

m

WHODOC 3/2

Malaria - research - reviews
Technologies, Med - reviews
WHO/MAL/83.1003



WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

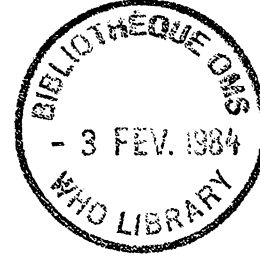
ENGLISH ONLY

a 66608

RECENT ADVANCES IN SOME LABORATORY TECHNIQUES AND PROSPECTS FOR THEIR APPLICATION IN MALARIA FIELD RESEARCH¹

by

J. Földes²



CONTENTS

	<u>Page</u>
1. Microscopy	1
2. Sampling and preservation of blood	3
3. Serological methods	4
3.1 <u>In vitro</u> inhibitory activity of sera	4
3.2 Antibody and antigen detection	5
3.3 Identification of <u>Anopheles</u> bloodmeals	6
3.4 Identification of species of sporozoites	7
4. Testing of the drug sensitivity of <u>Plasmodium falciparum</u>	7
5. Culture of <u>Plasmodium falciparum</u>	8
6. Clinical pathology	9

1. MICROSCOPY

Evaluation of fluorescent microscopy for the identification of malarial parasites
(Midha, N., Ichhpujani, R. L., Singh, P., Arora, D. R., Arora, B. & Chugh, T. D.,
Journal of communicable diseases, 13 (4), pp. 241-243, 1981)

Fluorescence microscopy was evaluated for the identification of malaria parasites. Acridine orange stain was found to be marginally superior to the conventional Romanowsky stain and more useful for field studies as it is less time consuming.

¹ Paper presented at the Tenth Meeting of the Steering Committee of the Scientific Working Group on Applied Field Research in Malaria, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Geneva, 25-28 April 1983.

² Formerly: Medical Officer, Research and Technical Intelligence, Malaria Action Programme, World Health Organization, Geneva, Switzerland.

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation sans l'autorisation de l'Organisation mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

Malaria blood cultures: a rapid fixation method to prevent sloughing of the thick film (Smrkovsky, L. L. & Rodrigez, C. S., Transactions of the Royal Society of Tropical Medicine and Hygiene, 75 (6), pp. 901-902, 1981)

A new method of fixation has allowed the laboratory to make its clinical laboratory findings (in particular, in vitro response of the malaria parasite to chloroquine) rapidly available to the physician after the patient is admitted and diagnosed as having Plasmodium falciparum malaria. In order to prevent the film from sloughing, it is air dried for 30 minutes, then fixed in methanol (1 second) and air dried. After this the slide is fixed (1 second) in acetone and air dried. It is then stained with a 2% Giemsa, at pH 7.1 for 30 minutes, rinsed, air dried and examined.

Rapid detection of malaria and other bloodstream parasites by fluorescence microscopy with 4'6 diamidino-2-phenylindole (DAPI) (Hyman, B. C. & MacInnis, A. J., Journal of parasitology, 65 (3), pp. 421-425, 1979)

Fluorescence microscopy of intact cells stained with DAPI reveals remarkably clear distributions of intracellular DNA. DAPI was thus the obvious choice for use as a diagnostic probe for bloodstream parasites. All operations were carried out at room temperature. Blood smears were fixed in 70% ethanol for 5 minutes. Smears were air dried then incubated in a 1.0 µg/ml solution of DAPI for 15-30 minutes. The slides were examined directly by fluorescence microscopy, without coverslip.

A fluorescent method for counting Babesia, Anaplasma, Plasmodium and Trypanosoma following DNA staining with 33258 Hoechst (Bisbenzimidazole) (Rodwell, B. J. & Howard, R. J., Annals of tropical medicine and parasitology, 75 (2), pp. 123-129, 1981)

Glutaraldehyde-fixed blood incubated in dye solution was dispensed as 1 mm³ droplets. Fixation and staining procedures were as follows: 0.02 ml of blood was added to 0.98 ml of 1.0% glutaraldehyde in 0.1 ml phosphate buffered saline, at pH 6.8; a 25 x 10⁻⁶ M solution of 33258 Hoechst stain in distilled water was prepared, filtered and stored in a dark bottle at 4°C; the fixed cell suspension was diluted 1:20 in the stain solution and then incubated at 4°C for 30 minutes in the dark. Using a direct counting technique, accurate estimates of parasitaemia were then obtained. Two other counting techniques were used for comparative observations.

