Research Report

NHRC/WHO/ Rockefeller Young Biomedical Scientist -2003 Award

Use of Polymerase Chain Reaction (PCR) for the diagnosis of malaria in Nepal

2003

Submitted by

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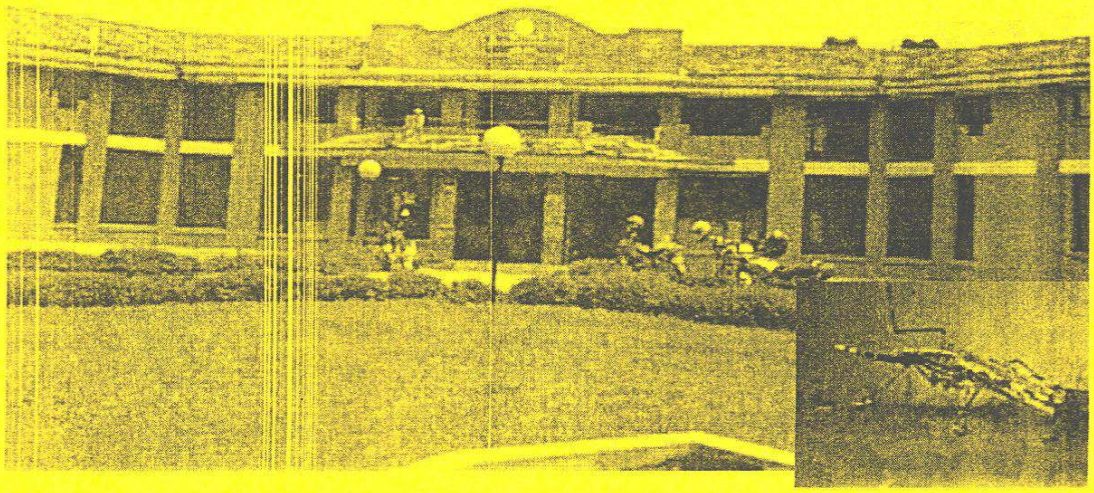


Introduction

Malaria has probably had a greater impact on world history than any other infectious disease. It has been responsible for the outcome of wars, population movements, and the growth and development of various nations throughout the world. More than 300 million individuals throughout the world are infected with malaria, and more than 1 million people a year, most of whom are children, are being killed by the disease. It is still a very common disease in many parts of the world, particularly in tropical and subtropical areas. Of the four most common species that infect humans, *P. vivax* and *P. falciparum* account for 95% of infections. *P. vivax* has the widest distribution, extending throughout the tropics, subtropics and temperate zones. *P. falciparum* is generally confined to the tropics. There has been a definite increase in the number of cases of *P. falciparum* malaria reported, which may be related to increased resistance to chloroquine. Malaria prevention is difficult, and no drug is universally effective. Vaccine development studies are ongoing, but malarial vaccines are not yet in general use (Garcia *et al*, 1997).

Worldwide, the number of cases of malaria caused by *Plasmodium falciparum*, the most dangerous species of the parasite, is on the rise. Drug resistant strains of *Plasmodium falciparum* are spreading rapidly. Further more, mosquitoes are becoming increasingly resistant to insecticides, and in many cases, have behavioral adaptation to avoid insecticide-treated surfaces altogether.

Malaria cases among tourists, travelers, military personals and migrant workers in malarious areas have been increasing steadily during the last several years, posing new concerns that the disease would be introduced to currently non-malarious areas of the world. Recent epidemics have claimed tens of thousands of deaths in Africa, and there is an increasing realization that malaria, as it was in the past may pose as a major impediment to socioeconomic development in many countries.

In many developing countries, resources for malaria diagnosis are sparse or unavailable. Small numbers of trained microscopists and microscopes often limits local clinics examining blood smears from a large number of patients suspected of having malaria.

Consequently, malaria diagnosis is often made only on the basis of clinical symptoms although this is, at best, 50% accurate (WHO, 1994).

Specific diagnosis of malaria is, usually made by microscopic examination. However, microscopic examination even by expert microscopists is time consuming, labor-intensive, and it is difficult to diagnose mixed *P. falciparum* and *P. vivax* infections, later being present predominantly in ring stages. Microscopic examination will not be cost-effective in malaria control in an area with very low but persistent transmission, because the cost to diagnose one positive slide will be high. There is therefore a need to develop alternative diagnostic methods to detect cases especially those with low-grade parasitemia to supplement & perhaps to replace microscopy in malaria control programs. (Tharavanij S, 1990)

A key feature of the new WHO Global Malaria Control Strategy (WHO, 1991) is the rapid diagnosis of malaria at the village and district level, so that effective treatment can be administered quickly to reduce morbidity and mortality. The need for rapid diagnosis of *P. falciparum* is most acute because of the severe nature of this infection and its non-specific symptomatology.

Diagnosis is the cornerstone for proper management of clinical cases, prevention of serious complications, institutions of specific antimalarial therapy and institution of effective control measures for eradication of disease.

The emerging problems associated with increased incidence of disease and death, resistance of malaria parasites and the vectors, socio-economic impediments, along with the lack of new, simple and sensitive diagnostic modalities, have led to a decreased capacity to optimally use the existing tools to combat the disease.

Microscopy still remains the mainstay in the diagnosis. However this is time consuming requires high expertise and its sensitivity is low in cases of low parasitemia, cerebral malaria, transfusion associated malaria and in malaria associated Tropical Splenomegaly Syndrome.

PCR detection method has been found to be more sensitive for *P vivax* (91%) than *P. falciparum* (89%). Level of parasite detection has been reported to be as low as 1 to 10 parasites per µl of blood in patient sample. Diagnosis by PCR has also been reported to be species specific for all four species of human Plasmodium (Kimura M. *et al,* 1995; Tirasophon W *et al,* 1994). However, problems with contamination from parasitic DNA, not present originally in the sample and difficulties in the specimen processing need to be resolved, before PCR can be used routinely as a diagnostic tool along with the high quality expertise development of the handling personnel.

Nepal is more or less a rectangular area in between china (North) and India (South) with following east-west ranges and regions starting from north to south i. e. high Himalaya (23%), Mountains (20%) Midhills (30%), Siwalik range area (13%) and plain terai areas (14%). Malaria situation in the country upto 1977 was maintained at the level between 10000 and 14000 cases annually. However from 1978 there was a real deterioration of situation in the country when malaria cases reached to 14212 from 11615 in 1977. With steady increase every year the number of cases reached to 16719 in 1983. There was a steep rise to 29188 cases in 1984, which again escalated to 42321 in 1985 with major outbreaks in Far western region, and minor ones in many parts of the country. The epidemiological outbreaks were combated with massive indoor residual spraying operation and by 1986 the cases started declining. During 1987 and 1990 the number of malaria cases fluctuated between twenty-two to twenty-six thousand cases. In 1991, there were 29135 cases with exacerbation of malaria in Eastern, Western and Central regions. The number of cases decreased to 9609 by 1995 (excluding malaria cases recorded in refugee camps).

Out of the 75 districts in the country, 64 districts have been identified as malarious. Further, out of those 64 malarious districts, 26 districts (Ilam, Jhapa, Morang, Sunsari, Saptari, Siraha, Panchthar, Taplejung, Dhanusha, mahottary, Sarlahi, Bara, parsa, Rautahat, Chitwan, Kapilvastu, Rupandehi, Nawalparasi, Dang, Bankey, Bardiya, Kailali, Baitadi, Dadeldhura, Kancharpur, Darchula) have borders with 4 states of India i.e. Bihar, U.P., West Bengal and Sikkim.

