Ann. Soc. belge Méd. trop.
1970, **50**, 5, 613-634

The diagnosis of human African trypanosomiasis (*T. gambiense*) by the use of fluorescent antibody test

1. Standardization of an easy technique to be used in mass surveys

BY

M. WERY, S. WERY-PASKOFF and P. VAN WETTERE

Summary — The indirect fluorescent antibody test makes a rapid mass diagnosis of the african sleeping sickness possible, by examining dried blood samples.

Polymorphic strains of *T. gambiense*, or better *T. brucei*, are maintained in albino rats for the production of antigen. Parasitized blood is taken from the heart and mixed with a heparine-glucose mixture. Thin films are made and must contain between 5 and 50 parasites per field (obj 25 ×, ocul 10 ×). They can be preserved for as long as 3 to 6 months at − 15 °C in polythene bags containing silicagel. Fixation is not necessary. Six blood samples can be examined on one slide.

Fluorescein labelled anti-human serum from « Institut Pasteur » of Paris, used at a 1/100 dilution, or from Nordic Pharmaceuticals, used at a 1/15 dilution produced regularly reproductible results.

T. brucei antigens taken from rats have some important advantages : strain less dangerous for the worker; regularly increasing parasitemia so that preparation of the antigen can be planned for a given day; good sensitivity of the test, serum from sleeping sickness patients infected with *T. gambiense* give frequent positive reactions at 1/320, while healthy controls are usually negative at 1/20.

In half a day, a non specialized laboratory technician can perform the reaction on a hundred of blood samples. Appreciation of the fluorescence takes 10 to 30 seconds for one sample, doubtful reaction are scanty.

Introduction

Fluorescent antibody test has been used for many years for diagnostic purpose in trypanosomiasis. The first tests were made in 1959 by Fife and Muschel for the diagnosis of Chagas disease. Culture forms were chosen by these authors as antigens.

Anderson *et al.* (1961) were the first to publish hopeful results in the diagnosis of african trypanosomiasis by immunofluorescence, using thin blood films made from experimentally infected laboratory animals. Since then many workers have studied this system of diagnosis, using the direct method in which the suspected serum and the

fluorescein labelled serum are allowed to act successively on the antigen.

Many variations of this method were described, concerning chiefly the way of preparing the antigen : Sadun *et al.* (1963) found equivalent antigenic properties for *T. gambiense, T. rhodesiense, T. cruzi* and *T. lewisi.* Thin blood films of these trypanosomes were fixed with a 10 per cent formaline solution added to rhodamine bovine albumine. Weitz (1963) preferred to use methanol fixed smears of *T. brucei* and *T. vivax.* Smears of *T. gambiense* made from infected albino rats were used by Courtois and Bideau (1966). These smears were fixed with a 5 per cent formaline solution. In East Africa, Bailey *et al.,* (1967) used *T. rhodesiense* from infected albino rats. Heparinised blood was taken by cardiac puncture diluted in P. B. S. (phosphate buffered saline) and centrifugated. Smears rich in parasites were made from the supernatant, and fixed with gentle heat. Thivolet *et al.* (1965) used *T. gambiense* for the search of anti DNA antibodies. Mouse blood films were fixed with acetone.

Other variations proposed by different workers chiefly concern the brand and dilution of fluorescein labelled anti-human serum, the time of action of the serum and labelled antiserum, the temperature at which the reaction takes place (37 °C or room T°), contrast staining and the means of elimination of non specific fluorescence, the mounting technique (drying or covering with buffered glycerine and coverslip), pH of washing and mounting fluids (pH 7.2 - 8).

Every author agrees that antigens, sera, and labelled antisera should be kept at — 20 °C, that the reactions should take place in moist atmosphere, that the intermediate washing should be made in P. S. B., that the preparations should be examined under blue or UV light within 24 hours, and that the measurement of green fluorescence should be classified in 4 or 5 degrees of intensity.

The patients' sera, may be collected in two different ways : supernatant after clotting of blood taken by vein puncture or dried whole blood absorbed on filter paper from finger puncture (Anderson *et al.,* 1961; Sandun *et al.,* 1961; Bailey *et al.,* 1967). The former technique allows antibody titration and a very long conservation of the serum at — 20 °C. The latter allows very fast blood collection for mass examination but, the conservation of these dried blood sampled is shortened to a few weeks at — 20 °C, or to a fortnight at room temperature. A single dilution of these sera can be examined, therefore, this method is only useful for screening test. The purpose of this work is to describe the details of the method worked out with material collected in the Congo Republic, near Kinshasa, and successfully used since November 1968.

We will discuss successively :

1° The dilution fluid.
2° The patients' sera.
3° The fluorescein labelled sera.
4° The reaction procedure.
5° The examination of the preparation in UV light.
6° The antigen preparation.
7° The comparison of different antigens.
8° The specificity of the reaction
9° The description of the images observed using the UV microscope.

1. *The dilution fluid*

Only one dilution fluid was utilised for all the manipulations :

Na Cl	8.5 g
NA 2 HPO4 — 12 H2O	3.54 g
Distilled water	1000 ml

The pH (7.2 - 7.4) of this phosphate buffered saline (PBS) remains constant for at least eight days after its preparation.

2. *The patients' sera*

A. Sera obtained after clotting of whole blood were distributed in polythene vials containing 0.5 ml. This method of storage avoids repeated thawing of a sample. Dilution of the sera was made in P. B. S.