Detection of exoerythrocytic stages of Plasmodium berghei in fixed liver tissue and cultured cells by an immunoperoxidase antibody technique (Hollingdale, M. R. & Leland, P., Transactions of the Royal Society of Tropical Medicine and Hygiene, 76 (5), pp. 624-626, 1982)

Resolution of the structure of the parasite was unsatisfactory by the indirect fluorescent antibody (IFA) technique. The paper describes the use of an indirect immunoperoxidase technique to identify exoerythrocytic (EE) stages of P. berghei in fixed liver section and cultured cells. EE stages of P. berghei in liver section fixed 12 hours after sporozoite inoculation and reacted with anti-red blood cell serum were clearly visible as dark brown bodies against unstained background cells. At 42 hours after inoculation, parasites were large. EE trophozoites in cultured W138 cells could be seen as early as three hours after sporozoite inoculation. At 48 hours, EE parasites were larger. Merozoites from mature EE parasites were produced in culture after 72 hours and stained clearly. The preparations are permanent and the parasites' structure could be seen more clearly than with IFA.

The reliability of the microscopic diagnosis of malaria in the field and in the laboratory (Collier, J. A. B. & Longmore, J. M., Annals of tropical medicine and parasitology, 77 (2), pp. 113-117, 1983)

A total of 470 thick and thin blood films was prepared from 129 villagers in the Solomon Islands. After staining with Giemsa, Leishman's and Field's stains, they were randomized and examined in the field. The specimens were then examined in the local central laboratory and by a microbiologist at a hospital in England. Films over which there was disagreement were examined by an expert at the Liverpool School of Tropical Medicine.

The rate of false negative diagnoses (for thick films) was 3% for the field workers, 9% for the malaria laboratory and 27% for the hospital in England. Field diagnosis was no less reliable than laboratory diagnosis ($P < 0.001$). Field's stain (Transactions of the Royal Society of Tropical Medicine and Hygiene, 34, pp. 195-202, 1942) was the most reliable stain for both thick and thin films. A new technique was developed for staining thin films with Field's stain. Fixation with methyl alcohol was followed by placing five drops of solution A at one end of the slide, and four drops of solution B at the other end. The two pools of stain were then drawn rapidly across each other using a glass slide, and staining took place for two minutes, before rinsing with a stream of buffer (pH 7.2). A miniature McArthur microscope was used in the field for examination of the slides.

2. SAMPLING AND PRESERVATION OF BLOOD

Evaluation of filter paper eluates for seroepidemiological studies on malaria in Peninsular Malaysia (Thomas, V. & Chan, W. C., Tropical and geographical medicine, 34, pp. 145-149, 1982)

The authors evaluated the usefulness of filter paper eluates in seroepidemiological studies on falciparum malaria as compared with the usefulness of sera, with particular reference to reproducibility, sensitivity and shelf-life. The fully soaked blood sampling paper absorbs 0.1 ml of blood (0.04 ml of serum). After drying at room temperature the coded filter paper strips were sealed in plastic bags. They could be stored either at -20°C or in the 4°C compartment of a refrigerator. For eluting the blood, the absorbent area was cut in two and soaked in 0.2 ml of a phosphate buffer solution, at pH 7.6. The dilution of such an eluate is 1:10. The serial dilutions were tested by the indirect fluorescent antibody test.

Evaluation of a finger-stick specimen collection method for seroepidemiology of antibody to hepatitis A virus (McCaustland, K. A., Bond, W. W., Bradley, D. W., Favero, M. S., Petersen, N. J. & Maynard, J. E., Journal of clinical microbiology, 13 (1), pp. 217-218, 1981)

The finger-stick swab method of specimen collection was found to be very useful for large-scale seroepidemiological surveys: it was rapid, economical and efficient. The finger-stick swab absorbed 0.2 ml of blood which was then eluted into a 1-dram, screw-capped vial containing 1 ml of 1% BSAS (bovine serum albumin in normal saline) with 0.1% sodium azide. The antibody titre was determined by radioimmunoassay. A series of laboratory and field studies were conducted for the evaluation of the method.