The country has not been able to completely adopt the Global Malaria Control Strategy especially to carry out early diagnosis and prompt treatment of clinical malaria cases, to use personal protection and feasible bioenvironmental methods, and to implement strategy for prevention and control of epidemic outbreaks. There is no long term planning and preparedness for insecticidal requirement. Lack of laboratory facility especially in hard core, *P. falciparum* resistant and epidemic prone health post / sub health post and lack of regular monitoring of drug sensitivity status of *P. falciparum* are the major problems and constraints in Malaria Control in Nepal.

Hence there is a need to employ a non-microscopic diagnostic technique, which should be sensitive and specific.

In a study done by Tirasophon W *et al,* (1994), PCR detection method has been found to be more sensitive for *P vivax* (91%) than *P. falciparum* (89%). Level of parasite detection has been reported to be as low as 1 to 10 parasites per microlitre of blood in patient sample. Diagnosis by PCR has also been reported to be species specific for all four species of human Plasmodium (Kimura M *et al,* 1995; Tirasophon W *et al,* 1994). However, problems with contamination from parasitic DNA, not present originally in the sample and difficulties in the specimen processing need to be resolved, before PCR can be used routinely as a diagnostic tool along with the high quality expertise development of the handling personnel.

There are few studies, which has employed and evaluated various primers for the diagnosis of various species of malarial parasites in their own countries context. The sensitivity and specificity of same primer were found different in different countries and context may be because of allelic diversity of the strain of same species of malarial parasite found in different countries. Feeling the acute need for the evaluation of different PCR primers for the rapid and accurate diagnosis of malaria in Nepal, we intend to evaluate the efficiency of the few primers used in the other countries for the diagnosis of malaria and access its use for the diagnosis of malaria from the blood of the infected patients, and compare it with the presently existing technique like Giemsa staining method.

Rationale / Justification

Diagnosis is the cornerstone for proper management of clinical cases, prevention of serious complications, institutions of specific antimalarial therapy and institution of effective control measures for eradication of disease.

The emerging problems associated with increased incidence of disease and death, resistance of malaria parasites and the vectors, socio-economic impediments, along with the lack of new, simple and sensitive diagnostic modalities, have led to a decreased capacity to optimally use the existing tools to combat the disease.

Microscopy is time consuming, requires high expertise and its sensitivity is low in cases of low parasitemia, cerebral malaria, transfusion associated malaria and in malaria associated Torpical Splenomegaly Syndrome. PCR detection method has been found to be more sensitive for *P vivax* (91%) than *P. falciparum* (89%). Level of parasite detection has been reported to be as low as 1 to 10 parasites per micro litre of blood in patient sample. Diagnosis by PCR has also been reported to be species specific for all four species of human Plasmodium. *Hence there is a need to evaluate a non-microscopic diagnostic technique (PCR), which should be sensitive, specific and may be available in central facilities of the country for evaluation of drug efficacy, drug resistance, to study genetic diversity of the parasite found in Nepal.*

Few protocols and primers for the diagnosis of malaria has been designed and evaluated by some of the workers in developed countries. The sensitivity and specificity of those were found different in different countries and context because of strain diversity. So, any diagnostic tests before introducing/accepting for diagnostic use, it has to be evaluated in own country's context in comparison with the existing diagnostic technique. The PCR primers designed and used in some malaria endemic countries may be more useful than the primers used in other continents because of the allelic diversity found by various researchers. PCR test may be as good as in other countries or may be more/ less sensitive in Nepalese strain.

Research Question:

Whether PCR test for the diagnosis of falciparum malaria is useful in Nepalese context?

OBJECTIVES

General

Standardization of PCR for malaria diagnosis in Nepal

Specific

- Conventional microscopic examination of peripheral blood smears to diagnose patients with suspected malaria cases.
- Standardization of PCR primers useful for the diagnosis of falciparum & vivax malaria in Nepal.
- Comparative evaluation of PCR with conventional microscopy for the diagnosis of malaria.

MATERIALS & METHODS

1. **Samples:** 283 -Blood samples collected in glass slide/ filter paper strips/ EDTA vial.

2. **Study Site:** Dhanusha and Kanchanpur districts, Nepal

During the study period (January-September 2003), blood samples from 289 suspected malaria patients from Dhanusha and Kanchanpur districts located in central and far western Nepal were collected and further analyzed as per study design.

Blood samples from patients with complain of signs and symptoms of malaria were collected aseptically by finger prick in clean and grease free glass slides / EDTA vials and filter paper strips prepared as per requirement.

Information including patients' age, sex and antimalarial treatment taken in the previous four weeks were collected from patient's brief interview. Formal oral consent to participate in the study was obtained from adult patients & guardians of young children. Patients less than a year of age were excluded due to the difficulty in obtaining an adequate blood sample.

Each study specimen was given a code number to minimize bios in the microscopic examination. A thin blood film was also prepared for parasite species identification and parasite count. EDTA blood was collected from few confirmed malaria cases for further analysis with PCR.

3. Transport of the sample

All the collected blood samples in EDTA vials/ slides/ filter paper strips were transferred to Central Department of Microbiology, Tribhuvan University, Kathmandu, with full precautions for transport of specimens. Ice box was used to transfer the samples for DNA extraction, where as slides with dried blood smears were transferred to CDM, TU as such within a week of collection.

Preparation of Thick & Thin blood films:

Thick blood film preparation & staining

1. Added approximately 5 µl of blood to a clean slide with an applicator stick / directly from the patients pricked thumb.
2. With the end of the applicator stick, quickly distributed the blood to make an even thick film about 1 square cm. Avoided excessive stirring of blood to make it possible to read fine print through a thick film.
3. The slide was labeled and allowed the thick film to air dry in a horizontal position.
4. Thus prepared thick blood film was dehaemoglobinised by dipping in buffered water pH 7.2 for 5-10 minutes.
5. Thus dehaemoglobinised slide was fixed with methanol & preceded for staining.
6. 3 % Giemsa Stain solution *in PBS pH 7.2* was prepared as per standard protocol.
7. The slide was flooded with diluted Giemsa stain for 20 minutes.
8. Removed the slides & washed in tap water in tap-water or buffered water pH 7.2.
9. Air dried the slide in a vertical position.

Thin blood film preparation & Staining with Giemsa stain:

1. A drop of blood was placed on one third of the way from the labeled end of the clean, grease and moisture free microscope slide and used a second slide held at 45^0 as a spreader to distribute the blood smoothly and rapidly to make a smooth thin film.
2. Air dried the blood film thoroughly.
3. Fixed the blood film in methanol for 1-2 minutes.
4. 3 % Giemsa Stain solution *in PBS pH 7.2* was prepared as per standard protocol.
5. The slide was flooded with diluted Giemsa stain for 20 minutes.
6. Rinsed the slide in tap water and air dried the slide in a vertical position.

Examination and Reporting of slides:

All the Giemsa stained slides were examined by using Nikon binocular microscope with built-in illumination at a magnification of 1000X (100X oil immersion x 10X x eye piece). All the three researchers, PG student, Research Assistant and the Investigator

himself examined the stained slides to avoid under/over reporting, without knowing the result of each other. The discrepant slides were reexamined and confirmed following standard photographic charts provided by WHO. Slides were reported negative only after examination of 200-300 fields systematically. Positive blood films were recorded as Parasites (*Rings+ Throphoziates+Schizouts*) per 100 white blood cells. The parasite count per µl of blood was obtained as summing up the WBC count of 8000/ul.

