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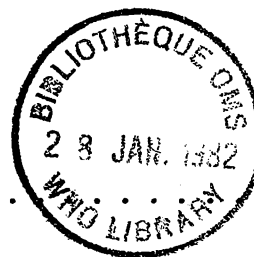
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ANTIGENS EMPLOYED IN IMMUNODIAGNOSTIC TESTS FOR THE DETECTION
OF MALARIAL ANTIBODIES^{1,2}

by

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CONTENTS



	<u>Page</u>
1. Introduction	2
2. Test antigens employed	2
2.1 Indirect fluorescent antibody (IFA) test	2
2.2 Indirect haemagglutination (IHA) test	3
2.3 Enzyme-linked immunosorbent assay (ELISA)	4
2.4 Gel diffusion tests (GDT)	5
2.5 Radioimmunoassay (RIA) test	5
2.6 Other tests	6
3. Discussion and conclusions	6
Summary	7
Acknowledgement	7
Résumé	8
References	9

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1. INTRODUCTION

The routine uses of malaria serology are the assessment of malaria experience in epidemiological studies, the laboratory confirmation of suspected cases in which a definitive blood-slide diagnosis cannot be made, and the screening of potential blood donors. Historically, in each of these applications, the test performed sought to detect or estimate malarial antibodies which, when present, often occurred at relatively low levels. Consequently, a major emphasis in development of serological tests for malaria was placed on achieving high sensitivity with less attention being given to the specificity of the underlying antigen/antibody reactions.

The following discussion will not include an evaluation of any of the tests, but will be restricted to consideration of the antigen preparations used as reagents to detect anti-malarial antibodies. The specificity achievable in any of the tests is largely a function of the antigen selected. Therefore, the discussion will centre on the availability and suitability of the antigen preparations which have been used. The tests themselves have been discussed recently by Voller & Houba (1981) and are described in detail in a WHO memorandum, "Serological testing in malaria" (WHO, 1974). No discussion of newer serological strategies which detect parasites, circulating antigens or immune complexes in blood has been included.

2. TEST ANTIGENS EMPLOYED

2.1 Indirect fluorescent antibody (IFA) test

Since the antigen used in the IFA test is the intracellular parasite, it is the most species-specific assay currently in use when the homologous parasite is selected as the test antigen. The IFA test is generally considered the standard against which other tests are measured (Spencer et al., 1979b). Antigen is prepared as thick or thin blood films of the parasitized blood, rich in schizonts, the most reactive stage. Blood infected with Plasmodium falciparum requires short-term incubation after being drawn to allow time for maturation to occur, since only young trophozoites (rings) are found in the peripheral circulation.

The obvious source of parasites for use in antigen preparations is an individual infected with circulating blood stages of an appropriate species of Plasmodium. This source, however, requires access to clinically ill patients and can be a serious problem for laboratories located in areas of low endemicity. As a substitute source, owl monkeys (Aotus trivirgatus) infected with an adapted strain of the appropriate species of parasite can be used. However, these monkeys are becoming exceedingly expensive and increasingly difficult to obtain. On the other hand, in vitro cultivation of erythrocytic stages furnishes an attractive alternative source of parasites to laboratories without access to active infections (Hall et al., 1978). Unfortunately, the in vitro approach is restricted at present to P. falciparum, since there is no system available for routinely growing the blood stages of other species of human parasites, although a preliminary report has appeared on the cultivation of P. vivax (Larrouy et al., 1981).

Total reliance on parasite cultures as a source of antigen for serological uses, however, may be impractical. Although continuous cultures of P. falciparum are routine in many laboratories, the procedure is expensive and time consuming, even when maintaining a single parasite species. In addition, the possibility of antigenic changes occurring in parasites maintained in culture for long periods cannot be ignored. Langreth et al. (1979) noted that there is a gradual loss of the knob antigen during long-term culture of P. falciparum.

