B) SUMMARY

Title

Evaluation of rapid whole blood immunochromatographic Pf/Pv assay for the diagnosis of *Plasmodium falciparum* and *Plasmodium vivax* malaria.

Research Institute

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Chief Scientific Investigator

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Period of Contract

Date of award - 5th September 2001

Date of completion - 30th April 2002

Scientific Background and scope/ Objectives of project

Microscopic examination of blood smears still remains the gold standard for malaria diagnosis, but is labour intensive and requires skilled operators. Hence, the development of rapid and specific diagnostic tests to identify individuals infected with malaria is of paramount importance to control the severe public health impact of this disease.

Studies have been conducted in Sri Lanka using the Parasight-F test based on the detection of HRP-2 antigen of *P.falciparum*. This has proved to be as sensitive and specific as thick blood film examination by microscopy in Sri Lanka (Kodisinghe, 1997). The major disadvantage with Parasight-F was that it could not detect *P.vivax* malaria which composed of a greater percentage (70%) of the infections in Sri Lanka .

In the Moneragala district the number of microscopist for the Year 2001 was 19 while in the North and East the total number of microscopists was 34 (27 and 7 respectively). In comparison there were 41 microscopists each, in Kurunegala and Anuradhapura districts which are also malaria endemic areas in Sri Lanka. Since 48% of the malaria cases have been reported from the Northern district where microscopists are few or not available (e.g.Mannar, no microscopist available in the Year 2000/2001) (Personal communication, Dr.Punsiri Fernando, Director, Anti Malaria Campaign) use of an alternative diagnostic methods would be very useful for effective malaria control. Currently, rapid diagnostic tests to detect both species of malaria are being used only in the private sector in Sri

Lanka. The results of this study would help to use such tests in areas of Sri Lanka where microscopists are deficient.

Objective

To evaluate the effectiveness of ICT Malaria P.f/P.v at the field level in detecting malaria infections in Sri Lanka as compared to microscopy

Specific Objectives

3) To determine the sensitivity and specificity of ICT Malaria P.f/P.v in the diagnosis of *P.falciparum* or *P.vivax* malaria as compared to microscopy

4) To assess whether ICT Malaria P.f/P.v can be used to give an indication of the parasite density and clinical severity of disease

3) To do a cost analysis of the Rapid Diagnostic Test as compared to Microscopy

Experimental methods

Prospective study conducted from May 2001 to March 2002. All persons above five years of age who presented to the Malaria Research Station, Kataragama or the Anti-malaria Clinic, Kurunegala, with a history of fever were recruited into the study. Thick and thin blood smears were examined for malaria parasites. All blood films negative in the field (100 fields) re-examined in a central laboratory (400 fields). The rapid diagnostic test (RDT), ICT P.f/P.v (AMRAD ICT, Australia) was performed

simultaneously by an independent investigator. The severity of clinical disease of all patients was evaluated. The unit cost for blood film examination and ICT P.f/P.v was calculated assuming that there are 290 working days per year. Normally a maximum of 65 slides are examined a day

Results

The study population comprised 328 individuals of which 126 (38%) were found to be infected, 102 with *P.vivax* (31.1%) and 24 with *P.falciparum* (7.3%). 202 individuals were negative for malaria. The RDT was found to be highly sensitive (100%) and specific (100%) for diagnosis of *P.falciparum* when compared with field microscopy Figure 1. The sensitivity for the diagnosis of vivax malaria was only 70%(Figure 2). When *P.vivax* parasitaemia is greater than 5000 parasites/ µl the RDT is 96.2% sensitive (Table 1). A significant association was seen between the band intensity on the dipstick and peripheral blood parasitaemia (Table 2) and clinical severity of disease in *P.vivax* ($p<0.001$ and $p=0.011$ respectively) (Table 3).

The cost incurred for blood film examination is shown in Table 4 and 5 while the unit cost for performing the rapid diagnostic test is given in Table 6. The total cost for carrying out a blood film examination was approximately Sri Lankan Rupees 27.00 while Rs. 367.00.

Figure 1. Validation of the immunochromatographic test for *P.falciparum* (100 microscopic fields examination)

	Blood film (Gold standard)	
ICT	21	3
	0	304

Sensitivity 100%

Specificity 99%

Figure 2. Validation of the immunochromatographic test for *P.vivax* (100 microscopic fields examination)

	Blood film (Gold standard)	
ICT	79	2
	29	226

Sensitivity 70%

Specificity 99%

Table 1. Sensitivity of ICT P.f/P.v assay by level of *P.vivax* parasitaemia

Parasites/μl	Samples Positive by blood film (100 fields)	Samples positive by blood film (400 fields)	Samples positive by ICT	Sensitivity (%) (100 fields)	Sensitivity(%) (400 fields)
≤500	0	4	0	0	0
501 to 1000	9	9	1	11.1	11.1
1001-5000	63	63	43	68.1	68.1
>5000	27	27	26	96.2	96.2

Table 2. Association between mean parasitaemia and band intensity of ICT P.f/P.v assay

Band intensity	Mean (± SD) Parasitaemia/ μl in patients with			
	n^{α}	*P.vivax*	n^{β}	*P.falciparum*
No band visible		1221.09 (611.42)		-
Faint	32	4266.66 (3060.00)		
Clear	45	7296.00 (4110. 40)	10	2642.50 (2702.57)
Strong	25	-	14	1389.28 (1020.59)
F-value		31.143		2.542
p-value		<0.001		0.125

$^{\alpha}$ Number of cases of *P.vivax*

$^{\beta}$ Number of cases of *P.falciparum*

Table 3. Association between mean total clinical score and band intensity of ICT P.f/P.v assay