PCR for malaria diagnosis

All the collected blood in filter paper strips/ EDTA vials were stored at -20^{0}C till use. Semi-nested multiplex PCR based on amplification of 18s small subunit ribosomal RNA (18sSSrRNA) gene was used to standardize the assay.

1. Sample Collection & Preparation

Whole blood samples collected from vein puncture and blood collected from finger prick/ vein puncture were kept in EDTA vial and also in filter paper strips for PCR assay. Each filter paper strips were kept in plastic bags and shipped to CDM, TU, Kathmandu. Whole blood were kept in cold box and shipped.

a. Filter paper strips
b. Whole blood

2. Preparation of DNA template

The isolation of DNA from whole blood and filter paper strips were standardized with the below mentioned all three methods.

a. Saponin lysis
b. Triton X-100
c. Boiling

3. Primer designing

Five PCR primers were designed by comparison of the published ssrRNA gene sequences obtained from Genebank. A reverse primer (UNR) was designed that would hybridize universally with all *Plasmodium* species and a wide range of vertebrates.

There were two forward primers, one designed to hybridize with mammals (HUF) and the other to all *Plasmodium* species (PLF). There were two other reverse primers, of which FAR hybridizes only with *P falciparum*, VIR only hybridizes with *P vivax*.

Semi-nested multiplex PCR

Detection and identification of malaria species were simultaneously performed using a set of two (semi-nested) PCRs, and the size of the products were estimated after electrophoresis on 2 % agarose gels stained with ethidium bromide.

a. First / Primary reaction:
   i. Reverse primer - Universal'- UNR
   ii. Forward primer- Plasmodium- PLF
   Mammals - HUF
   Product: 783-821 bp

b. Second reaction:
   i. Forward primer- Plasmodium genus specific - PLF
   ii. Reverse primer - Plasmodium genus specific -
      1. VIR - **Product 499 bp**
      2. FAR - **Product 395 bp**

Primer quantity determination

For this semi nested multiplex PCR,

First reaction mixture contained 10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$. 0.001%(w/v) gelatin, 1% glycerol, 200 µM of each dNTP, the PCR primers, 2.5unit of Tag polymerase and 41.1 µl of template DNA in the 50µl final volume of reaction mixture. The concentrations of the primers used were 25pmoles for UNR and PLF.

During second round of the reaction, 25 µl of reaction mixture contain 10mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5mM $MgCl_2$, 0.001% (w/v) gelatin, 1% glycerol and 200 µM each of dNTP and 1 unit of Tag polymerase. Template DNA was prepared by taking 2µl of the first reaction product in 1 ml of distilled water and for second reaction 2µl of it was taken. Primer concentration was determined empirically where PLF 25pmoles, FAR 15pmoles and VIR 2.5pmoles. The amount of template used was 2µl of the PCR product

of the first reaction diluted to a final volume of 1 ml with distilled water, and 2µl was added to the PCR.

PCR reaction cycles

A *Geneamp- 2400 PCR system* was used for both of the reactions. Reaction conditions were denaturation at 94^{0}C for 5 minutes followed by first reaction 40 cycles at 94^{0}C for 45 sec, 55^{0}C for 45 sec and 72^{0}C for 1 minute or multiplex PCR reaction 35 cycles at 94^{0}C for 20 sec, 62^{0}C for 20 sec and 72^{0}C for 30 sec. The final cycles followed by an extension time at 72^{0}C for 10 minutes.

c. Standard – 35 cycles – 94.....65-----72--------45.....4^{0}C

d. Specific – 94^{0}C for 5 minutes before other regular cycles
40 cycles – 94.....55.....72.....45.....4^{0}C

4. Visualization of PCR products - 2% Agarose gel

Visualization of the PCR products was done utilizing UV Gel Documentation Unit-Alpha Imager (Alpha Innotech)

a. First reaction **Product 783-821 bp**

b. Second reaction **VIR - Product 499 bp**
FAR - Product 395 bp

RESULTS

A total of 283 blood samples were collected from suspected malaria patients in Kanchanpur Disctrict, in far western Nepal during May- Nov 2003. Thus collected samples were analyzed utilizing conventional Giemsa stained malaria microscopy and PCR based on 18sssrRNA gene.

MICROSCOPY RESULTS

Out of the total 283 samples, only 66 (23.32%) samples were positive for malarial parasite (Plasmodium *falciparum*-26, *Plasmodium vivax*-40)

The prevalence of malaria in Kanchanpur district during the outbreak season 2003 was 23.32%.

Plasmodium falciparum malaria cases were 9.18% and *Plasmodium vivax* malaria cases were 14.13% of the total collected cases where as 39.39%, 60.60% of the total positive cases respectively. This rate is prevalence is much higher than the expected national prevalence rate.

Detail data- Annex

Plasmodium falciparum infection- Giemsa stained slide

PCR RESULTS

Results variation with Sample collection storage & processing

c. Filter paper strips

Two- three drops (100-150 µl of whole blood collected from finger prick was dropped in Whatman No-1 nitrocellulose filter paper. Dried and stored at room temperature till processing.

d. Whole blood

Whole blood collected in sterile vials with anticoagulant EDTA

Whole blood samples processed within 2 weeks of collection showed promising results while filter paper strip blood samples showed promising results even after few months of storage.

Preparation of DNA template

The isolation of DNA from whole blood and filter paper strips were standardized with the below mentioned all three methods.

- Saponin lysis
- Triton X-100
- Boiling

The Saponin lysis method could show promising & reproducible results and hence was utilized in the subsequent analysis.

Primer designing

Five PCR primers designed by comparison of the published ssrRNA gene sequences obtained from Genebank.

Semi-nested multiplex PCR

Detection and identification of malaria species were simultaneously performed using a set of two (semi-nested) PCRs, and the sizes of the products were estimated after electrophoresis on 2 % Agarose gels stained with Ethidium bromide.

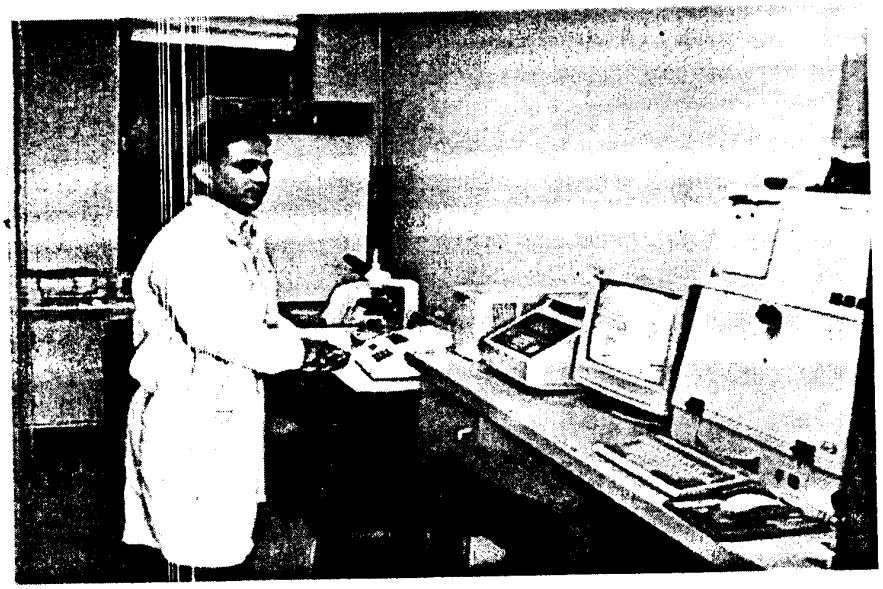
Primer	Sequence (5'-3')	Specificity	Product Size (Base pairs bp)
First Reaction			
Reverse Primer **UNR**	GACGGTATCTGATCGTCTTC	Universal	
Forward Primer **PLF**	AGTGTGTATCCAATCGAGTTTC	*Plasmodium*	783-821
Second Reaction			
Forward Primer **PLF**	AGTGTGTATCCAATCGAGTTTC		
Reverse Primers			
FAR	AGTTCCCCTAGAATAGTTACA	*P falciparum*	395
VIR	AGGACTTCCAAGCCGAAGC	*P vivax*	499

The primers we utilized in the above mentioned quantities gave promising results.