In the absence of a convenient source of human parasites, closely-related simian parasites have been substituted as the test antigen with some success. Examples of this type of substitution are P. fieldi (Collins et al., 1966) or P. cynomolgi bastianellii (Kubin & Voller, 1963) for P. falciparum, P. brasilianum or P. fieldi for P. malariae (Collins et al., 1966), and P. cynomolgi for P. vivax (Tobie et al., 1962). The monkeys required for maintaining infections with these species are more readily available than Aotus but are still expensive. More recently, substitution of a rodent parasite, P. berghei, for P. falciparum has been

suggested (Maier & Piekarski, 1979), although this choice would seem to be questionable in view of the considerable differences to be expected in the antigens to two such widely diverse species. However, as shown in patients with multiple previous infections (Collins et al., 1966), from data on malaria transmission rates (Draper & Voller, 1972) and from studies evaluating the sensitivity of the IFA test (Sulzer et al., 1969), substitution of a heterologous antigen for the homologous one does provide useful information, although such a substitution is usually accompanied by a decrease in the species specificity and sensitivity of the test.

Cross reactions among species do occur with the IFA test, so that ascribing responsibility to a particular parasite species for a given antibody titre requires that the antiserum be reacted with preparations of each species of parasite; that is, multiple testing becomes necessary. To circumvent the need for multiple testing, a single test antigen prepared from a mixture of cells, each infected with a different parasite species, has been used (Sulzer et al., 1973).

In summary, the main advantage of using the IFA test is the species specificity and sensitivity possible when using the intact intraerythrocytic parasites as the antigen. This type of antigen provides the natural binding site for induced antibodies which should be superior to antigens prepared from crude soluble extracts. Substitution of the homologous parasite with a closely related, but heterologous, simian parasite is possible as already mentioned; however, both reduced species specificity and reduced sensitivity may result. Furthermore, use of a heterologous antigen in seroepidemiological studies can cause difficulties in interpretation if more than one parasite species is present.

From a technical viewpoint, the main difficulty of the test lies in obtaining inexpensive and reliable sources of parasite antigen. This difficulty might be overcome in the case of P. falciparum by the creation and funding of a central facility charged with producing antigen in vitro for distribution to laboratories not having a readily available source. Microscope slides of parasitized blood films can be air-dried and stored at room temperature with a desiccant for several weeks, which facilitates transport (WHO, 1974). These antigen slides can also be frozen and stored for periods as long as 12 months at -70 °C (Sulzer et al., 1969; WHO, 1974).

For species other than P. falciparum, a facility with access to Aotus monkeys is urgently needed to produce antigen for distribution to laboratories routinely engaged in malaria serological work. Centralization of the production function for these other species would be an important conservation measure for Aotus monkeys awaiting the time that routine cultivation of the remaining species of human plasmodia is achieved and the production function can be assumed by a central culture facility.

2.2 Indirect haemagglutination (IHA) test

In the IHA test, antigen preparations consist of erythrocytes coated with crude, soluble malaria extracts. Although the test itself is relatively simple to perform, even under field conditions, the procedures for preparing the antigen and coating the carrier erythrocytes are a major undertaking and should only be attempted by experienced laboratories. Details of these procedures are adequately described elsewhere (Desowitz & Stein, 1962; Wellde et al., 1969; Kagan, 1972; Meuwissen et al., 1972; Mathews et al., 1975). It should be noted, however, that the source of the carrier erythrocytes (sheep or human "O"), the way they are handled (temperature and time stored, tanning and method of fixation), the species of Plasmodium (P. knowlesi, P. vivax or P. falciparum), the source of the infected cells (monkey or man), as well as the method of extraction of soluble antigen, may all affect the sensitivity or specificity of the test, which in turn will influence its reproducibility. For these reasons, large batches of antigen are prepared at one time and stored by lyophilization (Meuwissen & Leeuwenberg, 1972). Since the test requires very small amounts of antigen when microagglutination plates are used, one batch of sensitized erythrocytes is usually sufficient for completion of even extended seroepidemiological studies (Farshy & Kagan, 1973). However, lack of reproducibility, due to differences in reactivity of different antigen preparations, has been encountered in some long-term longitudinal studies, such as the 10-year study carried

out in Ethiopia by Armstrong (1972). Nonetheless, it must be conceded that the relatively small number of antigen-coated cells required per test and the large batches of antigen which can be prepared and stored at one time reduce the requirement for regular access to blood infections to prepare antigen as in the case of the IFA test.

