Parasite Biology

Malaria Parasite Bank

The Malaria Parasite Bank (MPB) was established in 1992. MPB is now functioning as a national resource facility and is involved in collection and characterization of field isolates of malaria parasites. Routine activities of the parasite bank include *in vitro* cultivation of *Plasmodium falciparum*, characterization of the isolates for susceptibility to antimalarials, cryopreservation, revival of adapted and non-adapted cultures, *etc*. Parasite isolates of *P. vivax* and *P. malariae,* malaria positive and negative sera, non-human malaria parasites in cryopreserved states and in their respective animal hosts, wherever possible, are also available in the bank. The details of malaria parasites and other biological material available in the parasite bank are given in Tables 1 and 2.

Table 1. Human malaria parasites preserved in the Parasite Bank

Parasite species	Collection sites		Years of collection			
	States	Districts	1992–2004	2005–06	2007–08	Total
P. falciparum	Andhra Pradesh	Visakhapatnam	12	—	—	
	Assam	Sonapur	20	—	—	
		Tezpur	6	—		
		Nalbari	—	1	—	
	Chhattisgarh	Jagdalpur	14	—	—	
		Bilaspur	—	26	—	
	Delhi		191	—	2	
	Gujarat	Anand	4	—	—	
		Kheda	7	—	—	
	Haryana	Gurgaon	25	—	—	
	Karnataka	Mangalore	0	14	—	
	Madhya Pradesh	Mandla/Jabalpur	14	—	—	
	Meghalaya	Tura	0	18	—	
	Mizoram	Kolasib	—	—	6	
	Orissa	Rayagada	29	—	—	
		Sundargarh	42	—	—	
	Rajasthan	Alwar	25	—	—	
		Bharatpur	35	—	—	
		Jaisalmer	38	—	—	
	Tamil Nadu	Chennai	0	4	—	
		Ramanathapuram	1	—	19	
	Uttar Pradesh	Baharaich	22	—	—	
		Gautam Budh Nagar	37	—	—	
		Ghaziabad	17	—	—	
		Allahabad	60	—	—	
	West Bengal	Kolkata	18	—	—	
		Midnapur	1	—	—	
		Total	618	63	27	708
P. vivax	Karnataka		0	6	—	6
	Delhi, Uttar Pradesh, Orissa		53	—	—	53
	Tamil Nadu		0	9	9	18
	Total		53	15	9	77
P. malariae	Orissa		4	—	—	4
	Delhi		1	—	—	1
Total			5	—	—	5
Total human malaria parasites						790

Malaria Parasite Bank is a National Resource for both Human and Non-human Malaria Parasites

Table 2. Total Non-human malaria parasites preserved in the Parasite Bank

Parasite species		Susceptibility to antimalarials
Simian malaria	*P. cynomolgi bastianelli* (CDRI)	Not done
	P. cynomolgi bastianelli (NICD)	–do–
	P. knowlesi (NICD)	–do–
	P. knowlesi (CDRI)	–do–
	P. fragile (CDRI)	–do–
Avian malaria	*P. gallinaceum*	Not done
	P. relictum	–do–
Rodent malaria	*P. berghei* (CDRI)	CQ-resistant
	*P. berghei**†	CQ-sensitive
	P. berghei	Quinine-resistant
	P. berghei ANKA	Not done
	P. berghei (NK65) (PGIMER, Chandigarh)	–do–
	P. chabaudi (Paris)	–do–
	P. vinckei petteri 279 BY	–do–
	P. yoelii nigeriensis (ICGEB)	–do–
	P. yoelii nigeriensis (CDRI)	Multi-resistant
	P. yoelii nigeriensis (LSHTM, London)**†	Not done
	P. yoelii yoelii (265 BY) (Paris)**	–do–

*Oocyst positive in *An. stephensi*; †Infective gametocyte producing strain; **Oocyst and sporozoite positive in *An. stephensi*

Screening of Drug Sensitivity Status

Since 1993, a total of 287 *P. falciparum* samples from different regions were tested for the sensitivity to chloroquine and 187 (67.03%) were found resistant to chloroquine (Table 3). Chloroquine (CQ) resistant and sensitive *P. falciparum* isolates were used for the studies on the role of *Pfmdr-1* gene in chloroquine resistance by studying the nucleotide changes at position 754, 1049, 3598, 3622 and 4234 in the coding region of *Pfmdr-1* gene and also to see whether any of the mutational changes can be used to study the detection of CQ resistance. The nucleotide changes in the said position were found ambiguous with strong association but incomplete correlation between