Parasitic diseases: testing with filter paper blood spots (Mathews, H. M., Laboratory management, 19 (2), pp. 55-62, 1981)

The collection of a small volume of blood on filter paper overcomes many of the problems of collecting, processing, and transporting blood samples for serological surveys. The stiff and strong rectangular piece of paper (1 x 3 inches) is imprinted with two 12 mm diameter circles. Approximately 100 μl of blood are required to fill each of these circles which are then placed in a slotted microscope slide box and allowed to dry completely at ambient temperature. The dried papers can be kept at 4°C or -20°C for several months. In the indirect haemagglutination test for malaria the paper discs were eluted with 0.4 ml phosphate buffer solution at pH 6.9, and for other tests at pH 7.2 and 7.4.

The cryopreservation of standard sera (Tosswill, J. H. & Ridley, D. S., Journal of clinical pathology, 34, pp. 76-77, 1981)

To determine the storage temperature needed for the preservation of standard sera for serodiagnostic tests, groups of sera were tested before and after storage at -70°C and -20°C for periods of up to five years. No definite advantage was found in storage at -70°C for the indirect fluorescent antibody test and certain other tests.

3. SEROLOGICAL METHODS

3.1 In vitro inhibitory activity of sera

The inhibitory effect of human immune sera upon the in vitro development of Plasmodium falciparum (Golenser, J., Miller, J., Avraham, H. & Spira, D. T., Tropical and geographical medicine, 35, pp. 15-20, 1983)

Sera from patients who had recovered from malaria were examined for their capacity to inhibit the growth of P. falciparum in vitro. Before being examined, the sera were first diluted to 10% in RPMI medium and dialysed against RPMI for 25 hours with one change of medium. This procedure was carried out in order to eliminate any residual drugs taken by the donor. Parasitized erythrocytes were then diluted to 0.5-1% in a suspension of non-infected erythrocytes. Aliquots of 100 μl of this suspension were dispensed into 96-well microculture trays. The supernatant was replaced by medium containing the different sera 60 minutes later, and then every 24 hours for 5-7 days. Parasitaemias were estimated daily from Giemsa stained smears.

IgG purified from inhibitory serum was found to be as effective as the original serum. There was no correlation between antibody titre measured by the indirect fluorescent antibody (IFA) test and the inhibitory capacity of the serum: some sera with high IFA titres did not inhibit the growth while some with low IFA titres inhibited the parasite. Autoantibodies directed against the erythrocytes, which were found in some sera, had no antimalarial inhibitory role and their absorption did not alter the inhibitory effect. Most of the antibodies produced were not related to protective immunity.

Plasmodium falciparum: rapid assay for in vitro inhibition due to human serum from residents of malarious areas (Jensen, J. B., Boland, M. T. & Hayes, M., Experimental parasitology, 54, pp. 416-424, 1982)

Tightly synchronized cultures of Plasmodium falciparum were exposed to various dilutions of human sera from healthy adults living in malarious areas before and after merozoite invasion was completed. All "immune" sera inhibited merozoite invasion. Additionally, most sera demonstrated moderate to severe retardation of parasite development, even when merozoite invasion was completed in nonimmune serum before developing parasites were exposed to immune serum dilutions. Both types of inhibition were discernible on Giemsa-stained thin films, and these observations were highly correlated with incorporation of (^3H)hypoxanthine. Large numbers of serum samples could be examined using microlitre quantities of immune serum in microtitre plate cultures processed with a cell harvester.

A microassay for detecting merozoite inhibition suitable for routine laboratory use (Cowen, N., Clancy, R., Cripps, A. & Alpers, M., American journal of tropical medicine and hygiene, 32 (1), pp. 6-10, 1983)

A micro semiautomated assay of merozoite inhibition was developed, based on the incorporation of radiolabelled isoleucine into parasite protein. The assay was validated using 44 sera from healthy blood donors in Madang, Papua New Guinea. In carrying out the assay, washed erythrocytes (8%) were added to a culture dish containing parasites, to reduce the red cell infection rate to between 0.7 and 1.4%. The culture was centrifuged and washed twice with RPMI 1640, supplemented with 10% pooled sera from healthy donors in Newcastle, Australia. Gentamycin was added to a concentration of 50 $\mu\text{g}/\text{ml}$. From a suspension adjusted to a 2% haematocrit, 90 μl aliquots were placed in flat bottomed wells of a microtitre tray. At 0 hours 5 μl of C^{14} -isoleucine, 10 μl of IgG or 15 μl of dialysed serum were added to each well. The microtitre plates were placed in a candle jar at 37°C and harvested at various

times on filter paper discs. The results, expressed in terms of incorporation of labelled amino acid, showed that inhibition of uptake of label occurred in 43.2% of tests, while an enhancement of uptake of label occurred in 4.7%. In addition, the assay of the IgG fraction of serum was performed and the sera were also examined by the indirect fluorescent antibody test. In conclusion, the merozoite inhibition test was considered to have "potential" for seroepidemiological studies, and for providing a mechanism of biotyping wild isolates.