The use of erythrocytic cultures of P. falciparum and more efficient separation and isolation methods (Kreier, 1977; Heidrich et al., 1981) could result in greater antigen uniformity and, consequently, greater reproducibility and sensitivity of the test. The impact on species specificity of using heterologous antigens in the IHA test has not been adequately investigated. In early seroepidemiological studies, P. knowlesi was frequently used as the antigen because of its greater availability as compared to P. falciparum (Mahoney et al., 1966; Rogers et al., 1968; Kagan, 1972; Mathews et al., 1970). More recently, the use of antigen prepared from P. falciparum or P. vivax appeared to increase both the sensitivity and specificity of the IHA test (Meuwissen et al., 1973; Mathews et al., 1975; Cornille-Brögger et al., 1978).

However, when it is used in seroepidemiological work, the IHA test frequently gives false negative results with sera from infected infants and young children even when their sera react at high titre using the IFA test (Meuwissen et al., 1973). In an effort to clarify the reasons for this discrepancy, Bidwell et al. (1974), using the P. falciparum/Aotus monkey system, showed that the IHA soluble antigen failed to detect antibody induced by the primary parasitaemia. Since these early antibodies were readily detected by the IFA test, it is probable that the antigens used in the two tests react with different antibodies.

The use of homologous antigens, improved antigen purification techniques, and standardization of reagents should measurably improve the quality and interpretation of data obtained with the IHA test.

2.3 Enzyme-linked immunosorbent assay (ELISA)

Although most of the basic reagents necessary for the ELISA test are commercially available, the test antigen must be locally prepared. Briefly, this is usually accomplished by preparing a soluble extract of heavily infected blood. The infected blood is sonicated and after centrifugation the antigen-rich supernatant is removed and used to coat polypropylene tubes or microtitre plates (Voller et al., 1975, 1976).

The source of the parasitized cells may be an infected animal (Aotus monkey for P. falciparum, rhesus monkey for P. knowlesi) or in vitro cultures of the parasite for P. falciparum (Spencer et al., 1979a). Although the species specificity of the ELISA has not yet been adequately tested, it is to be expected that the crude soluble antigen preparations would show no greater specificity than is found with the IHA test. On the other hand, the procedure for preparing the antigen and coating the tubes or microplates is far less complicated than in the IHA test and therefore subject to less variability which is a distinct advantage. Batches of ELISA antigen can be prepared ahead of time and stored in aliquots at -70 °C for later use in coating tubes or plates. In common with the IHA test, only very small amounts of antigen are required by the ELISA test, especially if microplates are used.

Ambroise-Thomas et al. (1981) demonstrated that soluble exoantigens contained in the filtered supernatants of 6 h 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; however, the potential of using these exoantigens in epidemiological work has not yet been determined. It should be noted, however, that production of merozoite-specific antigens requires extensive manipulation of parasite cultures and would complicate considerably the task of antigen preparation. Furthermore, this approach could conceivably reduce the sensitivity of the test since, by its very nature, it restricts the antigen reagent to those exoantigens released by a single parasite stage of a synchronous infection.

2.4 Gel diffusion tests (GDT)

The antigens used in precipitin tests for malaria serology, such as the GDT, are all of human origin and consist of two main types: placental antigens which are usually derived from a heavily infected placenta obtained from a woman infected with P. falciparum at the time of delivery (McGregor et al., 1966), or soluble antigens circulating free in the blood of a heavily infected person (Wilson et al., 1975).

The placental antigen, which is rich in large segmenting parasites, is prepared as a crude filtered aqueous extract of a finely minced placenta.

The large number of soluble antigens circulating free in the plasma are conveniently divided into 3 main groups: the L antigens, which are heat labile and are destroyed by temperatures of 56 °C; the R antigens, which are heat resistant and survive temperatures above 56 °C but are destroyed by a temperature of 100 °C; and the S antigens, which are completely heat stable and are not destroyed even at 100 °C. Each of these groups can be further subdivided, with at least 20 serologically distinct S antigens already identified (Wilson et al., 1975).