B. The dried blood samples were collected by a method described by Bailey *et al.* (1967), but slightly modified :

A blood drop was obtained by finger puncture and absorbed on Whatman n° 1 filter paper. Discs of 11 cm of diameter allowing the collection of 8 samples (1 - 2 cm of diameter) were used. The corresponding number of the patient was quoted on the filter paper.

Samples were preserved in polythene bags containing silicagel as dessicative. Each filter paper was isolated from each other by a piece of paper. For applying the test, discs of 5 mm diameter were punched out of each sample. Circles were made with nail varnish on the antigen slides. A small drop (0.03 ml) of P. B. S. was placed in each circle and on the top of each drop a dried blood sample was placed.

The dilution of the blood in the P. B. S., obtained by this way was found to be approximately equal to a 1/10 dilution of the serum (protein dosage).

The tests have been carried out with : sera from patients suffering from sleeping sickness at both stages of the disease, recently diagnosed, who have not yet been treated; sera obtained from Euro-

peans who had never travelled in tropical countries (those sera were collected in Belgium); sera from patients suffering from other parasitic diseases (amoebiasis; malaria; Filariasis *Loa-loa, Onchocerca volvulus* an *Dipetalonema perstans;* toxoplasmosis; schistosomiasis) as well as sera from syphilitic patients with a positive BW reaction; sera of patients suffering from lupus erythematosus, typhoïd fever and epidemic typhus.

3. *The labelled sera*

Three anti-human fuorescein-labelled sera were used :

1) Swine antihuman (Nordic Pharmaceuticals).
2) Rabbit antihuman (N. Ph.).
3) Rabbit antihuman (Inst. Pasteur, Paris).

The time of action for each of these labelled sera on the antigen was 30 minutes at room temperature, and different dilutions were tried. The labelled sera (1) and (2) were tested for dilutions of 1/5, 1/10, 1/15, 1/25, 1/50. It was the 1/15 dilution, recommended by the manufacturer which gave the best results. The labelled serum (3) was tested for dilution of 1/20, 1/25, 1/33, 1/50, 1/100. The resulting fluorescence given by any of these dilutions did not differ greatly. Therefore, we selected the 1/100 dilution as the most economical one.

Concentrated solutions of these labelled sera can be kept in good condition for several weeks at + 4 °C as well as — 15 °C.

4. *Description of the procedure*

A. Firstly, a drop of the diluted patient serum is placed in the circles made on the antigen slide. Those slides were left in a moist atmosphere for 30 minutes at room temperature. If dried blood samples were to be examined, the disc was placed on the top of a drop (0.03 ml) of P. B. S. placed in the circles and the contact time was prolonged to 1 hour in the same conditions described above in order to allow the elution of the dried serum in the drop of P. B. S.

B. Afterwards the slides were washed for 15 minutes in three consecutive Coplin jars containing P. B. S.

C. The second step of the reaction consisted of bringing into contact the right dilution of a labelled antihuman serum for 30 minutes.

The excess of washing liquid was previously removed with gauze, without the smear being dried completely.

D. Afterwards, the slides were again washed in the same way as in (B), and finally the slides were covered with buffered glycerine (glycerine 1 part, PBS 9 parts) and a coverslip. They were then ready to be examined.

5. *Examination of the preparation*

Examination should take place within 24 hours. If carried out later, the fluorescence may fade. The preparations were examined using a Reichert Zetopan microscope, equipped for U. V. light with a high pressure mercury lamp (HBO 200).

The primary filter was a BG 12/6 mm (UV + blue excitation light) and the secondary filter was composed of BG 9/1 mm + OG 1/1.5 mm.

A bright field condensator was used with fully opened diaphragm and a drop of immersion oil was placed on its frontal lens in order to assure a direct contact with the back of the slide to be examined. The optical combination used for reading was : obj. 25 x, eyepieces 10 x. The intensity of fluorescence in the dark room was appreciated in 5 levels from ± to + + + + as follows :

± : Trypanosomes hardly visible on the back ground.
\+ : Trypanosomes visible; but without any true fluorescence.
\+ + : Trypanosomes contrasting with the back ground and with pale fluorescence.
\+ + + : Trypanosomes showing brilliant fluorescence, on a dark back ground.
\+ + + + : Trypanosomes showing very brilliant fluorescence (Internal Structure does not show any more). Only preparations showing brilliant fluorescence were considered as positive (+ + + and + + + +).

6. *Preparation of the antigen*

The first antigen to be tested was prepared from a strain of *T. gambiense* recently isolated on a guinea pig from the blood of a sleeping sickness patient of the village of Bemba. This village is situated in the area in which our studies were made. The same area had been chosen by Binz *et al.* (1968) for their study of the estimation of IgM levels as screening test in sera of sleeping sickness patients.

This strain, kept in guinea pigs by monthly passages of infected blood injected intra peritoneally, gives a slowly raising parasitemia; the blood of the animals can be used for antigen preparation only 15-20 days, after inoculation :

As soon as parasitemia reaches 2 - 10 parasites per field (obj. 100 x, eyepieces 10 x) the anesthetised animal is bled by cardiac

puncture. The blood is collected by means of a 5 ml syringe containing 0.25 ml of the following mixture : Heparine 500 u. I./ml, 1 part; glucose 25 per cent, 1 part. Thin blood films are immediately prepared by putting a small drop of blood, using a Pasteur pipette, on slides cleaned with alcohol - ether.

