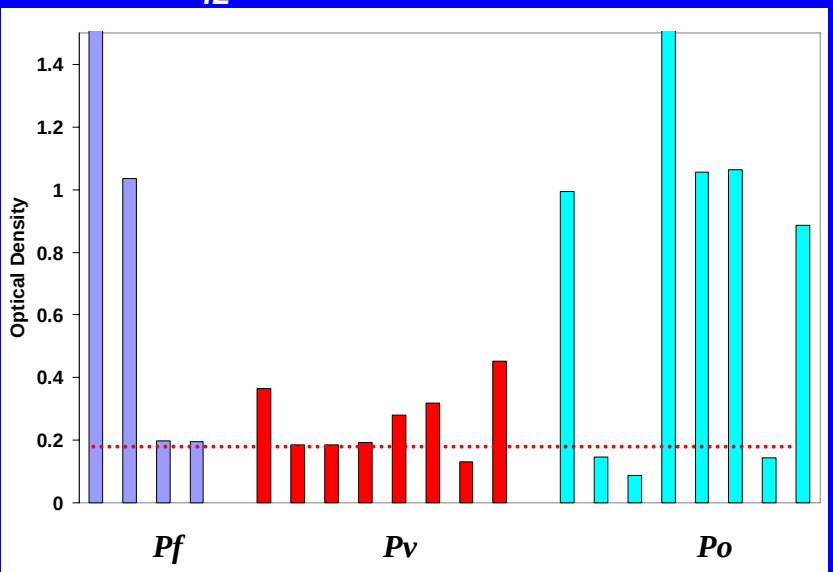
PfCSP



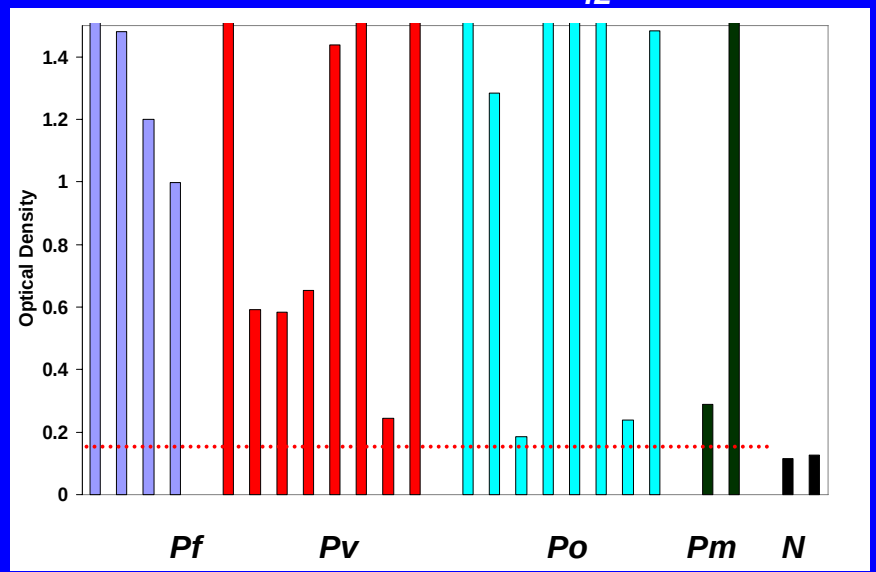
PfAMA1



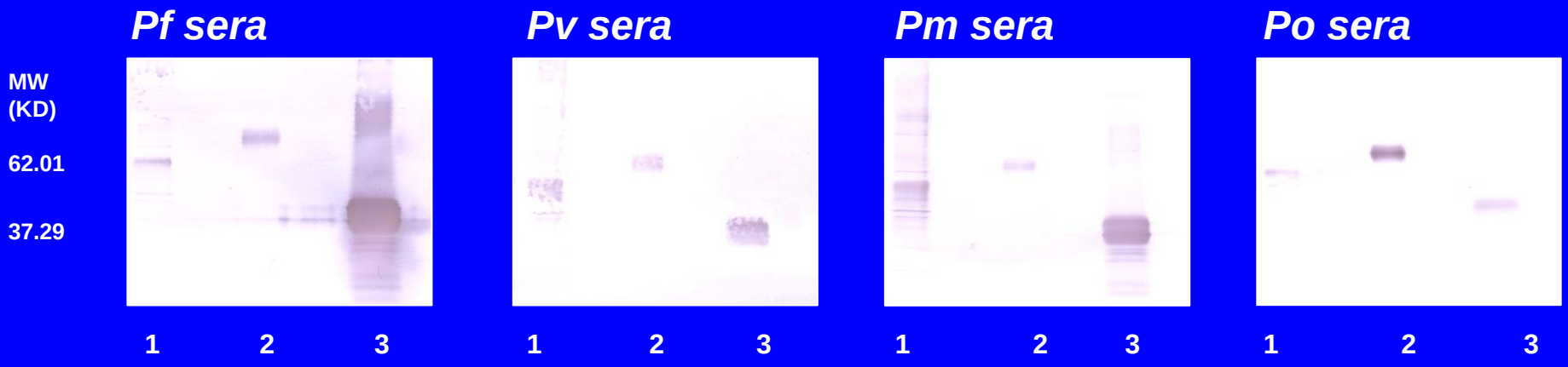
PfMSP1$_{42}$



PfCSP/PfAMA1/PfMSP1$_{42}$



Cross-species recognization of recombinant *P. falciparum* antigens with sera from *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* infected patients by western blot



Lanes 1: *rPfCSP*, 2: *rPfAMA-1*, 3: *rPfMSP1*$_{42}$

Acknowledgements

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