3.2 Antibody and antigen detection

Falciparum - Spot IF, BioMérieux. The kit is commercialized for the epidemiological assessment of malaria. The in vitro cultured Plasmodium falciparum schizonts are fixed on special slides by glutaraldehyde and stocked in plastic bags. The investigated sera are diluted and the indirect fluorescent antibody test performed and evaluated according to the attached instruction sheet.

Evaluation of cultured P. falciparum antigen in malaria indirect immunofluorescence test (Ray, K., Sharma, M. C., Sivaraman, C. A., Sulzer, A. J., Kagan, I. G. & Chowdhuri, A. N. R., Journal of communicable diseases, 14 (4), pp. 239-246, 1982)

Antigen prepared from a strain of Plasmodium falciparum (FAN-5) cultured in vitro was evaluated in the indirect immunofluorescence test using sera from normal individuals, individuals with bacteriological and parasitic infections other than malaria and patients suffering from patent malaria. On average, the antigen was found to have a nonspecificity of only 2.28% and its sensitivity in natural P. vivax infection was 82.4%. The titres were consistent on replicate testing. The titres obtained were comparable with those obtained with a reference Aotus P. falciparum antigen. Thus, the in vitro culture of P. falciparum using a simple technique has made available a new source for the production of malaria antigen in large quantities for serological purposes.

Preliminary field trial of a radioimmunoassay for diagnosis of malaria (Avraham, H., Golenser, J., Bunnag, D., Suntharasamai, P., Tharavanij, S., Harinasuta, K. T., Spira, D. T. & Sulitzeanu, D., American journal of tropical medicine and hygiene, 32 (1), pp. 11-18, 1983)

A radioimmunoassay (RIA) has been developed for the detection of P. falciparum in infected blood. The assay is based on the ability of solubilized, infected red blood cells (RBC) (P. falciparum "antigen") to combine with anti-P. falciparum antibodies and thus prevent the subsequent interaction of the latter with antigen-coated microtitre plates. The microtest plates were coated with the standard antigen (ST-Ag). The test sera were diluted and added to the coated plates. The bound antibody was reacted with ¹²⁵I labelled protein A (SPA) and the complex was solubilized with 0.1N NaOH. The contents of each well were transferred to plastic tubes and the radioactivity of the tubes was measured. Of the 108 blood samples tested, 23 were positive by microscopy and 39 by RIA. The proportion of false positives by RIA was low.

A highly sensitive solid-phase radioimmunoassay for Plasmodium falciparum antigens and antibodies (Avraham, H., Golenser, J., Gazitt, Y., Spira, D. T. & Sulitzeanu, D., Journal of immunological methods, 53, pp. 61-68, 1982)

A partially purified P. falciparum antigen preparation was obtained from in vitro cultured parasites enriched after gelatin sedimentation by sonicating the infected red blood cells and precipitating the proteins with 50% saturated ammonium sulfate. The precipitate was dissolved in buffer, ultracentrifuged and used to coat the microtitre plates by filling the wells with 0.1 ml of the antigen solution (i.e. enriched, sonicated, infected red blood cells). After incubation at 37°C for three hours, the plates were washed three times and 0.1 ml volumes of the test serum, diluted in 10% fetal calf serum (FCS), were added to the microplate wells and incubated at 37°C for four hours. The plates were then washed rapidly twice. Labelled staphylococcus protein A (SPA) was added to each well and the plate was left to stand for one hour at room temperature. After washing the wells three times, the material was solubilized in 120 µl aliquots of 0.1N NaOH. Finally, the contents of each well were transferred to plastic tubes and the radioactivity of the tubes measured.