The amount of blood is calculated to produce a thin film covering the whole surface of the slide, except an area of 1 cm for marking. The smears are then placed without any fixation in a dessicator at room temperature, for one hour. Afterwards, they are wrapped up in groups of five in thin paper (toilet paper rolls are suitable), the packets are dated, numbered, and enclosed in hermetically closed polyesther boxes containing silicagel : the boxes are kept in the freezer at — 15 °C.

Another recently isolated strain of *T. gambiense* from the same village was cultured in diphasic medium at 27 °C (Tobie, 1958). The liquid phase was collected from 3 tubes on the fifth day after inoculation, and centrifugated at 2,500 rpm for 15 minutes. The sediment is washed twice in P. B. S. The last sediment is suspended in 2 ml PBS. Drops of this suspension are placed on cleaned slides. When evaporation is sufficiently advanced, drying is completed in a dessicator, and the slides are wrapped up and placed in the freezer in the same way as for the blood smears.

Before use, packets of preparations are removed from the freezer and brought to room temperature in a dessicator. The slides are numbered and nail varnish circles are traced. Each circle will serve for one dilution of a serum or one sample of dried blood.

Smears made from blood taken from the tail of a rat or from the ear of a rabbit have in our experience two disadvantages :

1. The tickness, surface, cleaness and quality of the smears are variable because the blood coming out of the wound tends to coagulate after a while.

2. In our experience, these films lose their antigenic properties quicker than the smears made from heparinized blood in the same conditions of storage. (The titer, obtained with a well known serum, drops after 6 to 8 weeks, while smears of heparinized blood give a constant titer during 4 to 6 months).

The presence in the smear of red blood cells and platelets of the infected animal did not disturb the interpretation of the reactions. The red cells constitute a dark back ground, variable however with each serum. On this back ground dark green yellow, parasites sparkle when the reaction is positive (+ + + ou + + + +). If the reaction

is negative (± a +), trypanosomes may be difficult to find, even when more than 5 per field are present.

The same *T. gambiense* antigen collected from the infected blood of guinea pigs is very sensitive : sera of sleeping sickness patients who have not yet received treatment, give very bright fluorescence. The reactions are easy to read with the UV microscope, however, the negative control sera (from European subjects) at the 1/20 dilution give an image where the trypanosomes are still easily visible. Furthermore, the guinea pigs infected more than 20 days before the collection of the blood, contain an increased number of white cells which are stained by the procedure as well as the parasites and show a brilliant fluorescence with sleeping sickness patients' sera and weak fluorescence with healthy controls sera. This phenomenon may disturb the interpretation of the results.

Table 1 illustrates the comparison of the titers obtained with the same non fixed antigen collected from guinea pigs at different times of the infection. It is clear that the older the infection is, the more sensitive and the less specific will be the reaction. Negative European control sera may give doubtful result (+ +) with an antigen produced from a guinea pig infected 80 days before the collection of the blood.

N.B. It is possible to reduce the sensitivity of the reaction by treating the antigen smears with Evans blue at concentration of 1/15,000, for 3 to 5 minutes, after the contact with the labelled anti human serum. This procedure was abandonned, however, when we found more suitable antigens.

Fixation of the antigen smears 5 per cent formalin, aceton or methanol, removal of hemoglobine by HCl 0,3 N do not increase the specificity or the sensitivity of the antigens.

Because of the disadvantages shown earlier (slowly and irregularly increasing parasitemia in the guinea pig, production of antibodies by the infected animal, increased number of leucocytes in the blood) other strains of *T. gambiense* were isolated in guinea pigs, and adaptation of these strains to albino rats were attempted.

Furthermore, maintaining pathogenic strains of *T. gambiense* in rudimentary laboratories being dangerous for the worker, other species of trypanosomes were tested for their antigenicity. Williams *et al.* (1963) found that sera of animals experimentally infected with *T. gambiense* and *T. rhodesiense* would react with a *T. lewisi* antigen in the same way as with the homologous antigen. Sadun *et al.* (1963) showed cross reactivity between *T. gambiense, T. rhodesiense* and *T. cruzi.*

The comparisons of different strains and species will be treated in the following paragraph.

7. *Studied strains and species of parasites*

7.1 *Trypanosoma brucei* (EATRO 1125) is a polymorphic strain, kept in guinea pigs and albino rats. In the guinea pig, parasitemia developed around the 4th day after inoculation and became very high around the 10th day (more than 20 parasites per field in a smear examined at the 1000 x magnification), but the animal did not die and the number of parasites dropped in the blood. Relapses occurred regularly.

In the rat, parasites could already be found in the blood smears on the second day after inoculation, parasitemia increased regularly until the 6th or 7th day, when the animal died.

7.2 *Trypanosoma brucei* « HOARE » showed a behaviour comparable to that of *T. brucei* EATRO in guinea pigs. In albino rats, the infected animal died 7 or 8 days after inoculation. Mice only survived for five days.

This strain has been kept in laboratory animals for more than 10 years. Only slender forms can be found in smears, the strain is thus monomorphic.

