REVIEW OF LITERATURE
Parasitic infections generally elicit specific antibody responses in the host. These antibodies, thus, provide an indirect evidence for the presence of these infections and form the basis of a number of serologic tests widely used in the specific diagnosis of parasitic diseases (WHO, 1980). For serodiagnosis of malaria, too, a number of serologic tests have been described by the earlier workers and a brief review of the current status of these tests in the field of serodiagnosis of malaria, is presented below.

**Indirect Haemagglutination Test (IHA)**

First developed by Desowitz and Stein (1962) the indirect haemagglutination (IHA) test has been extensively used in the serodiagnosis and seroepidemiology of human malaria (Kagan et al., 1969a; Kagan 1972a; Kagan 1981; Brito, 1973; Meuwissen et al., 1972, 1973, 1974a,b; Meuwissen, 1981; Lobel et al., 1976; Lobel, 1981; Bagchi et al., 1978; Mamtani et al., 1979; Mahajan et al., 1981; Agarwal et al.,
Using human 'O' erythrocytes coated with *P. knowlesi* antigen, Rogers *et al.* (1968) obtained positive IHA results among 98% of the malaria patients. The test was found to be highly specific since only an insignificant proportion (less than 1%) of the healthy subjects yielded positive IHA titre.

Bagchi *et al.* (1978) determined antibody titres in 425 subjects, 341 of whom were clinically diagnosed as suffering from benign tertian malaria and 189 having confirmed *P. vivax* infection. Ninety healthy subjects and fourteen cases of proved enteric fever were included in the study as controls. A seropositivity of 84.12% in parasite proven cases, 38.15% in the parasitologically negative clinical cases of malaria, 14.44% in healthy controls and 14.28% in enteric fever cases was obtained with IHA test using *P. knowlesi* antigen. Mamtani *et al.* (1979) who used *P. cynomolgi* antigen in the IHA test, reported 21 to 36% seropositivity among the serum samples collected randomly from a rural community near Delhi.

Chandonani *et al.* (1981) employed the IHA test for detection of malaria antibodies among 528 serum samples obtained from different age groups. They obtained positive IHA results among 41.3% cases of slide positive malaria patients (*P. vivax* infection). However, they also reported positive IHA results in 38.9% of the parasitologically negative febrile cases.

Gupta *et al.* (1979) using *P. falciparum* antigen coated human group 'O' erythrocytes found that out of 763 subjects,
38 were IHA positive at Hosadurga Taluk and 21 out of 100 subjects at Farhathabad Taluk.

Ray et al. (1981) collected 190 blood slides and filter paper blood samples of fever cases from four malaria endemic villages of Alwar district in Rajasthan in order to find out extent of agreement between the slide positivity and presence of malaria antibody. Of the 190 fever cases, 112 were slide positive for malaria and 78 slide negative. In addition 42 serum samples of individuals without any known exposure to malaria from malaria-free area of Kashmir were also included in the study. The malaria parasites found in the study were *P. falciparum* (60.7%), *P. vivax* (33.3%), *P. malariae* (3%) and mixed infection (6%). Employing antigen from *P. falciparum* growing in vitro in the continuous cultures, 94.1% of seropositivity was obtained by the IHA test in *P. falciparum* cases and 64.7% in the *P. vivax* cases. Overall, 81.2% of the parasite positive and 68% of parasite negative individuals, had the anti-malarial antibody. None of the sera from the healthy subjects had malarial antibody titre of 1:16 or above.

In a seroepidemiologic study on malaria among children in West Africa, Mathews et al. (1976) found that 78% of the slide positive cases were also positive by the IHA test. In another study, carried out by Draper et al. (1972a) in East Africa, the IHA test was found to be positive in only 44% of such cases.
The epidemiological studies were also conducted in Brazil, the United States of America, Argentina and Colombia (Kagan et al., 1969b), Tobag, the Philippines (Mathews et al., 1970a,b) and Ethiopia (Armstrong, 1972). These workers who employed human group 'O' erythrocytes sensitized with \textit{P. knowlesi} antigen found out that the IHA test was of significant use in the seroepidemiological studies of human malaria.

The seroepidemiological studies were further reviewed by Kagan (1972b) who used simian malaria parasite \textit{P. knowlesi} as a source of antigen and pointed out that indirect haemagglutination (IHA) test is well suited for the epidemiological assessment of malaria. Large number of specimens can be titrated rapidly and inexpensively and blood can be collected easily on filter paper by finger puncture. It has been further emphasized that IHA test can be used to measure the level of malaria endemicity in a given population, to delineate malarious areas, to detect small foci of malaria transmission and to detect seasonal changes of malaria transmission (Kagan and Roger, 1968; Kagan et al., 1969a; Bruce-chwatt, 1970).