Table 3. Susceptibility/Resistance status of *P. falciparum* isolates to chloroquine (CQ) during 1992–2007

Place of collection	No. of samples tested	Response to chloroquine*	
		Susceptible	Resistant (%)
Delhi	74	17	57 (77.03)
Jaisalmer (Rajasthan)	22	1	21 (95.45)
Shankargarh (U.P.)	10	2	8 (80)
Gurgaon (Haryana)**	66	44	22 (33.33)
Sonapur (Assam)	18	5	13 (72.22)
Baharaich (U.P.)	11	6	5 (45.45)
Visakhapatnam (A.P.)	4	—	4 (100)
Gautam Budh Nagar (U.P.)	33	14	19 (57.57)
Bissam Cuttack (Orissa)	16	—	16 (100)
Rourkela (Orissa)	4	—	4 (100)
Jagdalpur (M.P.)	5	1	4 (66.66)
Tura (Meghalaya)	10	2	8 (80)
Mangalore (Karnataka)	1	—	1 (100)
Kheda (Gujarat)	1	—	1 (100)
Bilaspur (Chhattisgarh)	4	—	4 (100)
Kolasib (Mizoram)	6	6	—
Ramanathapuram (T.N.)	2	2	—
Total	287	100	187 (65.16%)

*WHO methods/kits were used; **Out of 66 samples tested from Gurgaon we could preserve only 25 *P. falciparum* samples, hence the difference in numbers.

chloroquine resistance and allelic variation in *Pfmdr-1* gene.

Erythrocyte Invasion Studies

Collaborative studies with ICGEB, New Delhi, aims to define the cytoadherence phenotypes and the invasion profile of Indian field isolates of *P. falciparum*, collected and cryopreserved in the parasite bank. Erythrocyte invasion by malaria parasites is mediated by specific molecular interactions. Sialic acid residues of glycophorin A are used as invasion receptors by *Plasmodium falciparum*. *In vitro* invasion studies have demonstrated that some cloned *P. falciparum* lines can use alternate receptors independent of sialic acid residues of glycophorin A. It is not known if invasion by alternate pathways occurs commonly in the field. In this study, we used in vitro growth assays and erythrocyte invasion assays to determine the invasion phenotypes of 15 *P. falciparum* field isolates. Of the 15 field isolates tested, 5 multiplied in both neuraminidase and trypsin-treated erythrocytes, 3 multiplied in neuraminidase-treated but not trypsin-treated erythrocyte, and 4 multiplied in trypsin-treated but not neuraminidase treated erythrocyte; 12 of the 15 field isolates tested use alternate invasion pathways are thus commonly used by *P. falciparum* field isolates (Table 4).

Table 4. Details of characterized *P. falciparum* parasites

Adapted isolates susceptible to chloroquine	54
Adapted isolates resistant to chloroquine	52
NF-54, an infective gametocytes producing strain of *P. falciparum*	1
3D 7A : a clone of NF-54	1
A-4 : a clone with binding property to CD36	1
Dd2: a clone which can invade trypsin treated erythrocytes	1
Field isolates which can invade trypsin treated erythrocytes	3
Field isolates which can invade neuraminidase treated but not trypsin treated erythrocytes	3
Field isolates which can invade normal erythrocytes but not in neuraminidase or in trypsin treated erythrocytes	3
Field isolates which can invade both in neuraminidase-treated and in trypsin-treated erythrocytes	5
Field isolates which can form rosettes	3
Field isolate which can bind to CSA	1
Field isolates which can bind to CD36	9
Field isolates which can bind to ICAM-1	2
Isolates with isoenzyme profile of GPI, GDH, ADA and LDH markers	22
Isolates with MSP-1, MSP-2 and GLURP markers	40

The experiments on erythrocyte invasion inhibition using anti EBA-175 showed encouraging results. Purified anti-EBA-175 R.II (F_2) antibody was used to test its ability to inhibit invasion of erythrocytes by parasites. One laboratory isolate 3D7 and two field isolates, RKL-9 and JDP-8, collected and preserved/maintained in parasite bank, with known invasion properties were selected for these studies. This antibody raised against EBA-175 showed about 80% inhibition compared to controls, indicating that this antibody is highly effective in blocking erythrocyte invasion by these parasites. Four more isolates were characterized for their cytoadherence properties, of which one from Assam and another from Delhi showed high binding to ICAM-1.