7.3. Three strains of *T. gambiense* were isolated in guinea pigs and maintained in guinea pigs and albino rats. The strain « Bemba » was isolated in November 1968 from a child showing parasites in lymphnodes and blood, and maintained in guinea pigs only. Experiments were carried out on this strain between February and October 1969.

This being the first strain isolated in our laboratory, we used it as a reference antigen. Infected guinea pigs showed parasites in their blood 15 days after inoculation. Parasitemia increased to more than 5 parasites per field. It was adapted on albino rats by a series of rapid passages on young animals. On the fifth passage, parasitemia grew rich enough to produce a suitable antigen between the fifth and fifteenth day after inoculation. Parasitemia in albino rats grew, however, less regularly than with the *T. brucei* strains which were used in the same animals. The *T. gambiense* strain became extremely pathogenic in a very short time for albino rats, the animal dying on the third day after inoculation with an extremely high parasitemia. Passages of this strain on albino rats were abandonned.

7.4. The strain « Moerbeke 1 » was isolated in December 1968 from a 11 years — old child showing parasites in the thick blood film. The strain was passaged monthly in guinea pigs, the animals

became positive between the tenth and the fifteenth day with a parasitemia of more than five parasites per field (*). This strain was adapted on albino rats by repeated passages on young animals. The pathogenicity for albino rats did not increase and the strain could be maintained by weekly passages.

This strain was also cultured on Tobie culture medium, from the blood of a guinea pig, on the first subinoculation after the isolation. Experiments were carried out on this strain between February and October 1969.

7.5. Strain « Moerbeke 10 » was isolated in a guinea pig in May 1969 from an African woman at the nervous stage of the disease. (thick B. F. +, S. F 52 el/mm^3, alb. 50 mg per cent). The characteristics of the strain were comparable to others strains of *T. gambiense* studied. Adaptation to rats was only possible using splenectomised animals. In these splenectomised animals, parasites appeared in the blood on the fourth or fifth day after inoculation and parasitemia reached very high levels after eight days, the animal dying around the 20th day.

7.6. A strain of *T. congolense* (EATRO 1157) was maintained on albino rats.

Parasites appeared in the blood around the 6th day and the animal died 15 to 20 days after inoculation, showing a very high parasitemia.

All these antigens were tested without any previous fixation with sera from sleeping sickness patients and Europeans (see par. 2).

The results presented in tables 1 to 6 lead to the following conclusions :

1) The titers obtained are remarkably constant with any brucei group antigen with preserved polymorphism.

2) The number of parasites in the antigen smears has no effect on the brightness of the fluorescence. It is easier, however, to examine the reactions when the smears contain between 5 and 20 parasites per field.

3) The *T. brucei* « HOARE » strain, maintained in rats for many years by passages twice a week, and has become monomorphic, offers a much less sensitive antigen. The fluorescence observed

(*) The appreciations of the parasitemia were done on thin blood films examined with a Wild M 12 microscope equipped with an 100 × oil immersion obj. and 10 × eyepieces.

TABLE 1

T. gambiense **strain « Bemba »**

Sera	15 sleeping sickness patients									15 Europeans					
Titer	1/20			1/80			1/320			1/20			1/80		
Evaluation of fluorescence	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++
Guinea pig antigen															
14th day	0	0	15	0	3	12	3	7	5	13	2	0	14	1	0
45th day	0	0	15	0	2	13	2	6	7	11	4	0	14	1	0
80th day	0	0	15	0	2	13	3	5	7	9	6	0	12	3	0
Rat antigen															
5th day	0	0	15	0	3	12	6	6	3	15	0	0	15	0	0

TABLE 2

T. gambiense **strain Moerbeke 1**

Sera	15 sleeping sickness patients									15 Europeans					
Titer	1/20			1/80			1/320			1/20			1/80		
Evaluation of fluorescence	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++
Guinea pig antigen															
18th day	0	0	15	0	5	10	6	8	1	13	2	0	15	0	0
43th day	0	0	15	0	3	12	4	9	2	10	5	0	14	1	0
Rat antigen															
5th day (5 p.p.f.)	0	0	15	0	3	12	5	7	1	15	0	0	15	0	0
5th day (30 p.p.f.)	0	0	15	0	3	12	7	4	2	15	0	0	15	0	0
17th day (10 p.p.f.)	0	0	15	0	2	13	5	8	1	15	0	0	15	0	0

TABLE 3

T. gambiense **strain Moerbeke 10**

Sera	15 sleeping sickness patients									15 Europeans					
Titer	1/20			1/80			1/320			1/20			1/80		
Evaluation of fluorescence	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++
Guinea pig antigen															
20th day	0	0	15	1	5	9	5	5	5	12	3	0	15	0	0
8th day	0	0	15	0	5	10	3	7	5	5	10	0	12	3	0
Splenectomised rats															
5th day	0	0	15	2	3	10	10	2	3	15	0	0	15	0	0
15th day	0	0	15	2	2	11	8	3	4	15	0	0	15	0	0
Culture antigen	0	0	15	0	6	9	3	10	2	10	5	0	12	3	0

TABLE 4

T. brucei **Eatro No. 1125**

Sera	15 sleeping sickness patients									15 Europeans					
Titer	1/20			1/80			1/320			1/20			1/80		
Evaluation of fluorescence	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++
Guinea pig antigen															
7th day	0	0	15	0	2	13	6	4	5	15	0	0	15	0	0
Rat antigen															
4th day	0	0	15	0	1	14	6	3	6	15	0	0	15	0	0
5th day	0	0	15	0	1	14	6	3	6	15	0	0	15	0	0
6th day	0	0	15	0	1	14	6	2	7	15	0	0	15	0	0

