INTRODUCTION

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Malaria continues to be a major health problem in India and other tropical and subtropical countries of the world. Before the national antimalaria programme was started in India in 1937, the number of malaria positive cases was 75 million in the country with as many as 8,00,000 deaths due to malaria. The antimalaria programme made spectacular progress in bringing the malaria scourge under control and in 1958 our National Malaria Eradication Programme (NMEP) was launched in collaboration with the World Health Organisation (WHO) with the objective of eradication of malaria from the country. This programme has the major responsibility of promoting and coordinating operation of research in malaria.

In 1963, the malaria problem in India was practically under control and no death due to malaria was reported during that year. The success of the chemotherapeutic measures adopted by NMEP was mainly due to normal sensitivity of the parasites to the standard antimalarial drugs. In addition,
the vectors (species of Anopheles) were also susceptible to the antimosquito measures which resulted in adequate control of vectors thus interrupting the transmission cycle of the parasite.

Unfortunately, the successful operational control measures were followed by complacency and slackening of the drive against malaria. The Dichlorodiphényl trichloroéthane (DDT) spraying was interrupted and the production and distribution of antimalarial drugs was curtailed drastically. Since 1970, there has been gradual resurgence of malaria in India as well as most of the adjoining countries. The incidence of malaria in India has again risen and the number of malaria positive cases during 1980 was 10-12 million.

The emergence of chloroquine resistance strains of *P. falciparum* in different parts of India is posing a serious threat to the malaria control programme. Studies conducted by NMEP have shown several foci of chloroquine resistance in states of Assam (Dist. Karbi-Anglong, Darrang, Kamrup and Goalpara) (Pattanayak et al., 1979); U.P. (Agra, Jhansi, Mirzapur) and Haryana (Gurgaon) (Dwivedi et al., 1979); Gujrat (Surat, Amreli), Maharashtra (Chandrapur) (De, C.M. et al., 1979); Nagaland, (Das et al., 1979); Meghalaya (Garo-hills, Khasi hills) (Chakravarty et al., 1979); Orissa (Keonjhar, Sambalpur, Kalahandi, Phalbom)
Multiple drug resistant strains of *P. falciparum* have emerged in South East Asia as well as in western Mediterranean and South American regions.

The potentially dangerous strains of malaria parasite are likely to spread to India and other neighbouring countries and they can cause a very high degree of mortality especially in children. A field study by the South East Asia Regional Office of the WHO has revealed that secular and intersecular waves of malaria epidemics have frequently decimated the population affected and particularly high mortality and morbidity rates have been recorded in South Korea, Mongolia, Nepal, Burma, Thailand, Indonesia, Sri Lanka and Maldives. Another equally disturbing development that has pushed the malaria control into rough waters is the resistance of the vectors to the conventional insecticides like Dichlorodiphenyl trichloroethane (DDT) and malathione (Kalra, 1979).

The UNDP/World Bank/WHO special programme for research and training in tropical diseases have, over the past several years sponsored the development of a new antimalarial mefloquine, a 4-quinoline methanol for the treatment of multiple drug resistant strain of the malignant tertian malaria parasite, *Plasmodium falciparum*.

The WHO (1974) have also urged the national governments to change the strategy from the "eradication of malaria to the containment of virulent malaria" in most of the countries.
of South East Asia. In addition, there is a problem of relapses of *P. vivax* because of the apparent failure of drug regimens used for radical cure programme in at least 8-10% of the treated *P. vivax* cases. The toxicity of primaquine specially methaemoglobinemia and haemolysis particularly in G-6-PD-deficient individuals entails a warning against its use for the radical cure (Thukston *et al.*, 1976).

On account of the resurgence of malaria, research in malaria has assumed a very high degree of urgency. Indian Council of Medical Research has formulated a comprehensive research programme covering different aspects of the disease viz. operational aspects of malaria control, ecology and control of vectors, chemotherapy, drug resistance by the parasite, serology of malaria, *in vitro* cultivation of the parasite and immunological aspects of malaria. In view of the worsening of malaria situation in India, NMEP has adopted a modified plan of malaria control (*ICMR, 1977*) with a strong emphasis on the development of reliable and reproducible serodiagnostic methods for the seroepidemiological assessment of malaria. The epidemiological situation can be assayed by delimitation and stratification of malarious areas and determination of transmission periodicity. A system for the detection of secondary cases and delimitation of secondary foci in the
area with a very high malariogenic potential when malaria has been eradicated is needed (Lopes, 1981). Kagan (1972) also emphasized the use of malaria serology as an epidemiologic method especially:

(1) To measure the level of malaria endemicity.
(2) To determine whether malaria transmission has been interrupted or reduced.
(3) To determine the role of migrants in the introduction of malaria from malarious endemic areas to receptive areas with little or no malaria.
(4) To delineate malarious areas.
(5) To detect the seasonal changes of malaria transmission.
(6) For an independent determination of the intensity and distribution of malaria since it is not necessary to depend on the recorded malariometric surveillance information.
(7) For identification of population groups with specially high rates of malaria infection.
(8) To assess the coverage of the standard surveillance methods which are used to measure the occurrence of malaria.
(9) For evaluating sera of malaria infected blood donors.
(10) For surveillance in areas of low endemicity.