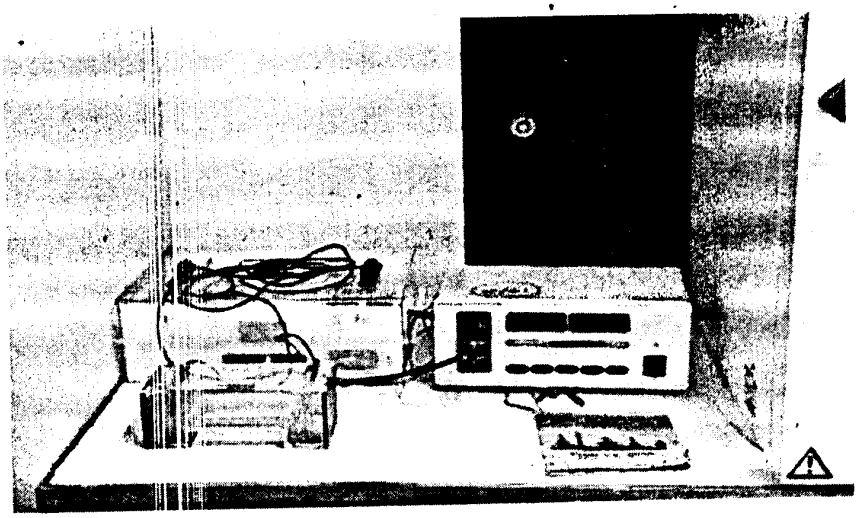
PCR reaction cycles

A *Geneamp- 2400 PCR system* was used for both of the reactions.

Denaturation at 94^{0}C for 5 minutes followed by first reaction 40 cycles at 94^{0}C for 45 sec, 55^{0}C for 45 sec and 72^{0}C for 1 minute followed by an extension time at 72^{0}C for 10 minutes, gave suitably visible results as below.



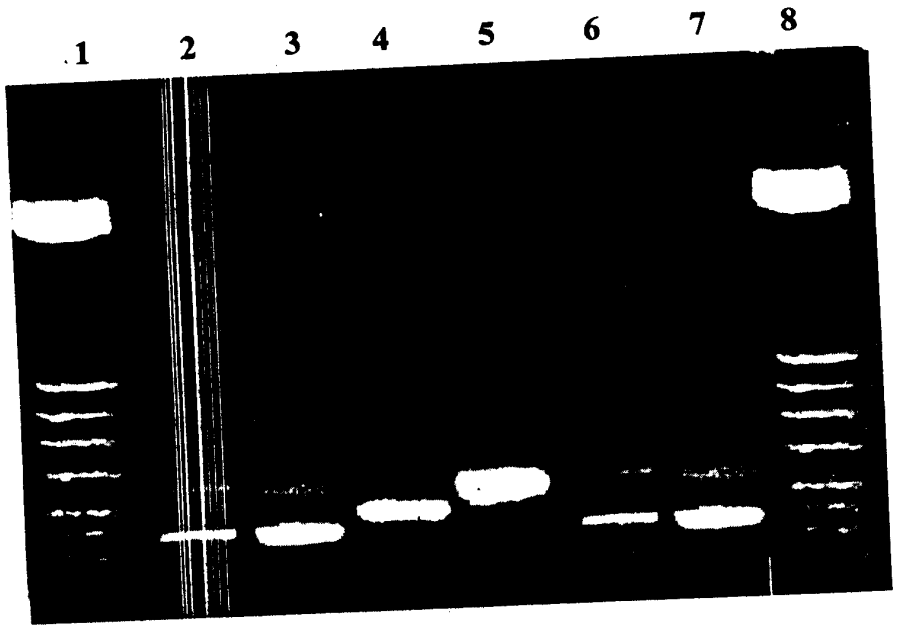
Processing of samples for PCR in Geneamp Thermal cycler, Gel documentation unit



Agarose gel-electrophoresis of PCR products for visualization of the PCR products

5. Visualization of PCR products - 2% Agarose gel

Visualization of the PCR products was possible utilizing UV Gel Documentation Unit- as below



LANES

1. MK- 50 bp marker
2. *Plasmodium falciparum* plus *Plasmodium vivax*
3. *Plasmodium falciparum* plus *Plasmodium vivax*
4. *Plasmodium falciparum*
5. *Plasmodium vivax*
6. *Plasmodium falciparum* plus *Plasmodium vivax*
7. *Plasmodium falciparum* plus *Plasmodium vivax*
8. MK- 50 bp marker

Comparative Evaluation of PCR and Giemsa stained blood smear microscopy method shows that PCR is almost as sensitive as Microscopy. PCR could detect 2 cases of falciparum malaria, which could not be detected by Microscopy, may be because of low parasitemia.

Limitations / Problems encountered

a. Sample collection - Instability & insurgency in country

b. Sample transfer - Instability & insurgency in country

- Postal system
- Electricity unavailability in collection centers
- Nepal Bandh

c. Procurement of reagents – No local stockiest

Transport doubtful for stability of reagents

Further improvement

Further improvement could be made following below mentioned measures

- Other primers with high specificity may be useful
- More samples representing whole country should be analyzed for calculating sensitivity, specificity and predictive values and generalization of the research results throughout the country.
- Strict sample transfer measures are essential as transfer of whole blood in room temperature had inhibitory effects on target materials.

RECOMMEDATIONS

- PCR is a useful diagnostic technique for malaria diagnostic research, especially in drug resistant and low parasitemic cases.
- The sensitivity of the PCR test is considerably high but sample collection, transport and storage conditions play a major role in improving the sensitivity & specificity of the test.
- It should be further standardized with other primers as well along with more representative samples collected throughout the country, for generalization of the test.
- PCR is a good and essential method for advanced research in drug resistance and diagnosis of difficult cases but may not be very fruitful in routine diagnosis because of its high cost.
- NHRC should promote other similar research with other primers detecting other genes and genes for antimalarial drug resistance in coming days as per the need of the country's health care problems.