TABLE 5

T. brucei **strain Hoare**

Sera	15 sleeping sickness patients									15 Europeans		
Titer	1/20			1/80			1/320			1/20		
Evaluation of fluorescence	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++
Rat antigen												
4th day (5-10 p.p.f)	0	10	5	3	11	1	15	0	0	15	0	0
5th day (50 p.p.f.)	0	15	0	5	10	0	15	0	0	15	0	0
Mouse antigen												
2nd day (5 p.p.f.)	0	15	0	5	10	0	15	0	0	15	0	0
3rd day (50 p.p.f.)	0	15	0	8	7	0	15	0	0	15	0	0

TABLE 6

T. congolense

Sera	15 sleeping sickness patients									15 Europeans					
Titer	1/20			1/80			1/320			1/20			1/80		
Evaluation of fluorescence	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++	± +	++	+++ ++++
Rat antigen															
9th day	1	9	5	7	8	0	15	0	0	15	0	0	15	0	0
12th day	3	6	7	5	10	0	15	0	0	15	0	0	15	0	0

with this old strain is one or two levels lower than with freshly isolated strains or strains preserved in liquid nitrogen.

4) Antigens prepared from rat blood have more regular characteristics and give unquestionable negative controls.

Antigens prepared from the blood of guinea pigs give still brighter fluorescence with positive sera. An excessively high degree of fluorescence however, is visible with these antigens after contact with negative sera.

This aspecific fluorescence is higher with antigens made from blood taken from animals which have been inoculated for some time. Moreover, in these antigens there is an aspecific fluorescence of the increased number of leucocytes.

5) *T. brucei* EATRO and *T. gambiense* antigens prepared from rats blood give similar results.

6) Antigens of *T. gambiense* prepared from culture are very sensitive, but less specific : negative sera often give doubtful reaction.

7) *T. congolense* antigen share some characters with antigens of *T. brucei* group. Numerous doubtful or even negative results however, are obtained after contact with sera from sleeping sickness patients.

8. *Specificity of the reaction*

In order to check the diagnostic value of the F.A.T., we tested the specificity of two antigens : *T. gambiense* Moerbeke 1 and *T. brucei* EATRO.

The following sera were chosen :

25 sera positive for toxoplasmosis (FAT).
16 sera positive for malaria (FAT).
10 sera from patients infected with *Loa loa*.
20 sera from patients infected with *Onchocerca volvulus*.
25 sera from patients with amoebic liver abscess. (FAT positive with amoebian antigen.)
10 sera from patients suffering from Schistosomiasis.
20 sera with positive B. W. reaction.
9 sera from *Lupus erythematosus* patients. (Six of them diagnosed by the research of Hargrave cells (*) and three by the FAT on the nucleus of leucocytes.)
50 sera from African patients chosen at random in different wards of the University Hospital of Lovanium. All these patients were treated for non parasitic diseases. As expected, however, the majority of these patients harboured in their faeces eggs of Ascaris, Trichuris, Taenia, Oxyuris, Ankylostoma, etc.

(*) Professor G. Van Ros from the Institute of Tropical Medicine in Antwerp kindly provided these sera.

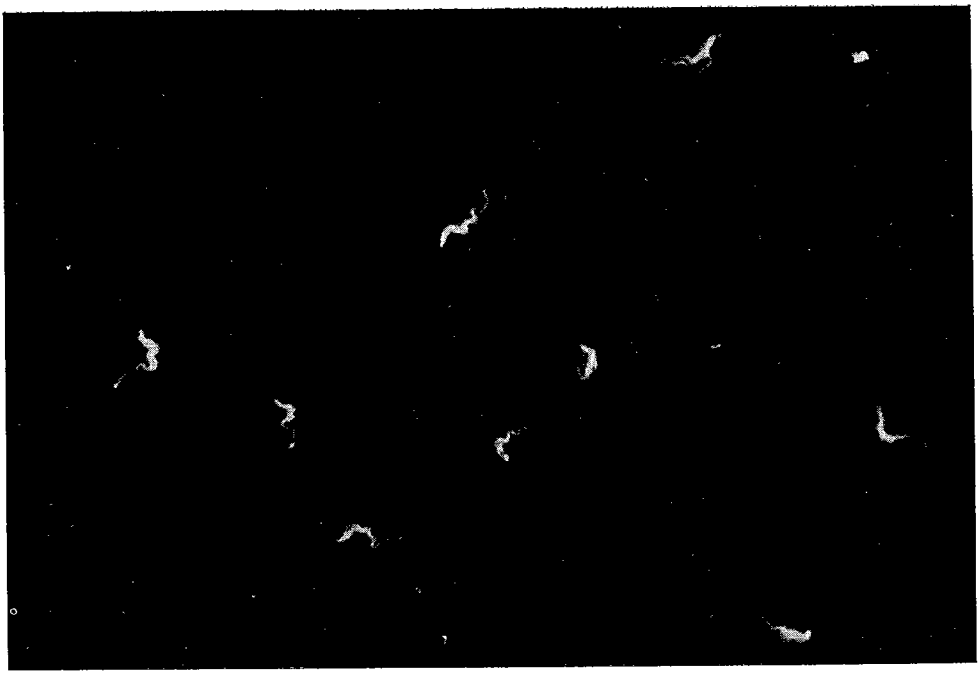

Plate 1

T. brucei Eatro 1125. Rat blood smear. Positive reaction (+++) given by a serum from a sleeping sickness patient. (Obj. 25 × ocul. 10 ×).

