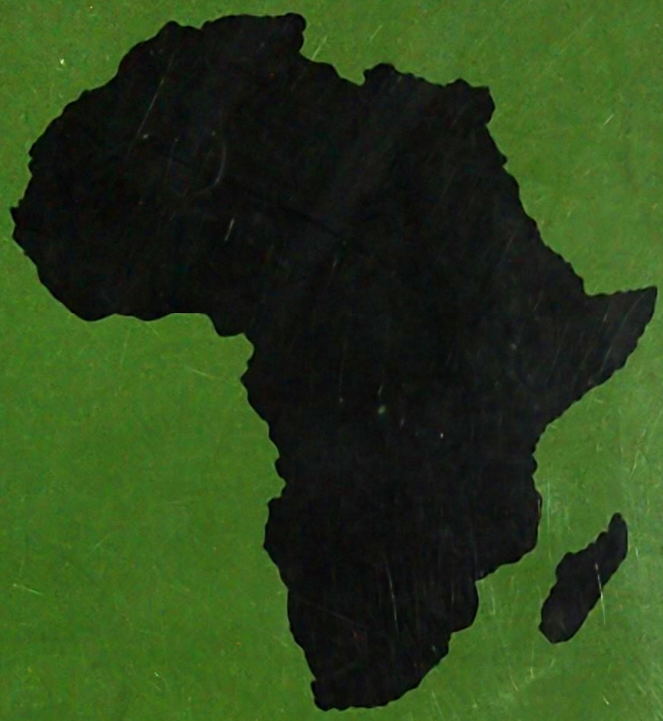
AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 23, NUMBER 1, MARCH 1994



EDITOR: B.O. ONADEKO
ASSISTANT EDITORS:
B.O. OSOTIMEHIN and A.O. UWAIFO

SPECTRUM BOOKS LIMITED
Ibadan • Owerri • Kaduna • Lagos

ISSN 1116–4077

Afr. J. Med. med. Sci. (1994) 23, 39-46

An evaluation of the reactivity of the card agglutination test for trypanosomiasis (CATT) reagent in the fontem sleeping sickness focus, Cameroon

T. ASONGANYI*, B.A. BEDIFEH*, S.S. ADE** and J.L. NGU*

*CUSS, University of Yaounde, Yaounde, Cameroon and **Autonomous Trypanosomiasis Sector, Fontem, Cameroon.

Summary

The Testryp[R] Card Agglutination Test for Trypanosomiasis (CATT) used for the serodiagnosis of gambiense trypanosomiasis is based on the variant antigen type (VAT) LiTat 1.3. This antigen is rarely expressed by trypanosomes in the Fontem focus of Cameroon, but the CATT has been used for serodiagnosis in the focus since 1985. We give here a summary of results obtained with the CATT in Fontem from 1985 to 1989. The CATT is specific for trypanosome antibodies since: (a) sera from persons with other parasitoses from areas non endemic for trypanosomiasis fail to react and (b) an enzyme-linked immunosorbent assay (ELISA) based on the detection of antibodies to somatic antigens of *T.b gambiense* from Fontem concorded with the CATT. CATT reactions in Fontem seem to be specific for the variant surface glycoprotein (VSG) since absorption of CATT reactive sera with formalin fixed bloodstream *T. gambiense* from Fontem and with culture produced procyclics of *T. gambiense* from Fontem failed to abrogate CATT reactivity. CATT on serum failed to confirm 37% of CATT positive cases on whole blood. Although immunoconglutinin (IK), anti-human red blood cell (RBC) antibodies and complement fixing immune complexes (ICs) were found in sera from Fontem, our results failed to incriminate immuno-conglutination of RBCs, reactions of RBCs with their autoantibodies and immune adherence hemagglutination as contributory factors in this lack of agreement between CATT on serum and whole blood. Further, comparison of whole blood and serum CATT results of parasitologically confirmed patients leads to the conclusion that screening with the CATT in the Fontem focus should be done on whole blood, not serum or plasma. CATT reactions in Fontem are based on cross-reactions with as yet undefined VATs.