Since the antigens required for the performance of the gel diffusion tests are obtained from heavily infected individuals, the present use of these tests is restricted to those laboratories having access to clinically infected people. Furthermore, these antigens are only useful in detecting P. falciparum infections. When the soluble plasma antigens are used, there is the additional problem that the specificity of the reaction may be excessive. All antigens are not present at one time, but may appear serially with the age of the infection and the corresponding circulating antibodies are reported to be short lived. Therefore the problem of standardization for routine general use would seem to present almost insurmountable difficulties. Nonetheless, S antigens would appear to be potentially useful in serotyping P. falciparum infections from various geographical locations (Wilson, 1981). The report (Wilson & Voller, 1972), that soluble antigens may be obtained from P. falciparum infected Aotus monkeys, makes a more convenient alternative source possible. Even so, reliance on infected Aotus monkeys as a source of antigen will remain a problem for the long term, in view of their cost and unavailability, as already emphasized in the section on the IFA test. The isolation from parasite cultures of soluble exoantigens detectable by the ELISA test (Ambroise-Thomas, 1981) suggests that the in vitro production of these antigens for use in precipitin tests like the GDT may be a viable alternative and should be evaluated.

The placental antigen used in the GDT can be prepared in relatively large batches, and is reported to be easily stored in the lyophilized state (McGregor et al., 1966). The soluble S antigens are also very stable (Wilson et al., 1973) and should easily be frozen and stored indefinitely at -70 °C.

Precipitin tests, such as the GDT, are considerably less sensitive than any of the other tests discussed. The GDT therefore require far more concentrated solutions of reagents in order to obtain an end point. These tests are thus relatively wasteful in terms of the limited antigen and antiserum resources.

2.5 Radioimmunoassay (RIA) test

Although the RIA test has not yet been used for seroepidemiological or serodiagnostic purposes, results of indirect methods using malaria antigen attached to sheep erythrocytes (Stutz et al., 1974) or to microtitre plates (Voller et al., 1977) have been reported. The antigens are prepared as soluble extracts of parasitized blood and are subject to the same advantages and disadvantages discussed under the ELISA test. As is to be expected, the sensitivity of the test is high, but the specificity, which depends on the purity of the antigen, is no greater than that obtained with ELISA.

2.6 Other tests

A number of other tests for detecting antibody against malaria parasites have been described (e.g., the schizont-infected cell agglutination test as well as a variety of in vitro growth and reinvasion inhibition tests). These tests were developed to fulfil specific research requirements and have not yet been adapted to meet the usual needs of seroepidemiological or serodiagnostic applications. In their present form they are either unsuitable or impractical for routine serological work.

3. DISCUSSION AND CONCLUSIONS

The antigens used in serological tests for detecting antibodies induced by malaria parasites fall into two broad categories: intact parasites, and soluble extracts prepared either from homogenates of parasites or from products (exoantigens) released by the parasite in the course of an infection. The major sources of these antigen preparations are humans or nonhuman primates infected with the appropriate human species of parasite. The availability of cultures of P. falciparum, and more recently P. vivax, should reduce reliance on the requirement for active mammalian infections with these two species to provide the antigens necessary for these serological tests.

The first requirement of a serological test for detecting antibodies in individuals infected with malaria parasites is unquestionably sensitivity, since these antibodies are often present at relatively low levels in the populations or individuals under study. Secondly, specificity of the reaction remains of considerable importance, since it has long been recognized that malaria parasites share antigens with other infectious agents (e.g., spirochaetes) and, more importantly, with each other.

There are four distinct species of parasite responsible for clinical infections in man which require specific identification. Since the specificity of any serological test for detecting antibodies can be no greater than the antigen used, it follows that great care should be exercised in their selection, processing, and standardization. Among the antigen preparations currently in use, the intact parasitized erythrocytes used in the indirect fluorescent antibody test should give a high degree of species specificity with the homologous human parasite. Serological tests that rely on crude extracts of soluble antigens tend, on the other hand, to be less species specific. Likewise, a species identification by any serological method will not be reliable if a heterologous antigen derived from a simian parasite is used in the test. At the opposite extreme, a test can make use of preparations such as S antigens which are so specific in their reactivity that general application of the test may be severely limited.