Plate 2

T. gambiense Moerbeke 1. Rat blood smear. Positive reaction (+++) given by serum from a sleeping sickness patient. (Obj. 65 × ocul. 10 ×).

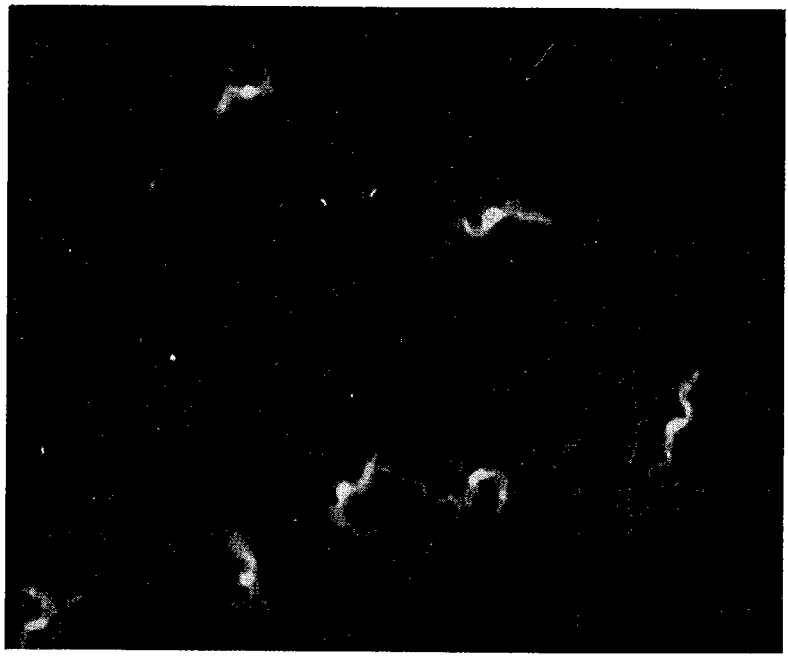

Plate 3

T. gambiense. Rat blood smear after contact with a serum from a sleeping sickness patient, giving a fluorescence of the parasites nuclei.

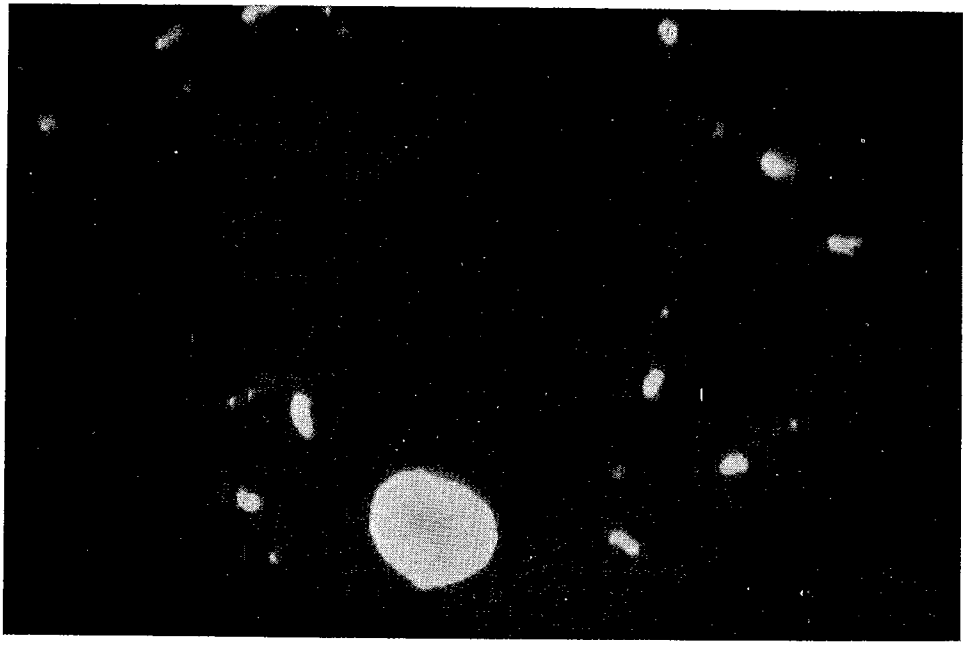

Plate 4

T. gambiense Moerbeke 1. Rat blood smear after contact with a serum from a Lupus erythematosus patient. Elective fluorescence of the parasite and white cells nuclei.

Only three serums from lupus erythematosus patients gave any positive fluorescence reaction (+++ or ++++) when diluted at 1/20.

Five per cent of those sera gave a dubious reaction, but the 1/40 dilution was used and the reaction became negative. Three of the 9 *Lupus erythematosus* sera gave a bright fluorescence (+++ or ++++) of the trypanosome nuclei, even when diluted to 1/300. But this fluorescence was accompanied by a comparable fluorescence of the leucocytes nuclei of the rat; this has never occurred with sera taken from sleeping sickness patients.