Lobel \textit{et al.} (1977) discussed the interpretation of the IHA titres for the study of malaria epidemiology and showed that serological profile of a population may provide an indication of the history and status of malaria.
Lobel et al. (1976) screened 1100 filter paper samples collected in two weeks from Guyana by epidemiological methods and reported the absence of malaria transmission in the interior jungle area of the country. In 1977, they again carried out seroepidemiological investigation of malaria in Guyana and demonstrated a gradual increase in the seropositivity rate as the age advanced. This supports the view that malaria antibody level which is low during childhood gradually increases throughout the adulthood. However, the high seropositivity rate in 0-1 year age group can only be attributed to the presence of maternal antibodies. According to Mathews and Donero (1981a,b) also, seropositivity rates increased with age and number of episodes of malaria. In younger children, the antibody titres tended to rise with the parasitaemia and fall in the absence of detectable parasites.

Meuwissen (1977a) stated that the interaction between the erythrocytes and antigen is not understood and the antigenic component that reacts with antibodies in the malaria samples are unknown.

It has been well established that homologous malaria antigens are superior to heterologous antigens in sensitivity. Several workers viz. Cornille-Brögger et al. (1978); Malineaux et al. (1978); Malineaux and Gramiccia (1980), who carried out a longitudinal seroimmunological study in
Garki, Nigeria, reported that antigen prepared from *P. falciparum* or *P. vivax* appeared to increase both sensitivity and specificity of the IHA test. Mathews et al. (1975) and Mathews and Dilworth (1976) prepared from *P. falciparum, P. vivax* and *P. brasilianum* and observed a sensitivity of 94.9% for *P. falciparum*, 91.4% for *P. vivax* and 85% for *P. brasilianum*.

Between 1962 and 1972, heterologous antigens such as *Plasmodium berghei, Plasmodium cynomolgi, Plasmodium coatneyi* and *Plasmodium knowlesi* were used extensively for the sero-diagnosis of human malaria because of their easier availability as compared to *P. falciparum* antigen (Mahoney et al., 1966). Due to the difficulties involved in preparing adequate human malaria antigen for the diagnosis, the use of this technique has been curtailed drastically particularly in the tropical countries where the need for such tests is maximum. Recently, Gupta and Bhat (1980), Beaudoin et al. (1981) demonstrated that antigens obtained from *in vitro* continuous culture of *P. falciparum* are satisfactory in the IHA test.

Various methods have been recommended for the isolation and purification of malaria antigen by different workers. It was Dulaney and D’ (1944) and D’Antonio et al. (1966a,b) who reported the hypotonic lysis as a method for release of schizonts from erythrocytes for the preparation of *P. knowlesi* antigen, whereas Schindler and Voller (1967) applied 0.3% saponin-saline treatment for the release of schizonts from
the erythrocytes following the method of Stauber and Walker (1946).

Zuckerman et al. (1969) have recommended the use of dextran treatment for the removal of leucocytes from the infected RBC and haemolysis of the schizont infected erythrocytes by using 0.3% saponin saline. The application of 3% high molecular weight of dextran to parasitized red blood cells for the removal of leucocytes debris has also been suggested by WHO (1974a). WHO (1974a) has also mentioned that parasitized cells in suspension should be disrupted either by sonication or less satisfactory by freezing and thawing. Lunde and Powers (1976) and Deans et al. (1978) compared several methods for the preparation of P. knowlesi antigen and recommended that saponin treated cells followed by freezing-thawing or French press separation appeared to be the method of choice in preparing malaria indirect haemagglutination (IHA) antigen.

Eling (1977) has further modified the method of schizont separation by using Ficoll gradient. This method holds promise for the separation of schizont infected erythrocytes from the malaria infected blood. According to Agarwal et al. (1981, 1982a) the separation of schizont infected erythrocytes by using Ficoll-Conray-420 and its subjection to 0.2% saponin saline treatment imparts greater sensitivity and reproducibility to the IHA test.
Lack of reproducibility is one of the inherent limitation of the IHA test. Hirata et al. (1969), have reported that the type of plastic tray, antiserum diluent and microtitration loops used, influence the reproducibility of IHA titres. Both uniformity of size and availability make red blood cells very useful for IHA test. However the variation in the different lots of red blood cells, namely, differences in the surface characteristics of various batches of erythrocytes (Paulik and Lauf, 1969), fragility and susceptibility to lysis (Danon et al., 1964) and physiologic and metabolic changes during storage (Marks and Johnson, 1958) are some of the difficulties associated with their use.