Anti-P. falciparum antibodies were detected by incubating antiserum dilutions in the coated wells and detecting the bound IgG with radioiodinated SPA. P. falciparum antigens were detected by their ability to inhibit binding of antibody to the coated wells. Sera from individuals with a history of P. falciparum infection contained antibodies detectable at a dilution of 1:75 000. P. falciparum infected red blood cells in vitro could be detected at levels of parasitaemia of the order of one parasite or less per 10^6 red blood cells.

Plasmodium yoelii: comparison of indirect immunofluorescence and radioimmunoassay for detecting monoclonal antibodies to malaria (Taylor, D. W., Munoz, P. A., Kim, K. J., Evans, C. B. & Asofsky, R., Experimental parasitology, 53, pp. 362-370, 1982)

Culture supernatants from 479 wells were examined. Approximately 9% of the wells were found to contain antibodies to P. yoelii by the indirect fluorescent antibody (IFA) test and 13% by radioimmunoassay (RIA) analysis. However, only 4.6% of the wells were positive by both IFA and RIA techniques. The minimal amount of monoclonal antibody required to give a positive reaction was higher for IFA than for RIA. Stage-specific hybrids were more easily detected in IFA than RIA and non-stage-specific antigens were more readily detected by RIA than IFA. Thus, it is important to use several serological methods if maximal efficiency in detecting antimalarial monoclonal antibodies is to be achieved.

Utilization of purified antigens for the serodiagnosis and epidemiological studies of human malaria. Advantages of the ELISA technique in evaluating specific IgG and IgM (Roffi, J., Lafabrie, B. & Stach, J. L., Bulletin de la Société de Pathologie exotique, 76, pp. 49-68, 1983)

Antigen was isolated and purified from red blood cells of Saimiri sciureus experimentally infected with Plasmodium falciparum. The enzyme-linked immunosorbent assay (ELISA) was carried out with the purified antigen. Two series of sera were used: one coming from an endemic area, the other from a control area. The results were compared with those obtained with the indirect fluorescent antibody test. There was a good correlation between the two tests when anti-IgG conjugate was used for ELISA but there was no correlation with anti-IgM conjugate. The ELISA allows a separate evaluation of specific IgG and IgM. Consequently distinction of recent and past infection would be possible.

3.3 Identification of Anopheles bloodmeals

Mosquito host bloodmeal identification: methodology and data analysis (Washino, R. K. & Tempelis, C. H., Annual review of entomology, 28, pp. 179-201, 1983)

The authors summarize the status of host bloodmeal identification studies with emphasis on new developments in basic methodology and host selection pattern studies. They give an account of the nonserological approaches and the serology. The review deals with the passive haemagglutination inhibition test (PHI) in detail. The PHI test and the enzyme-linked immunosorbent assay are recommended for bloodmeal identification. The PHI test is difficult to perform, but it is at present the only proven method for identifying bloodmeals as regards genus and species.

Application of enzyme-linked immunosorbent assay (ELISA) to identification of Anopheles mosquito bloodmeals (Edrissian, G. H. & Hafizi, A., Transactions of the Royal Society of Tropical Medicine and Hygiene, 76 (1), pp. 54-56, 1982)

Bloodmeals from Anopheles stephensi fed either on human volunteers or on guinea-pigs were smeared on filter paper by crushing the abdomen of the mosquito 1, 6, 12, 24 and 48 hours after feeding. The dried blood was eluted and investigated by the simplified ELISA technique. Results showed that ELISA was a quite specific and sensitive technique for the identification of the bloodmeals, even 24 hours after ingestion by anopheline mosquitoes.

An adaptation of the gel diffusion technique for identifying the source of mosquito bloodmeals (Collins, R. T., Dash, B. K., Agarwala, R. S. & Dhal, K. B., unpublished document WHO/MAL/83.992, 1983)

A modified gel diffusion technique has been designed which only requires a simple field laboratory for implementation. The bloodmeal smeared on filter paper is eluted and tested against human, cow and rabbit antisera by the gel diffusion technique.