9. *Description of the pictures observed using the fluorescence microscope*

As smears of parasitized blood were used as antigen, the appearance of the red blood cells, white blood cells, platelets and trypanosomes will be described successively.

a) Red blood cells constitute the background. They can be more or less visible, but never caused any difficulties for the appreciation of the fluorescence of the parasites.

The degree of their fluorescence depends on :

1) The animal from which the blood was taken. The erythrocytes of the albino rats are usually less visible than those of the guinea pig after contact with the same serum.

2) The serum to be tested. About 10 per cent of the sera (from sleeping sickness patients or others) give a slight fluorescence of the erythrocytes of rats and guinea pigs.

3) The purity of the labelled serum. The dilutions we employed gave excellent results. When more concentrated solutions are used, aspecific fluorescence of the red cells increases. We found no difference between conjugates prepared in pigs or in rabbits.

b) Platelets are usually invisible.

c) Leucocytes may show a true fluorescence (++) especially when :

1) the infection of the guinea pig is older than one month.

2) in blood of guinea pig and rat after contact with serum from lupus erythematosus patients.

d) Trypanosomes are the only elements showing bright specific fluorescence, only appearing after contact with sera from sleeping sickness patients. Only polymorphic strains of the brucei group, recently isolated or kept in the deep freezer give a brilliant fluores-

cence (plate 1), the whole parasite is fluorescent, but a higher dilution of the positive serum gives more detailed pictures, where only mitochondrial (?) sites shine (Plate 2).

We observed amongst sleeping sickness patients sera, two of which caused a true fluorescence of only nucleus and kinetoplast. The rest of the body of the trypanosome remained dark. (plate 3). We considered this picture to be similar to the one described by Thivolet *et al.* (1965). We obtained the same brilliant nucleus with sera of lupus erythematosus patients (plate 4).

Conclusions

The use of Indirect FAT for the diagnosis of African sleeping sickness *(T. gambiense)* may prove very useful as a screening test, more especially as diagnosis is made possible when clinical symptoms are absent.

The technique described in this work is easy to apply in small laboratories. Only a fluorescence microscope and the possibility of maintaining a strain of *T. brucei* on albino rats are necessary.

The examination of dried blood samples is very rapid. A non graduated technician can perform the reaction on at least 100 samples in half a day. The interpretation on one sample takes about 20 seconds.

The method can thus be used as a rapid and sure screening test of the whole population of a sleeping sickness region, and could replace the usual method in which thick blood films and lymph are examined only in individuals with engorged lymphnodes.

This method has proved to be least as fast as the IgM dosage, and the preliminary results show that the FAT is more specific than the IgM dosage. These results will be published shortly.

Résumé — **Le diagnostic de la Trypanosomiase humaine à *T. gambiense* par immunofluorescence.**

1. Standardisation d'une méthode facile applicable à des enquêtes de masse.

L'immunofluorescence indirecte permet un diagnostic rapide de la trypanosomiase africaine à *T. gambiense* sur grande échelle, grâce à la technique de prélèvement des échantillons de sang sur papier filtre.

L'antigène est fabriqué à partir d'une souche de *T. gambiense* ou mieux de *T. brucei* ayant gardé leur polymorphisme, entretenues sur rats albinos. Le sang parasité, prélevé au cœur sur héparine glucosée est étalé sur des lames porte-objet. Les frottis d'antigène doivent contenir entre 5 et 50 parasites par champ microscopique à l'objectif 25 ×, oculaire 10 ×; ils peuvent être conservés à — 15 °C en sachets de plastique contenant du silicagel et conservent leurs propriétés antigéniques pendant 3 à 6 mois. Ils sont utilisés sans fixation aucune. Chaque frottis permet l'examen de 6 échantillons de sang séché ou de dilutions de sérums.

Le conjugué fluorescent antiglobulines humaines de l'Institut Pasteur (Paris) nous a donné des résultats fidèlement reproductibles à la dilution de 1/100, de même que le conjugué fluorescent de Nordic Pharmaceuticals à la dilution de 1/15.

Les antigènes brucei de rats présentent des avantages appréciables : souche moins dangereuse à manipuler, régularité de l'augmentation de la parasitémie et prévision aisée du jour où l'antigène pourra être préparé, bonne sensibilité du test, les nouveaux cas de maladie du sommeil à *T. gambiense* peuvent être positifs jusqu'à 1/320, tandis que les témoins indemnes de la maladie sont parfaitement négatifs au 1/20.

Un technicien de laboratoire non spécialisé peut effectuer la réaction sur une centaine d'échantillons de sang séché en une demi-journée.. La lecture au microscope à fluorescence demande 10 à 30 secondes par échantillon, les réactions douteuses sont rares.

Samenvatting — **De diagnose van menselijk trypanosomiasis veroorzaakt door *T. gambiense*, bij middel van de immunofluorescentietest.**

1. Standardisatie van een eenvoudige techniek toepasselijk voor massa onderzoek.

Met behulp van de Immunofluorescentietest en het verzamelen van bloedstalen op filterpapier, is het mogelijk, op grote schaal, een snelle diagnose te stellen van Afrikaanse trypanosomiasis, veroorzaakt door *T. gambiense*.