Besides this, the sheep erythrocytes and human group 'O' erythrocytes have also been seen to influence the sensitivity, specificity and reproducibility of the IHA test. Meuwissen et al. (1972) used fresh sheep erythrocytes in the IHA test for the diagnosis purpose and found that sheep red blood cells stored at 4°C for more than 10 days had deteriorated in their capability to adsorb antigen and thus became unsuitable for use in the IHA test. Stabilization of SRBC's could provide continuous reproducibility of the test preventing a batch to batch variation of the cells (Stavitsky, 1954; Butler, 1963; Daniel et al., 1963).

Different methods for the fixation of red cells have been reported (Stein and Desowitz, 1963; Bray, 1963; Desowitz and Saave, 1965; Desowitz et al., 1966; Hirata and Brandriss,
Stein and Desowitz (1954) carried out formalinized, tanned, sheep red cells haemagglutination test using *P. berghei*, *P. vivax*, *P. coatneyi* and *P. cynomolgi* antigen against sera from patients with *P. vivax* infection and interpreted that sera from these patients gave highest titre up to 1 in 25600 with *P. cynomolgi* and *P. vivax* antigen. Positive haemagglutination reactions were obtained with *P. berghei* antigen but in low titres. *P. coatneyi* antigen cross-reacted with the few sera from patients with *P. falciparum* infection.

Gold and Fudenberg (1967) has also devised a chromic chloride method of binding antigen and antibodies to red cells for passive haemagglutination reaction and demonstrated that this method is very simple and rapid. Moreover, the serological sensitivity and immunological specificity are retained. This method has not been properly evaluated for diagnosis of human malaria.

The method for the preparation of stabilized sensitized cells has been further modified by Farshy and Kagan (1972, 1973): Mathews et al. (1973, 1975); Farshy and Healy (1974). These workers treated the human 'O' red cells with 2% pyruvic aldehyde; then the erythrocytes were tanned and treated with 2% glutaraldehyde and stored in refrigerator. This method is considered to impart greater stability to sensitized red cells which can be supplied to different laboratories.
Stabilization of red cells with formaldehyde (Stein and Desowitz, 1963) and pyruvic aldehyde in combination with glutaraldehyde (Farshy and Healy, 1974), however, resulted in vague agglutination pattern and indistinct end-points.

It was Bing et al. (1967) who suggested that sheep cells fixed in 1% glutaraldehyde solution can be used successfully approximately for up to one year with reproducible results. The glutaraldehyde method for preservation of red cells appeared to fulfil the desired requirements. A shorter time of treatment with the fixing agent resulted in stable, uniform preparation of erythrocytes which were insensitive to freezing and thawing and changes in pH and osmolarity. A method of storing the red cells after fixation with chilled 1% glutaraldehyde for a period of over 3 months has also been reported by Meuwissen and Leeuwenberg (1972); Meuwissen et al. (1973) and Meuwissen (1974), so that the same batch could be used in the tests done with the different batches of sera.

According to Meuwissen et al. (1973), nearly 90% of reproducibility of the IHA test was recorded using two batches of glutaraldehyde fixed sheep cells sensitized with different batches of P. falciparum antigen.

Meuwissen (1974) has also suggested that the IHA test is very simple, sensitive and reproducible and can be successfully used under field conditions, which is quite evident from the results reported by WHO (1974). It has been shown
that red cells fixed with glutaraldehyde, tanned and then sensitized with *P. knowlesi* antigen and lyophilized were found to be stable for at least one year at ambient temperature without any deterioration.

The IHA test using the lyophilized sensitized RBC can now be used as a field test. Meuwissen and Leeuwenberg (1972, 1977) and Cornille-Brogger and Mathews (1972) prepared lyophilized sheep erythrocytes sensitized with *P. falciparum* antigen and evaluated its practical importance under field conditions. According to these workers the use of lyophilized cells of standardized reactivity clearly adds to the simplicity and rapidity of the IHA test and justifies its recommendation for the seroepidemiological assessment of malaria under field conditions.

**Latex Agglutination Test**

Various investigators were able to detect antibodies against heterologous malaria infection, employing serum of chicken infected with *P. gallinaceum* or extracts of chicken cells infected with *P. gallinaceum* organism, to sensitize polystyrene (0.81µ) latex particles. Employing serum as antigen but not infected erythrocytes, antibody to human malaria was measurable (Todorovic *et al.*, 1968). Lazowski (1971) used a latex test on sera from 61 African children with acute malaria and on 96 control sera. He obtained only
27.9% positivity in the malaria group and 8.3% nonspecific positivity in controls using *P. berghei* antigen. Principally, the latex particle tests have been used in the detection of rheumatoid factors, which is an IgM antibody. The test is also sensitive in detection of IgG antibody.