APPENDIX

Central Department of Microbiology
Tribhuwan University
Kirtipur, Kathmandu
Malaria Research Group
Kanchanpur District-Malaria (2003)

S.N.	Sl N.	Name	Age	Se-	Address	Clinical Notes										Other Clinical Notes	Lab Finding				Samp Coll.		
						Fever		C & R	N/M	Sw	Ha	An	Mup.	Sm.	Hm.		Giemsa Microscopy		PCR	Para.F	EDTA	FP.	Slid
						C	I										Species of Plasmodium	Count					
1	0	Manwati Devi Thagunna	22	F	Tilakpur		√	√	√	√	√	√	√	√	√	..Fever back 5 days ..lower limbs pain.					×	×	×
2	0	Ram Bdr Kunwar	2.5	M	Barlkuna		√	√	√	√	√	√	√	√	√	..allergy through out the body					×	×	×
3	0	Pretty Sunwar	2	F	Khairbadi		√		√	√	√		√		√	..Diarrohea wth stomach upset,cough					×	×	×
4	0	Lu-man Joshi	15	M	Ultakham		√	√	√		√	√	√	√		..cough 2 days back,weakness					×	×	×
5	0	Sarswati sharma	11	F	Pipladi	√			√	√	√			√		..Diarrohea wth vomitting					×	×	×
6	0	Dhana Devi	38	F	Gadda C		√	√	√	√	√	√	√	√		..Gastritis,Severe headache.					×	×	×
7	0	Maheswri chettri	14	F	Bangaun		√		√	√	√		√			..Fever back 2 days ..1000F.					×	×	×
8	0	Deepak Luhar	4	M	Salghari		√	√	√	√	√		√			..cough					×	×	×
9	0	Lu-mi D Joshi	29	M	Haldukhal			√	√	√	√					HA from long time					×	×	×
10	0	Sagar Bohara	9m	M	Bagphata	√		√	√	√	√		√			.Fever 5 days (100F).					×	×	×
11	0	Khagendra luhar	18	M	Tilakpur			√	√	√	√		√			..Waist Pain					×	×	×
12	0	Henamta Shresta	7	M	Gobraia		√	√	√		√		√			..					×	×	×
13	0	Sheela Chaudhary	44	F	Imlia		√	√	√		√		√			..Diarrohea twice since morning					√	√	√
14	0	prakash D Badu	10	M	Nimbukheda		√		√		√		√			..Polypepsia,Chest beating abnormally	P. viva-				×	×	×

15	0	Prem Shreth	1.5	M	Nimbukheda		√		√		√		√			Cough					×	×	×
16	0	Usha K.Thapa	14	F	Barakuntha		√		√		√		√								×	√	✓
17		Toya Raj Joshi	32	M	Suda		√		√		√		√			..Sarotum swelled,Gastritis					×	×	×
18	18	Gagan Buda	25	M	M.Nagar		√		√		√		√			Involuntary Muscle Cramping					×	√	√
19	23	Heera Pant	33	F	Sripur		√			√	√	√	√								×	×	×
20	24	Padam Singh Rana	15	M	Rampur		√	√	√		√	√	√								×	×	×
21	26	La-mi Rana	5	F	Rampur		√			√	√										×	√	√
22	25	Mandari Sunar	5	F	Rampur		√	√	√		√	√	√	√		..cough 2 days back,weakness					×	√	√
23	27	Rabindra Chaudhary	5	M	Rampur	√										..Vomiting					×	×	√
24	28	Dalli Rokaya	50	M	Rampur		√									..Cough					×	√	√
25	29	Bindu Chaudhary	20	F	Shreepur		√		√	√	√										×	×	×
26	30	Kemchan Chaudhary	10	F	Rampur		√				√		√			Cough					×	×	×
27	31	Kamalapati Bhatta	70	M	Rampur		√									..Cough ,Abdomen Pain					×	×	×
28	32	Deepak Raj Pant	1	M	Rampur		√														×	×	×
29	33	Kaushila Bhatta	64	F	Rampur		√		√		√	√	√								×	×	×
30	34	Nab Raj Bhatta	30	M	Rampur		√		√		√	√	√								×	×	×
31	35	Prakash Koli	3	M	Rampur		√		√		√	√	√								×	×	×
32	36	Dinesh Sunar	11	M	Rampur		√			√	√	√	√			Cough					×	×	×
33	37	Parvati Bhatta	18	F	Rampur		√				√	√	√			Vomiting ,Dizziness					×	×	×

34	38	Brinedra Rana	8	M	Rampur		√				√					..Vomiting					√	√	√
35	39	Radha Devi Bhatta	25	F	Rampur		√		√		√	√	√				P. viva-				×	×	×
36	40	Nanda Devi Bhatta	57	F	Rampur		√		√		√	√	√			..Back ache					×	×	×
37	41	Shanker Nepali	17	M	Rampur		√		√		√	√	√			Abdomen Pain					×	×	×
38	42	Prem Sunar	3	M	Rampur		√		√		√	√	√								×	×	×
39	43	Parvatee Thakurathi	38	F	Rampur				√	√	√	√	√			Bitter Test,Parathesia					×	×	×
40	44	Manoj Bhatta	4M	M	Rampur		√		√	√	√	√	√								×	×	×
41	45	Beena Bhatta	1.5 M	M	Rampur		√			√	√		√			..Cough					×	×	×
42	46	Manmati Thakurathi	43	F	Rampur		√		√	√	√	√	√			..Cough					×	×	×
43	47	Sunil Parki	2.5	M	Rampur		√		√		√	√	√			..Rash seen in the body					×	×	×
44	48	Pari Datta bhatta	40	M	Rampur		√				√										×	×	×
45	49	Janaki Bhatta	30	F	Rampur		√				√	√	√								×	×	×
46	50	Manoj Sunar	1	M	Rampur		√		√	√	√		√			..Cough					×	×	×
47	51	Jhuma Devi Budha	60	F	Pahariya		√	√	√		√	√	√			Gastritis					×	×	×
48	52	Dan Bdr.Mahara	48	M	Pahariya		√	√	√	√	√	√									×	×	×
49	53	Dhan Bdr.Mahara	18	M	Pahariya	√		√	√	√	√	√	√								×	×	×
50	54	Gita Chaudhary	11	F	Sundarifanta		√	√		√	√	√									×	×	×
51	55	Keshab Bdr.Thapa	45	M	Nigali		√	√	√	√	√		√								×	×	×
52	56	Gopal Singh Budha	50	M	Domilla	√				√	√		√								×	×	×

53	57	Dipak Saud	12	M	Pahariya	√		√	√	√	√		√									×	×	×
54	58	Dura Kumari Ayer	6	F	Pahariya		√															×	×	×
55	59	Durga Mahar	1yr	F	Nigali	√										Cough						×	×	×
56	60	Dinesh Saud	5	M	Nigali	√		√		√		√										×	×	×
57	61	Padma Devi Rawal	40	F	Kspur		√	√	√	√	√	√	√									×	×	×
58	62	Somi km Karki	11	F	Kspur		√	√	√	√	√											×	×	×
59	63	Ganesh Bdr.Rawal	11	M	Kspur	√				√	√											×	×	×
60	64	Kabita Chaudhary	20	F	Kspur		√				√		√			..Dizziness						×	×	×
61	65	Chaiti Chaudhary	19	F	Kspur		√		√		√	√										×	×	×
62	66	Krishna Rana	10	M	Kspur		√			√	√											×	×	×
63	67	Lalita Rawal	11	F	Kspur		√			√	√											×	×	×
64	68	Bijay Singh Thagunna	16	M	Rampur		√															×	×	×
65	69	Ashok Bhatta	2.5	M	Rampur Bilashpur		√		-													×	×	×
66	70	Bashanti Bhatta	30	F	Rampur		√		√	√						..Feelind cold only						×	×	×
67	71	Kamala Bhatta	23	F	Rampur		√				√		√			Cough ,Feeling weakness						×	×	×
68	72	Sanjita Rana	4	F	Rampur		√	√								..Vomiting						×	×	×
69	73	Chapha Sunar	19	F	Rampur		√	√		√	√											×	×	×
70	74	Sarswati Bhatta	3	F	Rampur		√	√								Cough						×	×	×
71	75	Rampati Chaudhary			Rampur	√										Dizziness,Addomen Pain						×	×	×