3.4 Identification of species of sporozoites

Monoclonal antibodies to circumsporozoite proteins identify the species of malaria parasite infected mosquitos (Zavala, F., Gwadz, R. W., Collins, F. H., Nussenzweig, R. S. & Nussenzweig, V., Nature, 299, pp. 737-738, 1982)

Two sensitive immunoradiometric assays were developed for detecting circumsporozoite (CS) protein antigen using monoclonal antibodies. The CS protein extracted from infected mosquitos is adsorbed on the plastic surface of the wells of microtitre plates and reacted with specific monoclonal antibody radiolabelled with ¹²⁵I. In the two-site immunoradiometric assay (IRMA) the wells of plastic plates are first coated with monoclonal antibodies to an epitope of the CS protein, then incubated with crude extracts of sporozoites and washed. The antigen specifically bound is measured using an excess of radiolabelled monoclonal antibody directed against another epitope of CS protein. The counts remaining in the well are directly proportional to the concentration of antigen in the extract. The value of these two immunoassays in epidemiological studies remains to be established.

4. TESTING OF THE DRUG SENSITIVITY OF PLASMODIUM FALCIPARUM

Visual in-vitro test for determining the drug sensitivity of Plasmodium falciparum (Rieckmann, K. H., Lancet, 1, pp. 1333-1335, 1982)

A new simple test was developed for determining the drug sensitivity of P. falciparum; it does not require the preparation of blood films or the use of a microscope. Five µl of parasitized blood are transferred into the wells of a microculture plate containing culture medium and various drug concentrations. Drug susceptibility of P. falciparum is determined from the extent to which the drug inhibits parasite growth during the period of incubation. After incubation, 25 µl of a freshly mixed preparation of 1 part 1N NaOH and 1 part of 1N NaCl were added to the wells of the microculture plate. Pigmented precipitates were observed in the drug free (control) wells. Precipitation in the chloroquine containing wells was noted only at the drug concentrations which failed to inhibit maturation to schizonts in parallel cultures. Field trial of the test is in progress.

A rabbit in-vitro system to evaluate drug action against Plasmodium falciparum (Mrema, J. E. K. & Rieckmann, K. H., Transactions of the Royal Society of Tropical Medicine and Hygiene, 77(1), pp. 130-135, 1983)

A new model system was developed for identifying potential agents against drug-resistant falciparum malaria, particularly compounds which are converted in vitro to their active metabolites or which exert a prolonged suppressive activity after drug administration. Serum samples, collected from rabbits at various times after drug administration, were incubated with synchronized ring form parasites using the microtest system. The extent to which the presence of drugs in the serum inhibited parasite growth was usually determined after 32 to 40 hours of incubation. Antimalarial activity was observed in sera obtained from rabbits which received chloroquine, mefloquine, pyrimethamine and cycloguanil but not in those which received 4-4'-diacetyldiaminodiphenyl-sulfone (DADDS). The effects against the drug-sensitive strain were more marked than against the drug-resistant one.

Characteristics of chloroquine binding to glass and plastic (Geary, T. G., Akood, M. A. & Jensen, J. B., American journal of tropical medicine and hygiene, 32 (1), pp. 19-23, 1983)

Chloroquine binds to glass to an extent which can seriously decrease the availability of the drug. Preparations of chloroquine in various solutions showed decreases in concentration of up to 40% in glass containers. Passage of solutions of chloroquine over columns of glass beads or glass wool decreased chloroquine concentrations by as much as 70%. Chloroquine was found to bind extensively to cellulose acetate filters, but showed little binding to polycarbonate filters or plastics of various types. Human serum at concentrations of 5 to 50% inhibited the binding of chloroquine to glass. Reduction in chloroquine concentration can alter the interpretation of chloroquine sensitivity studies.

5. CULTURE OF PLASMODIUM FALCIPARUM

Plasmodium falciparum cultivation in vitro with fresh rabbit serum (Guan, W. B., Zhou, Y. C. & Huang, W. J., Chinese medical journal, 95 (9), pp. 655-657, 1982)

P. falciparum has been successfully cultivated in vitro with fresh rabbit serum. Fifteen ml of rabbit serum were added to 100 ml of RPMI 1640 medium with sodium bicarbonate. Five ml of culture with 5% infected and uninfected red cells were incubated at 35.5 - 37°C. The infection rate was over 20% and the culture has been maintained alive for more than 500 days. The results of comparative studies with human serum suggest that rabbit serum may be used with advantage for the continuous culture of malaria parasites.