**Indirect Fluorescent Antibody Test (IFA)**

Ever since its introduction by Coons *et al.* (1942), the immunofluorescent staining technique has been widely used in serodiagnosis of several microbial and parasitic diseases (Cohen and Sadun, 1976). In the field of malaria, this test was first developed by Brooke *et al.* (1959) for the demonstration of *P. berghei* using fluorescent antibody. Subsequently, Ingram *et al.* (1961) employed this technique for demonstration of *P. cynomolgi bainianelli* followed by Tobie and Coatney (1961) who demonstrated *P. vivax* by direct fluorescent antibody test. Kuvin *et al.* (1962a) utilized the indirect method of immunofluorescence to study the antibody response in the experimental *P. vivax* infections among human volunteers. It became evident from these preliminary studies that indirect fluorescent antibody test possessed great degree of specificity and provided a sensitive means for measuring antibody titres in malarial infections.

Lunn *et al.* (1965) have shown that parasitaemia is completely suppressed by chemoprophylaxis. At the beginning of the infection, malaria antibody could not be detected,
however, after treatment was stopped and the parasitaemia allowed to rise, antibody became detectable within few days. Other workers have also observed the time lapse between onset of parasitaemia and antibody detection (Kuvin et al., 1962b; Collins et al., 1964a). The best serological agreement between the results of slide diagnosis and titres of serum samples occurred with serum drawn 15 to 60 days after onset of symptoms (Gleason et al., 1971). Only in 3.8% samples the infecting species could not be determined. Titres with homologous antigens were at least four-fold greater than those with the heterologous antigen in 75.2% of the cases. According to Wilson et al. (1970) out of 231 sera samples collected from 69 men who had clinical attack of *P. vivax* and *P. falciparum* at intervals up to one year and titrated with both *P. vivax* and *P. falciparum* thick smear antigens, 98.5% had positive reaction of 1:16 or above and the antibody titres ranged from negative to 1:4096 by 60 days after the onset of symptoms with homologous antigen. Serum drawn 6 months after radical cure had significant drop in titre of 16 fold or greater and 53% had no detectable antibody.

Subsequently the test has been extensively employed in the serodiagnosis and seroepidemiology of malaria as well as in screening the blood donors for the presence of occult malaria (Bray, 1962; Kuvin and Voller, 1963; Collins et al., 1964a, b, c; 1967a, b; 1968b; 1961; McGregor et al., 1965; Jeffery, 1966; Fisher et al., 1970; Sulzer and Wilson, 1971b;
Mouwissen, 1975b, 1978; Mahajan et al., 1981; Agarwal et al., 1981, 1982b). In several studies IFA has been employed along with other serological tests like IHA (Indirect haemagglutination test), CFT (Complement fixation test and ELISA (Enzyme linked immunosorbent assay).

Wilson et al. (1971) compared IHA and IFA tests for malaria antibody and demonstrated that the IHA titres for men who had experienced previous attack of malaria were significantly higher and persisted for a longer time than IFA titre.

Bidwell et al. (197?) also made a comparative study of IHA and IFA tests for malaria antibody in Aotus monkeys infected with P. falciparum and reported that the immunofluorescence titres were higher than the indirect haemagglutination titres in the primary phase of the infection but in later phase they tended to parallel each other, though precipitins were often not detectable even during episodes of parasitaemia. This study finds support from the work of Mouwissen et al. (1974b); Voller et al., 1974 and Collins et al. (1973) who elucidated that IFA test detected antibodies slightly more efficiently than the IHA test during the primary parasitaemia and after 7 months IHA detected antibodies somewhat more efficiently than the IFA test. They also detected significantly high malarial antibody levels in the patients who previously had malaria, but it was not observed with IFA test.
In a comparative study carried out with sera collected from the United Republic of Tanzania, IHA test was found to be insensitive in the detection of antibody in children under five years of age (Voller et al., 1974a). Mauwissen et al., (1974b) evaluated the specificity of IHA test and also made a comparative study of IHA and IFA with sera collected from Tanzania; they reported that only one out of seven hundred sera from individuals without malaria was reactive. In the endemic areas, the IFA test with human IgG conjugate detected antibody slightly more efficiently than the IHA test during primary phase of parasitaemia.

Wilson et al. (1975) compared complement fixation, indirect immunofluorescence and indirect haemagglutination tests for malaria using sera from the U.S. citizens and reported 83% positivity by indirect immunofluorescence and indirect haemagglutination tests and only 57.1% by complement fixation test. False positive reaction with the group were very high for complement fixation (76.6%) and for IHA tests (15.9%) but only 2% for IIF. An evaluation of complement fixation and immunofluorescent tests was also made by Voller and Schindler (1967) with *P. cynomolgi bastianelli* antigen in a study of malaria antibody levels on sera from northern Nigeria. According to them few children and 20% of the adults yielded positive complement fixation results and in contrast to this almost all the children and adults gave positive fluorescent antibody reaction.
Voller (1968) evaluating the various tests observed that the schizont infected cell agglutination test was the most sensitive and was able to detect strain specific antibodies. The complement fixation test was able to detect species specific antibodies, whereas IFA test could detect only group specific antibodies.