72	76	Khem Raj Bhatta	1.5	M	Rampur	√										Mumps					×	×	×
73	77	Kalawati Bhatta	30	F	Rampur		√				√					Abdomen Pain					×	×	×
74	78	Santosh Bhatta	3	M	Rampur		√														×	×	×
75	79	Dev Raj Pant	30	M	Rampur		√				√					Backache,Parathesia					×	×	×
76	80	Manojk Pd.Bhatta	17	M	Rsmpur		√				√					Bitter Taste					×	×	×
77	81	Gauri Bhatta	18	F	Rampur		√				√	√									×	×	×
78	82	Sunil Sunar	5.5	M	Rampur		√									..Mumps					×	×	×
79	83	Madhavi Bhatta	25	F	Rampur		√				√		√								×	×	×
80	84	Niraj Sunar	5	M	Rampur		√		√							Cough ,Vomiting					×	×	×
81	85	Jeevan Bhatta	3	M	Rampur		√									Vomiting ,Dizziness					×	×	×
82	86	Manoj Bhatta	4	M	Rampur		√		√				√			Anore-ia					×	×	×
83	87	Naresh Koli	6	M	Rampur		√				√					Fever(99 F)					×	×	×
84	88	Aarati Nepali	2	F	Rampur		√		√				√			Cough ,Malnourshied					×	×	×
85	89	La-man Sunar	5	M	Rampur		√				√					Cough					×	×	×
86	90	Sanu Sunar	6	M	Rampur		√				√										×	×	×
87	91	Jagannath Bhatta	50	M	Rampur											..Cough ,Parathesia					×	×	×
88	92	Radha Bhatta	45	F	Rampur						√					Cough (98 F)					×	×	×
89	93	Gaura Bhatta	50	F	Rampur						√					Fever(98F)					×	×	×
90	94	Mannati Thakurathi	23	F	Rampur		√		√							Bodyache					×	×	×
91	95	Harindra Thakurathi	12	F	Rampur		√		√		√					Skin infecture					×	×	×
92	96	Man Bar. Thagunna	17	M	Rampur		√									Fever(97.6 F)					×	×	×

93	97	Kaumati Thagunna	19	F	Rampur		√	√			√	√				Fever(97.6 F)					×	×	×
94	98	Desh Raj Dagaura	20	M	Rampur		√				√										×	×	×
95	99	Sita Sunar	25	F	Rampur		√				√	√				Parathesia(97F)					×	×	×
96	100	Nisha Sunar	8	F	Rampur		√				√					..Cough (102 F)					×	×	×
97	101	Dammari Sunar	5	F	Rampur	√					√					..Cough(101.2 F)					×	×	×
98	102	Punam Sunar	1	F	Rampur		√									Fever(98F)					×	×	×
99	103	Rangi Sunar	30	F	Rampur		√				√	√				Fever(97 F)					×	×	×
100	104	Dhana Bhatta	17	F	Rampur		√				√					..Cough (101F)					×	×	×
101	105	Janaki Luhar	5	F	Rampur		√									..Commoncold(97F)					×	×	×
102	106	Pratima Thagunna	1	F	Rampur		√									..Commoncold,Cough (102F)					×	×	×
103	107	Puja Thagunna	3	F	Rampur		√									..Commoncold,Cough (102F)					×	×	×
104	108	Nanda odd.	35	F	Rampur		√				√					..Cough ,Parathesia(97.4F)					×	×	×
105	109	Uttara Luhar	35	F	Rampur		√				√	√				Parathesia(97F)					×	×	×
106	110	Devaki Bhatta	50	F	Rampur		√				√					(Fever 97.4 F)					×	×	×
107	111	Tulsi Bhatta	35	F	Rampur		√			√	√	√				Parathesia,M.T.Taken (10 Days)					×	×	×
108	112	Manmati Joshi	40	F	Rampur		√			√	√	√				Parathesia(96F.)					×	×	×
109	113	Rabindra Rana	2.5	M	Rampur		√			√	√	√				Boil , Cough(102 F)					×	×	×
110	114	Rame Bhatta	4	M	Rampur		√		√	√	√	√	√			Skin infection.ear Infection					×	×	×
111	115	Karan Singh	16	M	Rampur					√	√		√			Cough(101 F)					×	×	×
112	116	Shidha Raj Joshi	41	M	Rampur		√				√	√				Bitter Taste (98 F)					×	×	×
113	117	Bisna Bhatta	16	F	Rampur		√			√	√	√				Fever (97 F)					×	×	×

114	118	Ishori P.Joshi	3	M	Rampur		√				√	√	√			Fever(98.5F)					×	×	×
115	119	Dhana Joshi	5	F	Rampur		√				√					Fever(98f)					×	×	×
116	120	Keshab R Joshi	10	M	Rampur		√	√		√			√			Cough(96F)					×	×	×
117	121	Manju Bhatta	9	F	Rampur						√	√	√			Fever(98F)					×	×	×
118	122	Radha Joshi	11	F	Rampur		√			√				√		Fever(98F)					×	×	×
119	123	Nab Raj Bhatta	11	M	Rampur		√	√		√		√				Fever(97 F)					×	×	×
120	124	Mina Joshi	3	F	Rampur		√	√			√	√	√			Fever(97.8F)					×	×	×
121	125	Sabtri Thagunna	35	F	Rampur		√				√	√	√	√							×	×	×
127	126	Prem karki	14	M	Domilla	√		√			√	√	√								•	•	•
128	127	Kanche Buda	86	M	Domilla		√					√	√			Fever Since 15 days back					•	•	•
129	128	Tilak Nagarkoti	12	M	Domilla		√	√													•	•	•
130	129	Manju Bhandari	16	F	Domilla											Taken a medicine 20 days back					•	•	•
131	130	Jayanti Bhandari	20	F	Domilla											Taken a medicine 20 days back					•	•	•
132	131	Jayan Buda	20	M	Domilla		√			√	√	√									•	•	•
133	132	Suman Bohara	7.5	M	Domilla		√				√										•	•	•
134	133	Bina Rawal	1.5	F	Domilla		√						√			Malnourished					•	•	•
135	134	Bindu K.Joshi	10	F	Domilla		√	√	√		√	√	√			Fever(100 F)					•	•	•
136	135	Tikeshwari Bohara	35	F	Domilla	√			√	√	√		√			Fever(98F)					•	•	•
137	136	Ramesh P.Bhatta	4	M	Domilla		√		√	√	√					Fever(101F)					•	•	•
138	137	Manju Kumari Giri	10	F	Domilla		√	√	√	√	√	√	√			M.T.taken but Vomitted					×	×	×
139	138	Kalpana Thag uri	63	F	Domilla	√		√		√	√	√	√								×	×	×