Cytoadherence

Cytoadherence refers to the ability of blood stage *P. falciparum* trophozoites and schizont to adhere to the vascular endothelium in the human host and bind to uninfected erythrocytes to form rosettes. Cytoadherence enables *P. falciparum* to avoid to passage through the spleen where infected erythrocytes are destroyed. The adhesion of *P. falciparum* infected erythrocytes in brain capillaries is implicated in the syndrome of cerebral malaria. The endothelial receptors used by *P. falciparum* for cytoadherence include CD36, ICAM-1, CD31, V-selectin, E-selectin and chondroitan sulfate –A (CSA). In our collaborative studies with ICGEB, we have screened few *P. falciparum* field isolates for their cytoadherence properties (Table 4). These studies will help in understanding the pathophysiological conditions during cerebral/complicated malaria.

Cultivation of Pre-erythrocytic Stage of *Plasmodium vivax in vitro*

For the first time in India, *P. vivax* pre-erythrocytic schizonts (liver stage) were developed in hepatoma cell line using the facilities of the parasite bank. National Institute of Malaria Research has well-established insectary facilities for the production of sporozoites in the laboratory. Mosquitoes were fed on infected blood through artificial membrane feeding apparatus and the fed mosquitoes were dissected on appropriate days for oocyst and sporozoites. These sporozoites from artificially fed mosquitoes were used for inoculating the hepatocytes/hepatoma cell line for the development of pre-erythrocytic stage parasites.

Cultivation of Erythrocytic Stage of *Plasmodium vivax in vitro*

Efforts have been made to cultivate and adapt erythrocytic stages of *P. vivax in vitro*, like *P. falciparum* in different combinations of media and culture conditions, with little success. A low-level parasitaemia could be maintained up to 52 days and growth of the parasites were observed for 2–3 cycles.

Table 5. Major Research Institutes/Universities which received biological material from the Malaria Parasite Bank

Andhra Pradesh
1. University of Hyderabad, Hyderabad

Assam
2. Defence Research Laboratory (DRL), Tezpur
3. Regional Medical Research Centre, Dibrugarh

Chandigarh
4. Post Graduate Institute of Medical Education and Research
5. Punjab University
6. Institute of Microbial Technology

Delhi
7. All India Institute of Medical Sciences
8. Department of Biochemistry, South Campus, University of Delhi
9. Department of Zoology, University of Delhi
10. Dr. B.R. Ambedkar Centre for Biomedical Research, University of Delhi
11. International Centre for Genetic Engineering and Biotechnology
12. Institute of Genomics and Integrative Biology
13. Jawaharlal Nehru University
14. Maulana Azad Medical College
15. National Institute of Communicable Diseases
16. National Institute of Immunology
17. Jamia Millia Islamia University
18. Jamia Hamdard University
19. Rapid Diagnostic Pvt. Ltd.

Gujarat
20. Medical College & SSG Hospital, Baroda
21. Sardar Patel University, V.V. Nagar, Anand
22. Veer Narmad South Gujarat University, Surat
23. Span Diagnostic, Surat

Haryana
24. Maharshi Dayanand University, Rohtak

Karnataka
25. Astra Research Centre, Bengaluru
26. Indian Institute of Science, Bengaluru
27. Regional Office for Health and Family Welfare, Bengaluru
28. University of Bangalore, Bengaluru
29. Banglore Genei, Bengaluru

Kerala
30. Cochin University of Science and Technology, Cochin
31. University of Calicut, Calicut

Madhya Pradesh
32. Defence Research & Development Establishment (DRDE), Gwalior
33. Department of Zoology, S.N. Jain Post Graduate College, Vidisha
34. University of Sagar (formerly University of Saugar)

Maharashtra
35. T.N. Medical College & B.Y.L. Nair Charitable Hospital, Mumbai
36. Wockhard Research Centre, Aurangabad
37. M.G. Institute of Medical Sciences, Sewagram

Orissa
38. Institute of Life Sciences, Bhubaneswar
39. Regional Medical Research Centre, Bhubaneswar
40. SCB Medical College, Cuttack

Punjab
41. Punjabi University, Patiala

Rajasthan
42. Birla Institute of Technology & Science, Pilani

Tamil Nadu
43. Manonmaniam Sundaranar University, Nagercoil, Kanyakumari
44. Bharathiar University, Coimbatore

Uttar Pradesh
45. Central Drug Research Institute, Lucknow
46. Central Institute of Medicinal and Aromatic Plants, Lucknow

West Bengal
47. Kalyani University, Kalyani
48. University of Calcutta, Kolkata
49. West Bengal University, Kolkata

This short-term culture system standardized in the parasite bank can be used for screening of anti-malarials *in vitro*.