In a recent study on the comparative evaluation of ELISA, IHA and IFA tests by Mahajan et al. (1981), 92% of slide positive acute cases were diagnosed with both IFA and ELISA tests. IFA detected additional 6% positive cases of acute malaria which were missed by the ELISA technique.

The usefulness of serological methods as tool for malaria epidemiological evaluation and assessment has been established by Lobel (1981). The serological indices were highly useful in delineating and characterizing malaria foci in Middle America (Warren et al., 1972a,b; 1976), South America (Sulzer et al., 1975), Guyana (Lobel et al., 1976, 1977; Lobel and Kagan, 1978). Mauritius (Bruce-Chwatt et al., 1973), Moldavian Soviet Socialist Republic (Lysenko et al., 1977), Tunisia (Ambroise-Thomas et al., 1976) and Greece (Bruce-Chwatt et al., 1975).

Najera-Morrondo (1979) has suggested that efforts should be made to identify the real positive localities and even the malarious houses so that available sources for malaria control can be used efficiently and improvement of immunological
surveillance techniques is an important research subject in the development of malaria control methods (Bruce-Chwatt, 1977).

IFA test has been successfully applied in the detection of present malaria infection especially in children by several workers. In the investigation of a recent outbreak of *P. malariae* on the Island of Grenada, where malaria had been eradicated, use of IFA test proved to be useful in detecting additional cases of malaria (Tikasingh et al., 1980). Ambroise-Thomas et al. (1972) showed the absence of malaria on the island of Corsica, France and were able to delineate two areas where malaria transmission had occurred even before cases could be detected.

According to Thomas and Dissanaike (1977), positive fluorescent antibody results were detected in 89% of 288 Orang Asli (Malaysian aborigines) with *P. falciparum* antigen and in 62% with *P. brasilianum* antigen, whereas Voller and Bruce-Chwatt (1968) who made a serological survey in Nigeria, reported 92% of IFA positivity out of 914 sera collected from the area using *P. cynomolgi bastianellii* antigen. Draper et al. (1972b) have demonstrated that the IFA titres reflect the malarial infection in a given population to a great extent than do the spleen rates especially at low level of endemicity. Eighty four percent of children between 1-2 years of age and ninety nine percent in the 2-4 years age group showed positive
Collins et al. (1977) made a survey of an area of El-Salvador moderately endemic for malaria and reported that using IFA test about 44% of the infants from mothers who had IFA titre of 1:20 or higher to *P. vivax* during the later phase of their pregnancy gave positive IFA response of 1:10 or higher to this antigen. None of the sera from infants were positive in the absence of some level of malaria response in mother.

IFA test has been extensively applied to study the production and persistence of antibodies to malaria. McGregor et al. (1965) documented the presence of passively acquired malaria antibodies in Gambian infants which declined up to 16 weeks of age. The persistence of antibodies to Colombian strain of *P. falciparum* up to 20 months after sporozoite inoculation has been well documented by Draper et al. (1972a; Ambroise-Thomas, 1974; Volker, 1975). According to Biggar et al. (1980) sera were collected at monthly intervals during the first fifteen months of life from newborn infants living in Accra, Ghana and examined in the IFA test with *P. falciparum*, *P. ovale* and *P. malariae* antigen. The disease history was also recorded during this period. It was reported that passively acquired maternal antibodies had a half-life of only 5 weeks. In areas endemic for malaria transplacentally acquired maternal antibodies are thought to provide partial protection during the first few months of life, after which active immunity is slowly developed as a result of repeated
exposure to malaria. Until immunity is established, malaria is considered to be a serious illness associated with the marked morbidity and high mortality especially when illness is due to *P. falciparum* infection. Campbell et al. (1980) also demonstrated that passively acquired malaria IFA in the new-borns from coastal El Salvador degraded with a half life estimated between 43 and 52 days. During follow up of infants up to 6 months of age, no protection from malaria resulting from passively acquired antibody could be demonstrated.