Plasmodium falciparum: modifications of the in vitro culture conditions improving parasitic yields (Zorg, J. W., MacLeod, A. J., Dickson, I. H. & Scaife, J. G., Journal of parasitology, 68 (6), pp. 1072-1080, 1982)

Irrespective of the isolates used, suspension cultures in glucose-enriched RPMI 1640 medium buffered with TES* yielded about twice the amount of parasites that could be obtained with static thin layer cultures with HEPES** -buffered RPMI 1640 without additional glucose. In suspension culture, 1 mg/ml methylcellulose was added to protect the erythrocytes. With a cloned isolate of P. falciparum a further stimulation of the final parasitaemia could be achieved by supplementing the medium with 50 µg/ml hypoxanthine and 600 µg/ml reduced glutathione. Hypoxanthine and glutathione were identified as two of the factors critical to the ability of human serum to support the growth of the parasites.

Plasmodium falciparum: one-step growth in a semi-defined medium and the stimulatory effect of human seric lipoproteins and liposomes (Nivet, C., Guillotte, M. & Pereira da Silva, L., Experimental parasitology, 55, pp. 147-151, 1983)

The ring stages of P. falciparum within red blood cells cultured with complete medium stop growing when transferred to a basic medium containing RPMI plus fatty acid-free bovin serum, albumin and dialysable factors from human serum. Growth and multiplication can be partially restored by the addition of lipoprotein fractions prepared from human serum. Synthetic liposomes mimic the effect of lipoproteins.

A labour-saving method for the in vitro culture of Plasmodium falciparum (Reber-Liske, R., Acta tropica, 40, pp. 39-43, 1983)

The in vitro cultivation of P. falciparum requires at least daily changes of medium. Addition of 50 mg/l of hypoxanthine to RPMI 1640 medium permits postponement of the change of medium for up to 72 hours. A single sub-culture step is required to remove unlabelled hypoxanthine prior to the use of the cultured material in ³H hypoxanthine incorporation assays. The method constitutes a considerable saving of time and medium.

* TES = N-tris (hydroxymethyl) methyl-2-aminoethane sulfonic acid.

** HEPES = N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid.

6. CLINICAL PATHOLOGY

A rapid semi-quantitative glucose-6-phosphate dehydrogenase screening test for the clinical laboratory (Sewrajshingh, G. S. S. & Schouten, H., Tropical and geographical medicine, 34 (3), pp. 272-274, 1982)

The modified glucose-6-phosphate dehydrogenase (G-6-PD) screening test will detect all samples with G-6-PD activity below 100 U%. Individuals at risk for drug induced haemolysis (G-6-PD <50 U%) can be detected with a sensitivity of 80% and a specificity of 100%. The fluorescent spot test of Beutler was modified by reducing the quenching of haemoglobin components. Less erythrocytes were added to the incubation mixture and the clear fluorescence was not covered by the dark haemoglobin pigmented background.

A sensitive new prenatal test for sickle-cell anaemia (Chang, J. C. & Kan, Y. W., New England journal of medicine, 307 (1), pp. 30-32, 1982)

Improved detection of the sickle mutation by DNA analysis (Orkin, S. H., Little, P. F. A., Kazazian, H. H. & Boehm, C. D., New England journal of medicine, 307 (1) pp. 32-36, 1982)

The initial methods for prenatal diagnosis of sickle-cell anaemia required fetal blood sampling. The new techniques for restriction-endonuclease analysis of DNA permit the use of amniocentesis. The drawback of the first enzyme assays was that the amniotic cells had to be cultured to provide a sufficient amount of DNA for the DdeI enzyme analysis. The new assay with the enzyme MstII is so sensitive that it can be applied to uncultured amniotic-fluid cells.

The DNA was extracted from white cells or amniotic-fluid cells. One to five µg of genomic DNA was digested with one unit of MstII per microgram DNA. Digested DNA fragments were separated by electrophoresis on a 1.2% agarose gel and transferred by blotting to a nitrocellulose filter. For the hybridization probe, a 1.15 kb (kilobase) isotope labelled DNA fragment was used, isolated from the cloned human beta globin gene. Since the MstII site at beta⁵⁻⁷ position is abolished in sickle DNA, the enzyme hydrolysis results in a 1.35 kb fragment.

The simple test makes prenatal diagnosis feasible in countries such as Ghana, where an estimated 140 000 prenatal tests would be required per million pregnancies. The laboratory test can be further simplified by replacing the isotope labelled probe with a stable fluorescent one.

= = =