Als antigeen wordt een op witte ratten onderhouden *T. gambiense*, of beter, een *T. brucei* stam gebruikt, welke haar polymorphische eigenschappen behielden. Dit antigeen wordt bereid door met trypanosomen bevattend rattenbloed uitstrijkjes te maken op zuivere draagglaasjes. Dit bloed wordt bekomen door hartpunctie en het wordt vermengd met een geglucoseerde heparine oplossing. Deze antigeen uitstrijkjes dienen tussen 5 tot 50 trypanosomen per microscopisch veld te vertonen (objectief 25 ×, oogstuk 10 ×). Ze kunnen bewaard worden gedurende 3 tot 6 maanden in plastieken zakjes welke silicagel bevatten, bij een temperatuur van — 15 °C.

De antigeen uitstrijkjes worden gebruikt voor de test zonder enige fixatie. Elk preparaat laat het onderzoek toe van 6 gedroogde bloedstalen of 6 serumverdunningen. Het fluorescerend conjugaat anti-menselijk globulinen van het Pasteur Instituut (Parijs) bij een verdunning van 1/100, of dat van Nordic Pharmaceuticals bij een verdunning van 1/15, gaven ons resultaten die bij het herhalen van de test identisch bleven.

Dit brucei antigeen heeft volgende belangrijke voordelen; de stam is weinig gevaarlijk voor het laboratoriumpersoneel, het vertoont een zeer regelmatige parasitaemie curve bij de rat en bijgevolg kan de dag waarop het antigeen dient bereid te worden gemakkelijk voorzien worden; het is zeer gevoelig aan de test : serum van nieuwe gevallen van slaapziekte veroorzaakt door *T. gambiense* kunnen positief zijn tot een verdunning van 1/320, terwijl het bloed van gezonde getuigen meestal negatief is bij een verdunning van 1/20.

Een niet-gespecialiseerd laboratoriumtechnikus kan de nodige reactie's voor het onderzoek van 100 gedroogde bloedstalen uitvoeren op een halve dag. De interpretatie met behulp van een fluorescentie microscoop vraagt 15 tot 30 seconden per staal. Twijfelachtige resultaten komen zelden voor.

M. Wéry and P. Van wettere : Institut de Médecine tropicale Prince Léopold, Laboratoire Lovanium, B. P. 747, Kinshasa XI, Rép. dém. du Congo.

S. Wéry-Paskoff : Service de Bactériologie, Université Lovanium, Kinshasa XI, Rép. dém. du Congo.

Received for publication on May 28, 1970.

BIBLIOGRAPHY

Anderson, R. I., Sadun, E. H. and Williams, J. S., A technique for the use of minute amounts of dried blood in the fluorescent antibody test for Schistosomiasis Exp. Parasit., 1961, **11**, 111-116.

Bailey, N. M., Cunningham, M. P. and Kimber, C. D., The indirect fluorescent antibody technique applied to dried blood for use as a screening test in the diagnosis of human trypanosomiasis in Africa. Trans. Roy. Soc. Trop. Med. Hyg., 1967, **61**, 696-700.

Binz, G., Timperman, G. and Hutchinson, M. P., Estimation of serum Immunoglobin M as a screening technique for trypanosomiasis. A field trial in the Democratic Republic of the Congo. Bull. Wlld Hlth Org., 1968, **38**, 523-545.

Courtois, D. et Bideau, J., L'immunofluorescence appliquée au diagnostic de la trypanosomiase humaine africaine. Valeur comparative avec le titrage de l'immunoglobine IgM. Bull. Soc. Path. Exot., 1966, **59**, 809-817.

Dutertre, J. et Boisson, P., La sérologie de la Trypanosomiase sur sang desséché. Essai de la méthode des confetti de Cunningham. Médecine Tropicale, 1968, **28**, 5, 649.

Fife, E. H. J. and Muschel, L. H., Fluorescent antibody technique for serodiagnosis of *Trypanosoma cruzi* infections Ex. Biol. Med., 1959, **101**, 540-543.

Mattern, P., Utilisation de la réaction d'immunofluorescence pour le diagnostic de la trypanosomiase. Rapport final de la 5e Conférence technique de l'Occgé, 1961, **1**, 309.

Mattern, P., Technique et intérêt épidémiologique du diagnostic de la trypanosomiase humaine africaine par la recherche de la B_2 macroglobuline dans le sang et dans le L. C. R. Ann. Inst. Pasteur, 1964, **102**, 415-421.

Sadun, D. H., Duxbury, R. E., Williams, J. S. and Anderson, R. I., Fluorescent antibody test for the serodiagnosis of African and American Trypanosomiasis in man. J. Parasitol., 1963, **49**, 385-388.

Thivolet, J., Monier, J. C., Lalain, F. et Richard, M. M., Recherche quantitative simultanée des anticorps antinucléaires totaux et anti-acide désoxyribonucléique sur frottis de sang de souris parasitées par *Trypanosoma gambiense*. Ann. Inst. Pasteur, 1965, **109**, 817-830.

Weitz, B., The specificity of trypanosomal antigens by immunofluorescence. J. Gen. Microbiol., 1963, **32**, 145.

Williams, J. B., Duxbury, R. E., Anderson, R. E. and Sadun, E. H., Fluorescent antibody reactions in *Trypanosoma rhodesiense* and *T. gambiense* in experimental animals. J. Parasit., 1963, **49**, 360.