Sulzer et al. (1975) employing IFA test elucidated the prevalence of *P. malariae* and *P. vivax* whereas *P. falciparum* malaria was absent in the Peruvian Amazon jungle. The fluorescent antibody technique was applied to detect the presence of malarial antibody in population living in three different ecological areas of Malaysia using *P. falciparum*, *P. vivax*, *P. malariae* and *P. fieldi* antigens. There was a great correlation between antibody response and active parasitism in an hyperendemic malaria. The percentage and intensity of responses increased with the age of individuals. Collins et al. (1968a) and Voller et al. (1971) using *P. fieldi* antigen also reported that in Tanzania the mean IFA titre increased with increasing age whereas increase in positive IFA response with age had also been demonstrated in Mato, Grosso and Brazil by Jeffery et al. (1972) using *P. falciparum* antigen.
Bruce-Chwatt (1956) has shown that the fluorescent antibody levels in African children rise as splenic size increases which may confirm the view that in malaria, the spleen, in addition to displaying intense phagocytic activity, plays an important role in specific antibody formation. Marked difference in the IFA response were found in people above and below the critical altitude for malaria transmis-

sion in United Republic of Tanzania (Voller et al., 1971) and in Ethiopia (Collins et al., 1971). Similarly Kagen et al. (1959g) reported a high prevalence in individuals living below 1300 m in Nepal, whereas in persons living above that altitude the IHA prevalence was low. Persons living at an altitude below 6000 feet show higher IFA response against \textit{P. falciparum}, \textit{P. malariae}, \textit{P. vivax} and \textit{P. ovale} antigen as compared to persons living at elevation of 6300 feet or above. Males were found to be more susceptible than females (Collins et al., 1971).

Although the homologous antigens appeared to give highest IFA end-point, as compared to heterologous antigen (Diggs and Sadun, 1965), the application of IFA test in tropical countries is limited due to the inadequate supply of human malaria parasite as a source of antigen. Different workers have used different antigens for the evaluation of malaria antibody titres (Collins et al., 1966b; Voller and O'Neill, 1971; Collins and Skinner, 1972; Giacometti, 1973; Manawadu and Voller, 1978a,b).
Tobie et al. (1962), Vollcr (1962) and Vollcr and Bray (1962) conducted cross-reaction experiments between the primate malarias *P. vivax*, *P. cynomolgi bastianelli*, *P. condonri* and *P. ommatidis*. They obtained strong cross-reaction which suggested that these parasites may share common antigens. Between chicken parasites *P. gallinaceum* and *P. justanucleare*, species specificity, but not strain specificity, was exhibited. Kuvin and Vollcr (1963) reported the use of *P. cynomolgi bastianelli* against *P. falciparum* infected sera whereas Kilman et al. (1970) and Giacometti et al. (1973) detected malaria antibodies in man by fluorescence antibody test using *P. gallinaceum* antigen. The substitution of a rodent parasite *P. berghei* for *P. falciparum* has been suggested by Mealor and Pickerski (1978), although the choice seems to be questionable in view of the considerable differences and low reactivity of rodent antigen as compared to human malaria antigen. Collins et al. (1966a) tested 11 serum samples employing IFA techniques against antigens of *P. falciparum*, *P. malarias*, *P. vivax*, *P. fieldi*, *P. condonri*, *P. inui*, *P. coatnawi*, *P. cynomolgi bastianelli*, *P. knowlesi* and *P. brasilianum* and elucidated that *P. fieldi* was the most suitable simian antigen for immunofluorescent estimation of *P. malarias* and *P. falciparum* antibody. The antiserum from the monkeys infected with *P. inui*, *P. shortti*, *P. brasilianum*, *P. fieldi*, *P. cynomolgi*, *P. coatnawi*, *P. condonri*, *P. knowlesi* and an undescribed *Plasmodium* species
from the Nilgiri area of India were titrated by Collins et al. (1965) against 10 homologous and heterologous antigen. They also observed the highest level of heterologous reaction to \textit{P. fieldi} antigen revealing the presence of common or generic antigen in the species. However, Collins and Skinner (1972) finally recommended the use of two simian malaria parasites particularly \textit{P. cynomolgi bastianellii} and \textit{P. fieldi} for a number of field studies. Not only this Meuwissen (1966, 1968) also found that \textit{P. fieldi} gave good reaction with sera from patients infected with \textit{P. ovale}. IFA test was performed using thin smear antigen by different workers (Voller, 1964; Collin et al., 1964a; 1965; McGregor, 1965) whereas use of thick smear antigen for IFA test was suggested by Sulzer and Wilson (1967), Sulzer et al. (1969), Targett (1970), Lopez-Antunano (1974), Thomas and Ponnampalam (1972). But thick smear antigen prepared from the whole blood was useless because of high level of non-specific fluorescence (Sulzer and Kagan 1967).