The availability of a system for producing P. falciparum in cultured erythrocytes provides a source of specific antigen for that species, and the recent preliminary report of successful cultivation of P. vivax (Larrouy et al., 1981) may provide an adequate future supply of this parasite for serological uses. Attempts to cultivate P. malariae and P. ovale should be supported. Once an adequate source of parasite material has been identified, there remains the question of purification. In a broad sense, purification includes isolation of parasites free from extraneous antigens and separated by developmental stage (see Kreier, 1977).

Chemical purification, including identification of antibody binding sites on specific antigens, appears to be achievable with the newer approaches available, such as the use of monoclonal antibodies as probes (Perrin et al., 1981). It seems probable that the key to increased sensitivity and specificity of serological tests for detecting malaria antibodies lies in better defined, more highly purified, standardized antigen reagents.

SUMMARY

Consideration of the subject is restricted to those antigen preparations used as reagents to detect antimalarial antibodies, with the discussion centering on the availability and suitability of the antigen preparation used.

The indirect fluorescent antibody test is considered the most species-specific assay now in use and it is highly sensitive when intact intraerythrocytic parasites are used as antigen. The main difficulty of the test lies in obtaining inexpensive and reliable sources of antigen.

Whilst recent improvements have increased the specificity and sensitivity of the indirect haemagglutination test, false negative results may occur with infants and young children. Its convenience is the small number of antigen-coated cells required per test and the large batches of antigen that can be prepared, lyophilized and stored at one time.

Very small quantities of antigen are required for the enzyme-linked immunosorbent assay (ELISA) but its species-specificity has not been adequately tested.

The antigens required for the performance of gel diffusion tests are obtained from heavily infected individuals, hence their use is restricted to those laboratories having access to clinically infected persons. The problem of standardization presents considerable difficulties; in addition the tests are relatively insensitive and require large quantities of the limited antigen and antiserum resources available.

The sensitivity of the radioimmunoassay test is high but its specificity is no higher than the ELISA.

It is emphasized that the first requirement of a serological test is sensitivity and the second specificity. It seems probable that the advent of increased sensitivity and specificity lies in better defined, more highly purified, standardized reagents.

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RESUME

ANTIGENES EMPLOYES DANS L'IMMUNODIAGNOSTIC DU PALUDISME POUR DECELER LES ANTICORPS ANTIMALARIQUES

L'auteur a limité son étude aux préparations d'antigènes utilisées comme réactifs pour la mise en évidence d'anticorps antimalariques, en se préoccupant principalement de la disponibilité et de la convenance de la préparation employée.

Parmi les épreuves actuellement en usage, celle que l'on considère la plus spécifique d'espèce est la réaction d'immunofluorescence indirecte, qui présente en outre une grande sensibilité lorsque l'antigène est constitué de parasites intra-érythrocytaires intacts. La principale difficulté qui s'attache à ce test est d'obtenir des sources sûres et peu coûteuses d'antigène.

Quant à la réaction d'hémagglutination passive, bien qu'on en ait récemment amélioré la spécificité et la sensibilité, il lui arrive de donner de fausses réactions positives chez les nourrissons et les jeunes enfants. Sa commodité tient, d'une part au faible nombre d'hématies recouvertes d'antigène ("coated cells") qu'exige chaque épreuve, d'autre part aux importants lots d'antigène qu'il est possible de préparer, de lyophiliser et de stocker en une seule fois.

La méthode ELISA (titrage avec immuno-adsorbant lié à une enzyme) présente l'avantage de n'exiger que des quantités minimales d'antigène, mais sa spécificité d'espèce n'a pas encore été solidement établie.

Pour ce qui est des épreuves de diffusion en gel, elles requièrent des antigènes prélevés sur des sujets intensivement infectés, de sorte qu'ils ne sont utilisables que par des laboratoires ayant accès à des porteurs d'infections cliniquement apparentes. Outre qu'ils soulèvent des difficultés considérables de standardisation, ces tests sont d'une médiocre sensibilité et sont fortement consommateurs d'antigènes et d'immunsérums dont on ne dispose qu'en quantités restreintes.