Resume

Le principe du test d'gglutination sur cate (Testryp CATT) pour le serodiagnostic de la Trypanosomiase Humaine Africaine à *T. gambiense* est basé sur l'agglutination de la variante antigenique (VAT) de type LiTat 1.3. Cependant les trypanosomes du foyer de Fontem expriment rarement cet antigène; neamoin, le test est utilisé pour le diagnostic dans ce foyer depuis 1985. Sont présentés ici les resultats obtenus avec ce test à Fontem de 1985 à 1989. Le Testryp CATT est specifique pour les anticorps antitrypanosomes pour deux raisons essentielles: d'abord parceque les sérums des personnes affectés par d'autres parasitoses et habitant les régions idemnes de la trypanosomiase n'agglutinent pas, ensuite le test immuno-enzymatique (ELISA) utilisant les antigènes somatiques de *T. gambiense* isolés à Fontem donne des résultats concordant avec le CATT. Les réactions d'agglutination sur carte à Fontem sont specifiques pour les glycoproteines variables de surface (VSG) parceque l'absorption des sérums agglutinants avec: (a) les formes trypomastigotes sanguines fixées avec du formol et (b) les formes procycliques de *T. gambiense* produit en culture, n'ont pas supprimés le pouvoir agglutinant des sérums. Par contre le CATT pratiqué sur les sérums au laboratoire n'a confirmé que 63% des resultats positifs obtenus sur le sang total. Cette discordance entre les résultats du CATT sur le sang total et sur le sérum n'a pas été liée è la réaction conglutinante des globules rouges, à la réaction des globules rouges avec leur autoanticorps ou encore à

* Correspondence: Dr. T. Asonganyi, CUSS, University of Yaounde, Yaounde, Cameroon.

l'hemagglutination par immuno — adherence. Par ailleurs les résultats des CATT sur sérum et sur le sang total des trypanosomés confirmés indiquent que le screening avec le CATT au foyer de Fontem devrait être réalisé sur le sang total et non sur le sérum. Enfin les réactions de CATT à Fontem seraient dûes aux réactions croisées avec les VATs dont l'identité reste à définir.

Introduction

The value of the card agglutination test for trypanosomiasis (CATT) developed by Magnus *et al.* [1] and marketed by Smith Kline-RIT (Rixensart, Belgium) for the serodiagnosis of Gambian sleeping sickness has been demonstrated by several authors[2,3,4,5,6,7]. The test is currently employed in eleven countries, including Cameroon for the mass surveillance of *Trypanosoma brucei gambiense* [8]; the test having been introduced in Cameroon in 1985.

The CATT is presently based on the variant antigen type (VAT), LiTat 1.3; the rationale of the test being that one of the early peaks of parasitemia in a *T. b. gambiense* infected person is made up predominantly of iso-VATs of LiTat 1.3 [9,10]. However, the test has been reported to have a relatively poor performance in the Fontem focus[11] and other nearby foci[12]. Recent reports seem to indicate that this poor performance is due to the rarity or complete absence of the LiTat 1.3 iso-VATs in the Fontem focus[13].

The CATT is performed in the field of Cameroon mainly on whole blood, although it can be performed on serum or plasma. Agreement between field results and laboratory results on plasma or serum is usually poor[12,14]. This lack of agreement has been thought to be due to autoagglutination of red blood cells[12,14].

Trypanosomiasis patients usually have a non-specific B cell activation[15], high levels of immunoconglutinin (IK) [16] and circulating immune complexes (ICs) [17]. Such ICs can bind to human red blood cells (RBCs) in a complement dependent[18] and independent[19] reaction. It is possible that IK can immunoconglutinate RBCs to which complement fixing ICs are bound, leading to false positive field CATT reactions[17]. It is also possible that the CATT reactions in Fontem are cross- reactions with antibodies to other coendemic parasites.

Further, since the CATT reagent is composed of formalin fixed, freeze-dried trypanosomes, it is possible that the reconstituted antigen used in the field has "common" trypanosome antigens exposed during formalin fixation and (or) during freeze-drying and reconstitution. Antibodies to such "common" antigens would agglutinate the trypanosomes during a CATT test although they would not be VAT specific.