Sulzer et al. (1969, 1970). Voller and O'Neill (1971); Manawadu and Voller (1978a,b) suggested the use of mixed human malaria parasites viz., \textit{P. falciparum}, \textit{P. malariae} and \textit{P. vivax} obtained from \textit{Aotus trivirgatus} (Owl monkey) for the detection and measurement of malaria antibody using IFA test to avoid variability of results in different laboratories. They also suggested that antigen slides kept at -70°C, -20°C and 4°C were reactive throughout a period of 4 months. Antigen kept at room temperature deteriorated slowly as compared to 37°C.
Sulzer and Wilson (1971a) also suggested the storage of the antigen at -20°C or below, if these were to be used on later occasions. Better results were obtained while storing the slides at -70°C (Sodeman and Jeffery, 1966; WHO, 1974a).

According to Agarwal et al. (1981, 1982b) also the maximum sensitivity and discriminatory capacity of malarial IFA was obtained when the slides prepared from schizont stage antigen were kept at -70°C.

Indirect fluorescent antibody (IFA) test has played a very important role in evaluating sera from malaria infected blood donors. Malaria induced by blood transfusion from asymptomatic carriers is a problem for blood banks (Brooks and Fry, 1969). Transfusion induced malaria is recognised in many countries where malaria transmission has been eliminated (Lepes, 1969; Duhanine and Zukova, 1968; Lupescu et al., 1967). In non-endemic countries malaria serology is used mostly in investigating induced cases of malaria. An infective blood donor can be identified by testing the sera of donor with a history of possible past exposure (Ambroise-Thomas, 1974; Bruce-Chwatt, 1974; Miller, 1976; Najem and Sulzer, 1976).

Application of the IFA test for evaluation of malaria antibodies in a group of Italian and Pakistani immigrants in Bradford revealed that about 40% of the cases showed positive IFA response at a low titre against *P. falciparum* and *P. vivax* antigen. In a group of potential blood donors
whose whole blood was not used for transfusion because of their possible previous exposure to malaria, only 24 were found to have a positive IFA test using *P. falciparum* and *P. malariae* antigen (Bruce-Chwatt et al., 1972). In the serological screening of 33 donors with a compatible history, two infective donors were identified, one of whom transmitted *P. malariae* 47 years after apparent clinical illness, which is the longest reported period between probable clinical malaria and subsequent transmission of that infection by transfusion (Maulitz et al., 1976).

**Gel Precipitin Test**

Powny (1918) was the first to introduce immunoprecipitation technique for the detection and measurement of malaria antibody. Double diffusion in agar gel has been used to detect soluble antigens which occur in the plasma during and shortly after malaria infection (McGregor et al., 1968; Williams and Houba, 1972). These antigens are more readily detectable in children with severe acute infections. Wilson et al. (1969) demonstrated the soluble antigens associated with *Plasmodium falciparum* infections and classified them according to their resistance to heating at different temperatures. The heat stable S-antigens were shown to have considerable serological diversity, with more than 20 distinct S-antigen reported; no single immune serum contains antibody
to all of them (Wilson et al., 1975b). It has also been reported by Wilson et al. (1975c) that in relapse infection the same S-antigens recurred whereas during separate infections in individuals exposed to frequent reinfection different S-antigens appeared. In recent studies, Wilson (1980) has shown that the S-antigen associated with different isolates of *P. falciparum* were unaltered after repeated passage in *Aotus* and *Saimiri* monkeys and in *vitro* cultures in human red blood cells.

**Enzyme-Linked Immunosorbent Assay (ELISA)**

The ELISA developed in recent years represents a significant addition to the existing serological tools (Engvall and Perlmann, 1972; WHO, 1976; Landon, 1977; Sandre et al., 1977; Ambroise-Thomas and Desgeroges, 1978). The ELISA test, developed as an alternative to radioimmunoassay methods is highly sensitive, simple and relatively expensive (Ekins, 1976; Watson, 1976). The test is well suited for mass screening of serological samples and justifies recommendation for its potential use under field conditions. Several of the earlier studies have been reported by Vollor et al. (1974b, 1975a,b, 1976, 1978, 1980). Recently Edrissian et al. (1979), Ouakyi (1979), Spencer et al. (1979a,b, 1981) have evaluated the use of *P. falciparum* antigen derived from *in vitro* cultures for ELISA test.
Using the Panama II strain of *P. falciparum* as antigen, the micro ELISA was used by Spencer et al. (1979a) to test serum samples from 50 persons from South Eastern United States and serum specimens collected weekly from 4 non-immune and 9 semi-immune patients infected with *P. falciparum*. None of 50 persons from the United States had ELISA antibody titres of 1:80 or above. The nine semi-immune patients had rapid ELISA antibody response i.e. titre of 1:2560 or above following patent parasitaemia. This titre remained elevated despite disappearance of patent parasitaemia and it declined gradually following curative antimalarial therapy. The ELISA responses observed in the four non-immune patients were non-variable though positive titres appeared rapidly with patent parasitaemia. Majority of titres were lower than those observed in sera of immune patients. When the test was applied to sera of patients from Honduras and Vietnam and the results were compared with those obtained by the indirect fluorescent antibody (IFA) technique, Spencer et al. (1979b) found a considerable number of negative ELISA results, especially in children with a parasitologically positive blood smear as well as positive IFA test. This was further investigated in a recent report providing the results of IFA and ELISA tests in over 800 individuals from El Salvador, all of whom were slide positive for *P. falciparum*. It was found that 88% had an IFA titre of 1:20 or above and 84% an ELISA titre of 1:160 or above. False negative IFA tests were seen in 11.7% cases.
and with the ELISA in 12.6% of the cases. When ELISA titers were 1:640, 99% were also positive by the IFA test; of those with IFA titre 1:80, 94% were also positive with ELISA (Spencer et al., 1981).

