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9. ABSTRACT

The use of thick-smear slides for the indirect fluorescent antibody (IFA) test for malaria appears to have distinct advantages over thin blood films which have been employed for this purpose. The technique for preparation of the thick antigen slides is as follows: 1) Draw blood from the donor with a heparinized syringe; 2) remove the plasma by centrifugation and wash the cells five times in buffered physiological saline (pH 7.2); 3) resuspend cells in buffered physiological saline, so that thick smears prepared from the suspension give at least 10 or more plasmodia to each high-power field; 4) prepare thick smears on microscope slides and air dry at room temperature; 5) store at -70 C until needed. The IFA test is performed as follows: 1) remove slides from storage, place in staining racks, and immerse in glass distilled water for 10 minutes on a slowly moving slide rotator; 2) drain and air dry at room temperature; 3) immerse in acetone at -20 C for 30 minutes, drain, and air dry (this step may be omitted); 4) place slides in moist chamber, overlay amounts with serum dilutions to be tested, and incubate 30 minutes at 37 C; 5) place in racks in buffered physiological saline, and wash on a slide rotator for 15 minutes; 6) drain, air dry, and replace in moist chamber; 7) overlay with the appropriate dilution of anti-species conjugate mixed with Evans blue, 1:500; 8) incubate 30 minutes at 37 C; 9) wash as in step 5; 10) drain, air dry, and mount with buffered glycerin and a cover slip; 11) read on a fluorescence microscope equipped with BG-12 (to locate plasmodia) and UG-2 (to evaluate specific fluorescence) exciter filters and an OG-1 (or equivalent) barrier filter. The several advantages ot this technique are discussed.

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The Use of Thick-smear Antigen Slides in the Malaria Indirect Fluorescent Antibody Test

The use of thick-smear antigen slides for the indirect fluorescent antibody (IFA) test for malaria appears to have distinct advantages over thin blood films which have been employed for this purpose. The technique for preparation of the thick antigen slides is as follows: (1) Draw blood from the donor with a heparinized syringe; (2) remove the plasma by centrifugation and wash the cells five times in buffered physiological saline (pH 7.2); (3) resuspend cells in buffered physiological saline, so that thick smears prepared from the suspension give at least 10 or more plasmodia to each high-power field; (4) prepare thick smears on microscope slides and air dry at room temperature; (5) store at −70 C until needed.

The IFA test is performed in the following manner: (1) remove slides from storage, place in staining racks, and immerse in glass distilled water for 10 min on a slowly moving slide rotator; (2) drain and air dry at room temperature; (3) immerse in acetone at −20 C for 30 min, drain, and air dry (this step may be omitted); (4) place slides in moist chamber, overlay mounts with serum dilutions to be tested, and incubate 30 min at 37 C; (5) place in racks in buffered physiological saline, and wash on a slide rotator for 15 min; (6) drain, air dry, and replace in moist chamber; (7) overlay with the appropriate dilution of antispecies conjugate mixed with Evans blue, 1:500; (8) incubate 30 min at 37 C; (9) wash as in step 5; (10) drain, air dry, and mount with buffered glycerin and a cover slip; (11) read on a fluorescence microscope equipped with BG-12 (to locate plasmodia) and UG-2 (to evaluate specific fluorescence) exciter filters and an OG-1 (or equivalent) barrier filter.

There are a number of advantages to this technique of performing the test. Washing the parasitized red blood cells removes the donor's gamma globulin, which reduces nonspecific staining. Blood specimens with a small percentage of parasitized erythrocytes can be used for antigen preparation. When cells are resuspended following the washing procedure, the original volume of blood may be reduced, which concentrates the parasitized cells. Blood specimens with heavy parasitemias may be diluted several volumes. Use of thick smears in place of thin smears results in many plasmodia in any given field so that searching for parasitized cells is not required. Smears are made in scribed circles, which facilitates locating parasites and reading of the test. The use of a counterstain further controls nonspecific reactions in the test. Reading of the slide is very rapid: processing two or more slides per minute is not unusual. Reactions are usually frankly positive or negative, and doubtful reactions are easily resolved because of the number of parasitized cells in each field.

Evaluation of the test with 46 sera for which blood slides were available for parasitologic evaluation indicated that the IFA test employing thick smears of *Plasmodium brasilianum* and *P. fieldi* had a high degree of specificity with the new antigen (Table I). Blood films from each patient were Giemsa stained and examined for malaria parasites. When tested with the simian antigens, one serum specimen of slide-positive *P. falciparum* infections gave negative results. When retested with its homologous antigen, this same specimen gave positive results. The serum was drawn early in the parasitemia; this might affect cross-reactivity with heterologous antigens.

Equivocal reactions, neither frankly positive nor negative at a dilution of 1:5 (the lowest dilution tested), occurred with only six of 46 sera; these six specimens are counted as serologically negative in the table. Blood slides from these individuals were negative. When clinical histories of the six patients were examined, two had definite histories of previous malaria. The other four were from seamen tested in a shipboard outbreak of malaria.

TABLE I. *Qualitative results of the malaria indirect fluorescent antibody test on sera, compared with parasitological examinations of blood slides, from 46 suspected cases of malaria.*

	Blood slide		
	Positive	Negative	Total
IFA positive	13	3	16
IFA negative	1	29	30
Total	14	32	46

These six individuals, therefore, might well have had malaria antibodies, and the readings, conservatively considered equivocal, may indicate this fact.

To date antigen has been evaluated for only *Plasmodium brasilianum* from the spider monkey (*Ateles* sp.) and *P. fieldi* from the rhesus monkey (*Macacca mulatta*). Preliminary trials using malaria parasites of human origin indicate that the washed-cell–thick-smear technique can be employed successfully. The evaluation of the human malaria antigen will be the subject of a later, more extensive report.

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