The present studies were carried out to gain information regarding the origin of CATT positive reactions in the Fontem focus where LiTat 1.3 iso-VATs are rare or absent and to evaluate the general performance of the CATT in the Fontem focus from its introduction in May, 1985 to the present.

Materials and methods

Study area: The Fontem trypanosomiasis zone has been described in detail by several authors[20,21,22]. Prior to 1985 when the CATT was introduced in the Fontem focus, serological tests for trypanosomiasis were based on either the indirect immunofluorescence test (IFT) or Cellognost^R trypanosomiasis test (Indirect hemagglutination: Behringwerke, A.G. Marlburg, W. Germany).

Screening with Testryp^R CATT: Sleeping sickness survey in the Fontem focus is based on a mobile team which works during part of the year and is able to screen up to 700 people a day.

In each community surveyed, individuals of all ages, sex and occupation are assembled at the chief's palace or a local primary school. In the field, the CATT is performed on whole blood essentially as recommended by the manufacturers (Smith Kline-RIT, Rexensart, Belgium). The results of the test are interpreted according to agglutination patterns as follows: +++ (very strong reactions: positive), ++ (strong reactions: positive), + (weak reactions: positive), ± (doubtful reactions: positive if confirmed by a second CATT, negative if not confirmed), — (negative reactions). Following such screening, persons with positive reactions are screened for trypanosomes using stained thick films, gland puncture or the lumber puncture, although the miniature anion-exchange/centrifugation test (mAECT)[23] was used during one of the tours in 1988.

Laboratory Studies

One hundred and nine serum samples (92 from whole blood CATT positive (W^+) and 17 from whole blood CATT negative (W^-, persons) collected during some of the tours and 37 other sera collected from persons from areas non-endemic for sleeping sickness, comprising 12 from malaria, 10 from onchocerciasis patients and 15 from Caucasians were analysed in the laboratory for antibodies against trypanosomes using qualitative CATT and ELISA. The sera were also analyzed for immunoconglutinins, for anti-human RBC antibodies and for complement fixing IC_s. For tests in which all the sera were not analyzed, samples with various CATT reactivities were randomly selected.

CATT on Serum: 5 µl of serum was used for the qualitative CATT essentially as described by the manufacturers. Following these tests, sera that reacted very strongly (+++) were titrated against CATT antigen following absorption with:

(a) Culture produced procyclic trypanosomes from two *T.b. gambiense* stocks (MHOM/CM/88/M001 and B018) isolated from patients in the Fontem area.
(b) Blood stream trypanosomes from two *T.b. gambiense* stocks (MHOM/CM/88/A010 and M001) purified from rat blood and fixed with cold formalin as described for the CATT antigen[1].
(c) Bloodstream trypanosomes from a *T.b. brucei* clone MiTat 1.2 fixed with a mixture of cold acetone and formalin in saline as described by Katende *et al.* [24].

10^8 fixed bloodstream trypanosomes were added to microfuge tubes and washed once with PBS. The pelleted trypanosomes were resuspended in 100µl of serum and incubated at 37°C for 1h, followed by 4°C overnight. The procyclics of *T.b. gambiense* produced at the Tsetse Research Laboratory, University of Bristol, UK[25] and freeze-dried in aliquots of 10^8 were resuspended at Centre Universitaire des Sciences de la Santé (CUSS) in 100µl of test serum and incubated as above. Serum without absorbent incubated at 37°C for 1 hour was used as a control. Following incubation the tubes were centrifuged at 15.000g for 15 min (Beckman J - 21B) and the sera diluted (1/2-1/128) and CATT tested.

ELISA for anti-trypanosome antibodies: This was performed using somatic antigens from a *T.b. gambiense* stock (MHOM/CM/88/A010) from Fontem. 10^9 bloodstream trypanosomes purified from rat blood by percoll gradient centrifugation[26] and filtration through a DEAE — cellulose column were suspended in PBS and subjected to 6 cycles of quick freezing and thawing (liquid nitrogen/37°C), centrifuged at 20.000g for 30 min at 4°C (Beckman J-21B) and the supernatant (somatic antigens) dialyzed against two changes of PBS — 1mMEDTA at 4°C. The protein content of the extract was estimated using the method of Bradford[27] with BSA as standard. The extract was used at 3µl/ml in ELISA experiments as described by Asonganyi *et al.* [28].