En ce qui concerne enfin le radio-immuno-essai, sa sensibilité est élevée mais sa spécificité n'est pas supérieure à celle de la méthode ELISA.

La première qualité exigible d'une séroréaction est la sensibilité, et la seconde la spécificité. Les progrès dans ce sens dépendront selon toute probabilité de la mise au point de réactifs standardisés mieux définis et d'un plus haut degré de pureté.

REFERENCES

- Ambroise-Thomas, P. et al. (1981) Mise en évidence, par une microméthode immuno-enzymologique (ELISA), d'antigènes métaboliques produits in vitro par Plasmodium falciparum en culture (Unpublished document WHO/MAL/81.930)
- Armstrong, J. C. (1972) Evaluation of the results of an indirect hemagglutination test for malaria in an Ethiopian population. Proceedings of the Helminthological Society of Washington, 39: 545-553
- Bidwell, D. et al. (1974) Application of indirect haemagglutination tests for malaria. VI. Comparison of indirect haemagglutination and immunofluorescence tests for malaria antibody in Plasmodium falciparum infected owl monkeys (Unpublished document WHO/MAL/74.824)
- Collins, W. E. et al. (1966) Fluorescent antibody studies in human malaria. IV. Cross-reactions between human and simian malaria. American journal of tropical medicine and hygiene, 15: 11-15
- Cornille-Brögger, R. et al. (1978) Changing patterns in the humoral immune response to malaria before, during and after the application of control measures: a longitudinal study in West African savanna. Bulletin of the World Health Organization, 56: 579-600
- Desowitz, R. S. & Stein, B. (1962) A tanned red cell haemagglutination test, using Plasmodium berghei antigen and homologous antisera. Transactions of the Royal Society of Tropical Medicine and Hygiene, 56: 257
- Draper, C. C. & Voller, A. (1972) The epidemiologic interpretation of serologic data in malaria. American journal of tropical medicine and hygiene, 21: 696-703
- Farshy, D. C. & Kagan, I. G. (1973) Use of stable sensitized cells in an improved indirect microhemagglutination test for malaria. Infection and immunity, 7: 680-682
- Hall, C. H. et al. (1978) Cultured Plasmodium falciparum used as antigen in a malaria indirect fluorescent antibody test. American journal of tropical medicine and hygiene, 27: 849-852
- Heidrich, H.-G. et al. (1981) Free-flow electrophoresis isolation of intracellular parasites (Plasmodium falciparum) from culture. Separation of the free parasites according to stages. Journal of parasitology (In press)
- Kagan, I. G. (1972) Evaluation of the indirect hemagglutination test as an epidemiologic technique for malaria. American journal of tropical medicine and hygiene, 21: 683-689
- Kreier, J. P. (1977) The isolation and fractionation of malaria-infected cells. Bulletin of the World Health Organization, 55: 317-331
- Kuvin, S. F. & Voller, A. (1963) Malarial antibody titres of West Africans in Britain. British medical journal, 2: 477-479
- Langreth, S. G. et al. (1979) Plasmodium falciparum: Loss of knobs on the infected erythrocyte surface after long-term cultivation. Experimental parasitology, 48: 213-219
- Larrouy, G. et al. (1981) A propos de l'obtention par culture in vitro de formes intra-erythrocytaires de Plasmodium vivax. Comptes Rendus des Séances de l'Académie des Sciences Série III: Sciences de la Vie, 292: 929-930
- Mahoney, D. F. et al. (1966) The preparation and serologic activity of plasmodial fractions. Military medicine, 131: 1141-1151
- Maier, W. A. & Piekarski, G. (1978) Zur serodiagnostik der malaria. Plasmodium berghei und P. falciparum als antigen für den indirekten immunofluoreszenz - test. Immunität und Infektion, 7: 75-82
- Mathews, H. M. et al. (1970) A seroepidemiological study of malaria in the Republic of the Philippines by the indirect hemagglutination test. American journal of epidemiology, 92: 376-381