Supply of Biological Materials

Providing malaria parasites to the scientific community has been one of the major activities of the parasite bank. Following biological materials were supplied to various institutes, universities and other research organizations (Table 5).

- Adapted and non adapted cryopreserved parasites isolates.
- Parasite isolates resistant/sensitive to chloroquine.

- Parasite isolates with rosetting properties.
- Parasite isolates with different cytoadherence properties.
- Isolates with different erythrocyte invasion properties.
- Sera/plasma from malaria positive patients.
- Different stages of parasites such as merozoites, ring forms, gametocytes from culture.
- Sporozoites harvested from artificially fed mosquitoes.
- Different species of avian, simian and rodent plasmodia.
- Rodent plasmodia infected rats/mice.
- Sera/plasma from respective vertebrate hosts.

Screening of Medicinal Plant Extracts for their Antiplasmodial Activity

As part of new drug (antimalarial) development, the parasite bank is involved in the primary screening of medicinal plants (extracts) collected from different parts of India. About 159 plant extracts/compounds were screened for their antiplasmodial activity. For this purpose the parasite bank is maintaining chloroquine resistant and sensitive *P. falciparum* and *P. berghei* isolates. Out of 159 samples tested, 39 showed antiplasmodial activity. The details of the samples tested are given in Table 6.

Table 6. Details of plant extracts/compounds screened for their antimalarial properties *in vitro*

Source	Total samples screened properties	No. of samples with antimalarial
NIMR, Delhi	20	9
RMRC, Dibrugarh	8	2
DRL, Tezpur*	40	12
DRDE, Gwalior	16	7
Kerala University**	72	6
Guru Nanak Dev University, Amritsar	3	3
Total	159	39

*Collaborative project (DRDO), **Collaborative project (DBT)

Human Resource Development

Imparting training is one of the mandates of NIMR. Expertise available for providing training in different techniques is listed in Table 7. Several scientists/research scholars were given training in parasite bank for one week to four months in different techniques.

A workshop on "Establishment of malaria parasite and screening of antimalarials" was conducted by late Dr. C. Usha Devi and Dr. C.R. Pillai at the Institute of Endemic Diseases, University of Khartoum, Sudan during 28 May to 12 June 2005 (17 scientists participated in the workshop). The details of training imparted from 1993–2008 are given in Table 8.

Table 7. Training facilities available in the parasite bank

- Collection, cryopreservation, revival and transportation of malaria parasite isolates/strains
- *In vitro* cultivation of erythrocytic stages of *P. falciparum*
- Short-term cultivation of *P. vivax* and other species of plasmodium
- *In vitro* cultivation of exo-erythrocytic stages of *P. vivax*
- *In vitro* testing for sensitivity of *P. falciparum* isolates to antimalarials
- *In vitro* and *in vivo* screening of medicinal plant extracts for antiplasmodial properties

Table 8. Training imparted to scientists/students (1993–2008)

Year	Indian	Foreign	Total
1993	1	—	1
1994	3	1	4
1995	2	4	6
1996	4	—	4
1997	4	—	4
1998	4	1	5
1999	2	1	3
2000	5	4	9
2001	—	—	—
2002	4	4	8
2003	8	1	9
2004	14	—	14
2005	4	17	21
2006	16	—	16
2007	17	2	19
Total	88	35	123

Cell Lines Available at the Parasite Bank

- Hepatoma cell line: Hep G2 A16 used in the *in vitro* cultivation of exo-erythrocytic stage malaria parasites
- Myeloma cell line: SP2
- Hybridomas: 2A 10 (anti-*P. falciparum* sporozoite antibody secreting cells)
- 2 F2 1 A7 (anti-*P. vivax* sporozoite antibody secreting cells).

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