140	140	Amar Karki	10	M	Domilla		√				√	√										×	×	×
141	141	Rekha Buda	12	F	Domilla		√	√			√					Fever (99 F)	P.viva-					×	×	×
142	142	Binu Buda	4.5	F	Domilla	√		√			√		√			Fever(101 F)						×	×	×
143	181	Sher Bdr. Chaudhari	25	F	Domilla		√	√	√	√	√		√			Fever(98.5F)						×	×	×
144	182	Parvati Joshi	2	F	Domilla		√	√		√	√		√			Fever(98.5F)						×	×	×
145	183	M.B. Khatri	40	M	Domilla		√	√			√	√	√			M.T. taken one day back						×	×	×
146	184	Bhagrathi Ayer	45	F	Domilla		√	√	√	√	√	√	√				P.viva-					×	×	×
147	185	Jumli Devi	40	F	Domilla		√		√		√	√	√				P viva-					×	×	×
148	186	Bashu Devi Buda	38	F	Domilla		√	√		√		√										×	×	×
149	187	Panchu Rana	15	M	Domilla		√	√	√	√	√						P.falciparum					×	×	×
150	188	Mina Parki	11	F	Domilla		√	√		√	√		√									×	×	×
151	189	Chanti Chaudhary	19	F	Domilla		√	√		√	√											×	×	×
152	319	Manisha Bhatta	1	F	Domilla	√											P.falciparum					×	×	×
153	321	Dinesh Raj Bhatta	20	M	Domilla	√					√		√									×	×	×
154	323	Hari Chand	22	M	Domilla	√							√									×	×	×
155	324	Ajab Bdr. Bist	60	M	Jhalari	√				√	√	√										×	×	×
156	327	La-mi Shakya	24	F	Jhalari	√	√	√	√	√	√		√	√	√							×	×	×
157	329	Bisna Bhatta	18	F	Jhalari								√									×	×	×
158	330	Lok Bdr.Bohara	16	M	Jhalari	√				√			√									×	×	×
159	338	Man Singh Kuwar	40	M	BaraKunda	√		√		√	√	√	√									×	×	×
160	339	Maina Devi Luhar	30	F	BaraKunda	√					√		√									×	×	×

161	343	Roshani Singh	14	F	Aithpur		√	√	√			√										×	×	×
162	344	Khandra Bist	25	M	Suda		√															×	×	×
163	345	Tara Sunar	30	F	Ultakham		√															×	×	×
164	346	Purna Bdr.Sunar	25	M	Khairbhatti		√															×	×	×
165	347	Shagr Bist	30	M	Tilkpur	√				√	√		√	√								×	×	×
166	348	Shanker Bist	25	M	Tilkpur		√				√											×	×	×
167	349	Nanda Sharma	20	F	Pipladi	√		√	√		√		√				Typhoid					×	×	×
168	350	Ram lal Chaudhary	26	M	Pipladi																	×	×	×
169	351	Rajesh Bist	24	M	Tilkpur	√			√		√		√									×	×	×
170	352	Dhana Bhatta	25	F	Tilkpur		√															×	×	×
171	353	Durga Devi Thagumna	26	F	Beldadi																	×	×	×
172	354	Anish Okheda	18	M	Beldadi																	×	×	×
173	355	Bishnu Bajhal	14	M	M.N.P.Bazar	√				√	√		√									×	×	×
174	356	Ramlal Chaudhary	25	M	Beldadi																	×	×	×
175	357	Lok Raj Bhatta	26	M	Latamantad	√				√	√		√									×	×	×
176	358	Hari Pd.Joshi	18	M	Ultakham		√			√												×	×	×
177	359	Budhi Bdr.K.C.	16	M	Bhagatpur		√		√	√	√		√									×	×	×
178	360	Jit Bdr Gautam2	20	M	Bhagatpur			√	√		√											×	×	×
179	361	Shanti Bhatta	22	F	Chadani	√			√	√	√		√									×	×	×

180	362	Rakesh suanana	22	F	Chadani		√		√	√	√		√									×	×	×
181	363	Jhanak B Rawal	23	M	BaraKunda		√	√	√	√												×	×	×
182	401	Nada D Bhatta	62	F	Suda		√			√	√		√									×	×	×
183	402	Manni D Damai	35	F	Suda	√		√	√	√	√	√	√									×	×	×
184	403	Rakesh Damai	2.5	M	Suda		√			√												×	×	×
185	404	Ramesh Shaha	30	M	mahulia	√		√	√		√	√	√									×	×	×
186	405	Dev sing Bhandari	11	M	Ultakham	√	√			√		√										×	×	×
187	406	Dev R Bhatta	14	M	Tilkpur		√	√		√												×	×	×
188	407	Nab Rjoshi	28	M	Darchula		√		√	√	√		√									×	×	×
189	408	Bahadur Luhar	24	M	Gaddahchauki		√	√		√	√											×	×	×
190	409	Sunil Rana	3	M	Bagphata		√	√	√	√		√			√							×	×	×
191	410	Surendra Kunwar	7	M	Sundarpur		√			√	√		√									×	×	×
192	411	Haru D Joshi	35	F	Suda	√			√	√	√		√									×	×	×
193	412	Rajendra Chaudhari	15	M	Mushapani		√	√	√		√	√	√	√								×	×	×
194	413	Kalu Chudahari	2	M	Gaugi			√		√	√		√									×	×	×
195	414	Punata Rana	3	M	Suda	√			√	√	√											×	×	×
196	415	Nabeen Dhami	11	M	Suda	√					√	√										×	×	×
197	421	Dhanjeet Bohara	26	M	Tilkpur	√		√	√	√	√	√										×	×	×
198	422	Maya K Bista	3	F	Tilkpur		√	√	√	√				√								×	×	×
199	423	K R Joshi	33	M	Routela	√		√	√	√	√	√	√									×	×	×
200	424	Durga Devi Bista	72	F	Tilkpur		√		√		√		√									×	×	×

201	425	Karan B Bishta	52	M	Tilkpur		√		√		√		√										×	×	×
202	426	Jyanti K Joshi	14	F	Haldukhal	√		√	√	√	√												×	×	×
203	427	Ganesh Okheda	25	M	Haldukhal		√	√		√		√			√								×	×	×
204	428	Devendra B Singh	18	M	Haldukhal		√	√	√														×	×	×
205	429	Sarda D Pal	25	F	Haldukhal		√	√		√													×	×	×
206	430	Prem Bhul	17	M	Haldukhal		√		√		√		√										×	×	×
207	431	Kamala Singh	5	F	Haldukhal		√	√	√			√	√										×	×	×
208	432	Lu-mi Bhatta	40	F	Haldukhal	√		√	√	√				√	√								×	×	×
209	433	Ganga Dhami	40	M	Haldukhal	√			√	√	√	√		√									×	×	×
210	434	Dabal S Bohara19	19	M	Tilkpur		√		√	√	√												×	×	×
211	435	Dil B Bohara	19	M	Tilkpur		√		√		√												×	×	×
212	441	Lu-mi Bhatta	4	F	Daijee		√	√		√	√												×	×	×
213	442	Radhika K unwar	17	F	Daijee		√	√		√	√	√											×	×	×
214	443	Ram Bohara	30	M	Daijee		√	√			√		√										×	×	×
215	444	Narad pandey	20	M	Daijee	√		√	√	√				√									×	×	×
216	445	Neshi Bhatta	17	F	Daijee		√	√	√			√		√									×	×	×
217	446	Bishna Khetri	40	F	Daijee	√		√		√	√				√								×	×	×
218	447	Bashu Sunar	13	F	Daijee		√		√	√	√												×	×	×
219	448	Ganesh Nepali	16	M	Daijee	√		√	√	√													×	×	×
220	449	Lu-mi Nepali	12	F	Daijee		√	√	√	√	√												×	×	×
221	450	Harani Damai	40	F	Daijee	√		√	√	√	√	√											×	×	×