- Mathews, H. M. et al. (1975) The indirect hemagglutination test for malaria. Evaluation of antigens prepared from Plasmodium falciparum and Plasmodium vivax. American journal of tropical medicine and hygiene, 24: 417-422
- McGregor, I. A. et al. (1966) Demonstration of circulating antibodies to Plasmodium falciparum by gel-diffusion techniques. Nature (London), 210: 1384-1386
- Meuwissen, J. H. E. T. & Leeuwenberg, A. D. E. M. (1972) Indirect haemagglutination test for malaria with lyophilized cells. Transactions of the Royal Society of Tropical Medicine and Hygiene, 66: 666-667
- Meuwissen, J. H. E. T. et al. (1972) Studies on various aspects of the indirect haemagglutination test for malaria. Bulletin of the World Health Organization, 46: 771-782
- Meuwissen, J. H. E. T. et al. (1973) Application of the indirect haemagglutination test for malaria: reproducibility with Plasmodium falciparum-sensitized cells. Bulletin of the World Health Organization, 49: 317-319
- Perrin, L. H. et al. (1981) Antigenic characterization of plasmodia using monoclonal antibodies (Unpublished document, WHO/MAL/81.938)
- Rogers, W. A., jr et al. (1968) A modified, indirect microhemagglutination test for malaria. American journal of tropical medicine and hygiene, 17: 804-809
- Spencer, H. C. et al. (1979a) The enzyme-linked immunosorbent assay (ELISA) for malaria. I. The use of in vitro-cultured Plasmodium falciparum as antigen. American journal of tropical medicine and hygiene, 28: 927-932
- Spencer, H. C. et al. (1979b) The enzyme-linked immunosorbent assay (ELISA) for malaria. II. Comparison with the malaria indirect fluorescent antibody test (IFA). American journal of tropical medicine and hygiene, 28: 933-936
- Stutz, D. R. et al. (1974) Estimation of antimalarial antibody by radioimmunoassay. Journal of parasitology, 60: 539-542
- Sulzer, A. J. et al. (1969) Indirect fluorescent-antibody tests for parasitic diseases. V. An evaluation of a thick-smear antigen in the IFA test for malaria antibodies. American journal of tropical medicine and hygiene, 18: 199-205
- Sulzer, A. J. et al. A multi-species malaria antigen for use in the indirect fluorescent antibody test. Transactions of the Royal Society of Tropical Medicine and Hygiene, 67: 55-58
- Tobie, J. E. et al. (1962) Fluorescent antibody studies on cross reactions between human and simian malaria in normal volunteers. American journal of tropical medicine and hygiene, 11: 589-596
- Voller, A. & Houbba, V. (1981) Malaria: In: Practical methods in clinical immunology, Vol. 2, Immunologic investigation of tropical parasitic diseases, Edinburgh, Churchill Livingstone
- Voller, A. et al. (1975) New serological test for malaria antibodies. British medical journal, 1: 659-661
- Voller, A. et al. (1976) Enzyme immunoassays for parasitic diseases. Transactions of the Royal Society of Tropical Medicine and Hygiene, 70: 98-106
- Voller, A. et al. (1977) A comparison of isotopic and enzyme immunoassays for tropical parasitic diseases. Transactions of the Royal Society of Tropical Medicine and Hygiene, 71: 431-437
- Wellde, B. T. et al. (1969) An indirect hemagglutination test for malaria using an antigen from the lysate of parasitized erythrocytes. Military medicine, 134: 1284-1293
- Wilson, R. J. M. & Voller, A. (1972) A comparison of malarial antigens from human and Aotus monkey blood infected with Plasmodium falciparum. Parasitology, 64: 191-195

- Wilson, R. J. M. et al. (1973) The stability and fractionation of malarial antigens from the blood of Africans infected with Plasmodium falciparum. International journal for parasitology, 3: 511-520
- Wilson, R. J. M. et al. (1975) Occurrence of S-antigens in serum in Plasmodium falciparum infections in man. Transactions of the Royal Society of Tropical Medicine and Hygiene, 69: 453-459
- Wilson, R. J. M. (1981) Methods for the biological characterization of malaria parasites: Characterization of Plasmodium falciparum with L, R and S-antigens (Unpublished document, WHO/MAL/81.937)
- WHO (1974) Serological testing in malaria. Bulletin of the World Health Organization, 50: 527-535

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