In addition, there are many other problems being faced by the malaria control program such as vector resistance to
insecticides, exophilic behaviour of vectors, population movement and human behavioural patterns which call for improved methods of and approaches to malaria control. It is, therefore, necessary to develop new, effective, reliable and reproducible means of controlling malaria which are simple, cheap and easy to perform (Wernsdorfer, 1981).

However, application of serologic tests for diagnosis of malaria in tropical countries has suffered because of non-availability of adequate standard malaria antigens. The experimental host (Aotus trivirgatus) of Plasmodium falciparum from which antigen can be obtained, is restricted in its distribution to the New World, and no such host is available in India. The in vitro methods for cultivation of malaria parasites are still at exploratory stage in India. As an alternative, simian Plasmodium spp. have been found to be useful source of antigen in certain studies on human malaria. Thus, P. knowlesi has been used as antigen in the IHA (indirect haemagglutination assay), IFA (indirect fluorescent antibody) and ELISA (Enzyme linked immunosorbent assay) tests (Collins, et al., 1966; Kagan et al., 1969; Meuwissen et al., 1972; Lobel et al., 1973; Voller et al., 1975).

Further studies are needed to ascertain the usefulness of antigens prepared from different simian Plasmodium spp., viz. P. knowlesi, P. cynomolgi and P. coatneyi, etc.
Specific antigen with a very high degree of consistently reproducible sensitivity is one of the most important prerequisites for any serological study.

It has been reported that only schizont stage antigen should be used if maximum sensitivity and discriminatory capacity of malarial IHA, IFA and ELISA is to be achieved. Different methods have been employed for harvesting parasitized erythrocytes from infected blood for preparation of antigen viz.,

(1) Differential centrifugation
(2) Discontinuous ficoll density gradient
(3) Ficoll Metrizamide (hypaque) gradient

Storage of antigens at different temperatures and for different intervals of time have been shown to influence the quality of the antigen used for serodiagnostic tests.

Different serodiagnostic tests like IHA (Indirect haemagglutination assay), IFA (Indirect fluorescent antibody), ELISA (Enzyme linked immunosorbent assay), gel-diffusion precipitation, counter immunoelectrophoresis, latex agglutination and complement fixation tests are recommended as an aid to diagnosis, to establish the prevalence of malaria in a given population, to aid in the evaluation of control measures or to screen potential donors for blood transfusion (WHO, 1974).

Examination of stained blood smear is still the best technique to detect infection in individuals. However, this
method has its own limitation when a given population has attained immunity and the level of parasitaemia in the blood is very low.

Under these circumstances serological methods could provide additional evidence of malaria experience, endemicity and immune status of a given population (Field et al., 1963).

The immunodiagnosis of malaria is still at a formative stage in India in spite of the great progress which has been achieved during recent years. Of the many serologic tests which have been developed IHA, IFA and ELISA possess the desired degree of sensitivity, specificity and reproducibility (Sadun, 1972). The IHA (Indirect haemagglutination assay) and IFA (Indirect fluorescent antibody) tests have been used for the detection of antibodies against malaria. In IFA test, homologous antigens have been preferred, since they give higher level of response than do the heterologous antigens (Collins and Skinner, 1972). The malaria passive haemagglutination test formerly known as the IHA (Indirect haemagglutination assay) has been proved to be a useful addition to the serologic tests used for this disease (Farshy and Kagan, 1972; Meuwissen and Louwenberg, 1972; Bidwell et al., 1973; Meuwissen et al., 1973; Meuwissen, 1974; Coirnille-Meuwissen, et al., 1974a; Voller et al., 1974a; Brögger and Mathews, 1975; Mathews et al., 1975; Bagchi et al., 1978).

Various workers (Tohie et al., 1962; Collins et al., 1964a; Sulzer and Wilson, 1971a; Hall et al., 1978) using
the IFA (Indirect fluorescent antibody) test demonstrated the production and persistence of specific human malaria antibody.

These methods for the most part utilize serum obtained by venipuncture. These procedures are unpopular in underdeveloped countries and are difficult to perform in young children as they require equipments and safeguards against contamination frequently unobtainable under field conditions. Furthermore, storage and transportation of serum at high ambient temperatures frequently render it unsuitable for critical diagnostic work. To obviate these difficulties, methods based on the principle of testing small amounts of blood obtained from finger prick have been employed for the seroepidemiology of malaria.

The present study was designed in the attempt to evaluate the comparative behaviour of these tests with regard to their specificity and sensitivity in diagnosing slide positive cases of malaria, fever cases of varied origin, normal healthy subjects and random hospital patients.

IHA test was performed employing:

(i) Sheep red blood cells (SRBC) fresh
(ii) SRBC treated with 1% glutaraldehyde solution
(iii) SRBC treated with chromic chloride
(iv) SRBC treated with formaldehyde

Studies have been made to assess the degree of
reproducibility and the specific threshold titres and to investigate the effect of storage of SRBC's subjected to different treatment as outlined above.