222	451	Namila Joshi	12	F	Daijee	√		√																				×	×	×		
223	452	Damesh Bhatta	15	M	Daijee	√		√	√		√																	×	×	×		
224	453	Karan Bhandari	28	M	Daijee		√	√			√																	×	×	×		
225	454	Deepak Air	15	M	Daijee		√	√	√			√																×	×	×		
226	455	Janaki Bista	25	F	Daijee	√			√		√		√															×	×	×		
227	461	Durga K Bista	17	F	Bangaun	√				√	√	√		√														×	×	×		
228	462	Dhanwati Sunar	55	F	Bangaun	√				√	√		√															×	×	×		
229	463	Ram Luhar	16	M	Tilkani	√					√																	×	×	×		
230	464	Sunare Parki	22	M	Tilkani	√							√															×	×	×		
231	465	Birmati Negi	24	F	Tilachaur		√	√		√			√															×	×	×		
232	481	Jaya Sunar	18	M	Haldukhal	√		√	√	√	√		√															×	×	×		
233	482	Lal Bahadur	78	M	Tilakpur	√		√		√	√		√															×	×	×		
234	483	Surendra Sunar	14	M	Tilakpur	√			√	√	√		√															×	×	×		
235	484	Kalu Damai	23	M	Haldukhal	√		√	√		√			√														×	×	×		
236	485	Sarswati Damai	21	F	Haldukhal	√		√	√	√	√		√															×	×	×		
237	486	Raju Damai	18	M	Tilachaud		√		√	√		√																×	×	×		
238	487	Mangla Pahari	24	F	Gaddahchauki	√			√		√		√															×	×	×		
239	488	Anita K Pharai	7	F	Gaddahchauki	√			√	√	√		√															×	×	×		
240	489	Anil Pharai	41	M	Gaddahchauki	√			√	√	√	√		√														×	×	×		
241	490	Dasharath Luhar	20	M	Gaddahchauki	√		√	√	√	√		√															×	×	×		
242	491	Gop S Nayak	30	M	Gaddahchauki	√		√	√	√	√		√															×	×	×		

243	492	Karan Khadaka	25	M	Gaddahchauki	√		√	√	√	√		√										×	×	×
244	500	Pavrvati Bista	19	F	Gaugi	√					√	√				Malari diagnosed 15 days							×	×	×
245	501	Chandra Chaudhari	23	M	Daijee	√			√				√										×	×	×
246	502	Durga Nath	51	M	Jubeda		√	√	√		√		√										×	×	×
247	503	Basanti Bist	19	F	Baildadi	√		√	√	√		√											×	×	×
248	504	Puja Debua	72	F	Jubeda		√	√		√	√	√											×	×	×
249	505	Govinda Gari	21	M	Bhagatpur		√	√	√	√	√		√										×	×	×
250	506	Sarada Chaudhary	22	F	Pipladi	√		√	√		√	√		√									×	×	×
251	507	Dhan B Malla	64	M	Jimmua		√			√	√		√										×	×	×
252	601	Ganga Singh Dhami	25	M	Jhalari,2		√	√	√	√	√	√	√			Fever since 3 days Back							×	×	×
253	602	Ganesh D Bhatta	11	M	Jhalari,3		√	√	√	√	√	√	√			Fever since 3 days Back	P.viva-						×	×	×
254	603	Suresh Bista	12	M	Jhalari,4				√	√	√	√	√			Malaria med taken 3 days ag	P.viva-						×	×	×
255	604	Deb Bd Kaini	24	M	Jhalari,9		√	√	√	√	√	√	√			Diarrohea since 2 days	P.viva-						×	×	×
256	605	Gorakh S Dhami	35	M	Jhalari,2		√	√		√	√						P.viva-						×	×	×
257	606	I S Mahara	22	M	Jhalari,7		√		√	√	√	√				Burning sensation ,Fev(5)							×	×	×
258	607	Arjun Mahara	4	M	Jhalari,8		√	√	√		√		√				P.falciparum						×	×	×
259	608	Ama B Chaudhary	8	M	Jhalari,9		√	√		√		√				Fever around 101F							×	×	×
260	609	Bheeba k Thapa	10	F	Jhalari,10		√	√	√	√	√		√			Fever sincce 7or8 days back							×	×	×
261	610	Naresh Sunar	10	M	Jhalari		√	√					√			convulsions,stomach pain	P.falciparum						.	.	.
262	611	Kishori Joshi	40	F	Jhalari		√	√					√			Insomania,Stomach pain	P.viva-						.	.	.

263	612	Rita Kumari Bhatta	8	F	Jhalari		√				√		√									.	.	.
264	615	Bhuwan Saud	24	M	Jhalari		√	√				√				..His Sister Diagnosed MP.						.	.	.
265		Prakash Kuwar	20	M	Jhalari		√				√	√				..1 Months long mild fever	P.viva-					.	.	.
266	617	Deepak Ayer	28	M	Jhalari		√	√		√	√	√	√									×	×	×
267		Asha Devi Patali	60	F	Jhalari		√	√			√		√			Medicine given but not taken	P.falciparum							
268		Jayanti Joshi	12	F	Jhalari		√		√		√		√				P.falciparum					.	.	.
269	619	Lok Singh Saud	16	M	Jhalari		√	√																
270		Ram Bdr.Bist	20	M	Jhalari		√	√			√													
271		Ram Rani chaudhary	13	F	Jhalari		√						√											
272	620	Bhakta Khati	40	M	Jhalari		√	√			√		√									×	×	×
273	621	Hari P Kandel	27	M	Jhalari		√	√					√									×	×	×
274	622	Parvati Bogati	36	F	Jhalari		√	√		√	√	√										×	×	×
275	623	Chana D Thapa	25	F	Jhalari		√	√	√		√		√			Malaria med taken 7 days ag						×	×	×
276	624	Ghanshyam Air	6	M	Jhalari		√		√		√		√									×	×	×
277	630	Lal Bahadur	29	M	Jhalari		√			√	√		√									×	×	×
278	631	Goma Sunar	30	F	Jhalari	√			√	√	√											×	×	×
279	632	Karuna Sunar	3	F	Domilla		√		√	√	√											×	×	×
280	633	Chandra B B.K.	45	M	Domilla		√		√	√	√		√									×	×	×
281	634	Naam Sara BK	40	F	Domilla		√	√		√	√		√									×	×	×
282	635	Anita Badayak	16	F	Jhalari		√	√			√	√	√				P.falciparum					×	×	×

283	636	Badhu R Chaudhary	18	M	Jhalari	√				√	√	√		√			P.viva-				×	×	×
284	637	Bhim B Mahara	28	M	Jhalari		√	√	√		√		√								×	×	×
285	638	Rama Chaudhary	18	F	Jhalari	√	√	√	√	√	√	√	√	√	√	Suspected of typhoid	P.falciparum				×	×	×
286	639	Bijaya KC	26	M	Jhalari	√					√		√								×	×	×
	A	Dhan Bdr.Bohara	60	M	Daijee		√			√		√		√		Blood taken from PHC	P.viva-				×	×	×
288	B	Amita Joshi	28	F	Daijee		√			√			√			Blood taken from PHC	P.viva-				×	×	×
289	C	Prabesh Rana	19	M	Daijee		√		√		√					Blood taken from PHC	P.viva-				×	×	×