Band Intensity	Mean (± SD) clinical score in patients with			
	n[β]	*P.vivax*	n[α]	*P.falciparum*
No band visible	32	8.469 (3.98)		-
Faint	45	9.377 4.23)		-
Clear	25	11.680 (3.49)	10	9.000 (2.44)
Strong		-	14	11.785 (3.68)
F value		4.749		4.323
p-value		0.011		0.049

[α] Number of cases of *P.vivax*

[β] Number of cases of *P.falciparum*

Table 4. Recurrent expenditure for the preparation of a blood film

Category	Cost per slide (Sri Lankan Rupees)
Laboratory staff	
Microscopist	6.15
Laboratory orderly	2.96
Laboratory labourer	3.18
Supplies	12.00
Maintenance	1.64
Total	25.93

Table 5. Capital Costs for the preparation of a blood film

Category	Cost per slide (Sri Lankan Rupees)
Equipment	0.76
Training personnel	0.17
Total	0.93

Table 6. Cost of Rapid Diagnostic test

Category	Cost per kit (Sri Lankan Rupees)
The cost of a single ICT P.f/P.v	= 360.00
Cost of lancet	= 2.00
Cost of supplies (Cotton wool Rs, 8700 per annum and alcohol Rs.28,420 per annum which is Rs.37,100.00 per year, Rs.127.93 per day)	= 1.96
Salary per annum of non-microscopist who is trained to carry out ICT Rs.62,400 per annum, Rs.215 per day	= 3.30
Total unit cost of performing and ICT P.f/P.v for diagnosis of malaria	= 367.26

Discussion

In Sri Lanka, in the remote villages and the north and east where there is a dearth of microscopists, control of malaria is a great challenge. If rapid diagnostics are being used in the field, these tests can be used for on the spot diagnosis and treatment of both *P.vivax* and *P.falciparum* infection by non-medical, trained staff and this could help to attain the goals of the Roll Back Malaria initiative. In view of this, the recently developed ICT

P.f/P.v has been tested at village level and compared with results of traditional blood film examination. *P.vivax* is the predominant species, in Sri Lanka, accounting for about 60-80% of all malaria infections. These tests can be used as an epidemiological tool as it can be used to identify the *Plasmodium* species infecting the patients and it allows the delivery of appropriate chemotherapy rather than treatment based on clinical diagnosis which is practiced in areas where there are no microscopists.

However, in areas with already established laboratory networks it is cheaper to use microscopy for diagnosis of malaria, providing there is prompt and accurate laboratory reporting.

Conclusions and recommendations

The ICT P.f/P.v test can be used in areas of Sri Lanka in the absence of microscopists, but it should not replace microscopy.

Papers published on work done under contract

D.Fernando, P.Fernando and N.D.Karunaweera (2002). Evaluation of rapid whole blood immunochromatographic Pf/Pv assay for the diagnosis of *Plasmodium falciparum* and *Plasmodium vivax* malaria. *Eleventh Annual Academic Sessions of the Sri Lanka College of Microbiologists, 7th to 9th June 2002.*

This communication also received the third prize which was awarded for free papers.

A copy of the abstract and certificate are attached herewith.

C) The MD Thesis titled "Evaluation of rapid whole blood immunochromatographic Pf/Pv assay for the diagnosis of *Plasmodium falciparum* and *Plasmodium vivax* malaria". submitted to the Post Graduate Institute of Medicine has been accepted. A copy of the thesis will be submitted for deposition in the library.

Annexure 1

ID NO

To be filled up after examination of blood

1. Is the blood firm positive for malaria parasite ?
   a) By examination of 100 microscopic fields Y/N
   b) By examination of 400 microscopic fields Y/N

2. Parasite species PV/ PF /MX

3. Parasitaemia % _____

4. Gametocytaemia %_____

5. R____/ A___/S___/ G____ (enter numbers)

6. Time of arrival of patient _____am/pm

7. Time the patient left the clinic _____am/pm

8. Time taken to examine the blood film and give the result___ Mins

9. Is the blood sample positive for malaria parasites by ICT Malaria P.f/ P.v Y/N

10. Parasite species PV/PF

11. Band intensity
    Faint – 1 / Clear- 2 / Strong - 3

12. Time taken to perform the ICT Malaria P.f/ P.v and give the result_____ Mins

13. Treatment

	No. of tablets
Chloroquine	_____
Primaquine	_____
Paracetamol	_____
Other	_____ (specify)

Annexure 2

ID NO

Clinical notes and other relevant information regarding the present illness

1.Name_____

2.Address_____

3. Date of birth __ /__ /__ (Day/Month/Year)

4. Date of first visit __ /__ /__ (Day/Month/Year)

5.1 Time since last malaria infection (days/months/years)_____

5.2 Species Pv/Pf/Mixed

6. Any drugs taken within the past one month? _____

7.How long is it since you fell sick and this visit to the clinic_____ hrs / days.

8.Regarding the present illness

a. Temperature at presentation _____ (C)(F) :

b.	Headache:	absent/ mild/ moderate/ severe	
c.	Myalgia;:	absent/ mild/ moderate/ severe	
d.	Arthralgia:	absent/ mild/ moderate/ severe	
e.	Shivering:	absent/ mild/ moderate/ severe	
f.	Cold:	absent/ mild/ severe	
g.	Sweating and hot spells:	absent/ mild/ severe	
h.	Nausea:	absent/ mild/ severe	
i.	Vomiting:	absent/ mild/ severe	
j.	Anorexia:	absent/ mild/ severe	
k.	Backache:	absent/ mild/ severe	
l.	Hypochondrial pain:	absent/ mild/ severe	R/ L

8. Our assessment of sickness well / sick / very sick.
9. Monthly family income

10. Occupation