Ouakyi (1980) did not observe a consistent relationship between parasite density and the value of ELISA readings, but she produced an evidence that a negative ELISA value was observed in non-immune patients with a low parasite density (<1%) with a stay of less than 6 months in an endemic area, whereas a positive ELISA value with a same low parasite density was seen in patients with a longer stay.

Voller et al. (1980) applied the ELISA technique in Garki, Nigeria, during a epidemiological field study and demonstrated the occurrence of ELISA negative infants with a proven parasitaemia. Ambroise-Thomas et al. (1981) demonstrated that soluble exoantigens contained in the filtered supernatants of 6 hour cultures of P. falciparum merozoites can be used to prepare test plates for ELISA and may indeed increase the specificity of this test if applied to the detection of infections with this species.

Bidwell and Voller (1981) applied two ELISA methods for detection of malaria parasites in an experimental test using blood samples of P. falciparum infected Aotus monkeys. In the double antibody sandwich ELISA method, polyvinyl microplates were coated with immunoglobulins of malarious sera, infected blood was added and after the usual washing peroxidase...
labelled antimalarial serum and substrates were introduced. Blood with a density of one parasitized cell per 10^3 erythrocytes gave positive reaction by this method.

Recently, Mackey et al. (1982) have described an ELISA inhibition test, based on a previously developed RIA, for the detection of P. falciparum in human blood; parasites were detected at a level of 8 parasites per 10^5 RBC.

Countercurrent Immunoelectrophoresis (CIEP)

This method has been used for the detection of malarial antigens and antibodies in serum and plasma and possesses greater sensitivity than gel precipitation, besides being quicker. Seitz (1975) applied CIEP in the experimental rodent (malaria infected) for the detection of antigen and antibodies and found that antigen could be detected in serum at dilutions 8-16 times higher than by double diffusion. Seitz (1975) reported encouraging preliminary results using human plasma samples containing malaria antigen and antibodies. The method was used to study the kinetics of antigenemia in experimental rodent malaria models (Seitz, 1976; June et al., 1979) in which it was observed that the level of detectable soluble antigen correlated with the level of parasitemia. Perrin et al. (1979) used CIEP to detect antigen quantitatively in the human sera from patients with acute malaria infection and followed the level of antigen before, during and after treatment. Peak levels of antigen were found before the start of treatment, after which
there was a rapid diminution. However, the demonstration of antigen up to two weeks after the disappearance of parasites from the blood was still observed.

Radioimmunoassay (RIA)

This immunodiagnostic technique was first introduced by Stutz et al. (1974) for the detection of malarial antibody. Avraham et al. (1980), detected P. bicons infection in an inhibition test using radio-labelled staphylococcal protein A to register the binding of specific antibody in parasite antigen-coated tubes. He suggested that the inhibition of antibody binding occurred at dilutions corresponding to parasitaemia of 8 parasites/10⁵ RBC. Mackey et al. (1980a) also developed a similar method employing radio-labelled anti-IgG in the second stage, and using washed, lysed RBC from infected mice in a serial dilution and detected parasites at a level of 1 parasite/10⁶ RBC.

The methodology developed in murine model was subsequently modified and applied as a microassay to the detection of P. falciparum infection in human blood (Mackey et al., 1980b; Mackey, 1981). The supernate from sonicated parasites recovered by saponin lysis of infected RBC from in vitro cultures of P. falciparum was used as a source of antigen for coating microtubes. Using specific antibody from the IgG fraction of sera from the immune Gambian and labelled anti-IgG in the second stage the parasites were detected at a level of 8
parasites/10^6 RBC. The findings suggested a significant degree of correlation between the results of RIA and microscopy while reporting 82% positivity from 100 microscopically proven cases.

Avraham et al. (1981) applied RIA using human immune serum and radio-labelled protein A for the detection of *P. falciparum* from in vitro cultures. Inhibition of antibody binding was shown by sonicated, infected RBC at a level of parasitaemia equivalent to 1 parasite/10^3 RBC.