ELISA for anti-human RBC antibodies: This was done using RBC membranes from human group O^+ RBC [29]. The membranes were used in ELISA experiments at a protein concentrations of 10µl/ml.

Immunoconglution (IK) assays: a hemagglutination assay for IK was performed as described by Hautenen *et al.* [30], except that for serial dilution of the sera, PBS was used instead of PBS containing 0.2% BSA. The result was read by sedimentation patterns as described by Lachmann[31].

Immune adherence hemagglutination assay (IAHA): The test was performed as described by Inada *et al.* [32], except that fresh human serum diluted 1/10 instead of guinea pig serum was used as a source of complement. Human group O^+ RBC_s were pooled from 10 donors to ensure the presence of an averagely high concentration of C_3b receptors. Hemagglutination was appreciated visually.

Statistical analysis: CATT prevalences according to age group, sex and year of screening as well as concordance between the CATT and ELISA results were compared using the X^2 (chi square) test. In each case, differences at the $p < 0.05$ level were taken as significant. Levels of anti-erythrocyte antibodies in groups with different profiles of field and laboratory CATT results were compared using Tukey's test (33), based on medians and their confidence intervals.

Results

Performance of the CATT from May 1985 to May 1989

Of 44,839 persons screened by CATT from 1985 to May, 1989, 709 were positive, giving a CATT positivity rate of 1.58% (Table 1). The CATT prevelance was significantly higher in females (1.80%) than in males (1.33%) (X^2 = 15.91 $p < 0.001$) (Table 2). It increased steadily with age, rising from 0.98% in the < 5 year old group to 2.62% in the > 44 year group (Table 2). The difference in the CATT prevalence in the different age groups is significant (X^2 = 81.53, df = 3, $p < 0.001$). The association between the CATT positive prevalence and age was significant in both males (X^2 = 34.42, df = 3, $p < 0.001$) and females (X^2 = 46.24, df = 3, $p < 0.001$). The prevalence of CATT positives varied greatly from year to year, ranging from 5.24% in 1985 to 0.64% in 1989. These yearly variations are statistically significant (X^2 = 258.18, df = 4, $p < 0.001$).

From 1985 to 1987, all CATT$^+$ persons were treated for trypanosomiasis. As from 1988, only parasitologically confirmed patients were treated. Of the 17,006 people screened in 1988, 116 (1.65%) were CATT positive and 38 (0.54%) were trypanosome positive. Also, in 1989 (up to May) 77 (0.64%) CATT positives and 17 (0.14%) trypanosome positives were got out of 12,040 persons screened.

Table 1: Distribution of prevalence of CATT positivity by year of screening in the Fontem focus

Year	Nb examined	Nb CATT+	% CATT+
1985	1775	93	5.24
1986	19363	298	1.54
1987	4655	125	2.68
1988	7006	116	1.65
1989 (to May)	12040	77	0.64
Total	44839	709	1.58

Table 2: Prevalence of CATT positivity by age group and sex

Age groups (Years)		Male	Female	Total
0 - 4	NE[a]	4645	4430	9075
	NP	43	46	89
	%	0.93	1.04	0.98
5 - 14	NE	11136	10912	22048
	NP	132	153	285
	%	1.19	1.40	1.29
15 - 44	NE	3545	6372	9917
	NP	59	154	213
	%	1.66	2.42	2.15
> 44	NE	1716	2102	3818
	NP	46	54	100
	%	2.68	2.57	2.62
Total	NE	21042	23816	44858
	NP	280	429	709
	%	1.33	1.80	1.58

a) NE = number examined; NP = number positive; % = per cent positive,

Specificity of CATT

Of 32 sera: 12 malaria, 10 onchocerciasis and 10 European tested in CATT, none gave a positive result, confirming that the CATT is specific for anti-trypanosome antibodies. A total of 141 sera were screened by ELISA. The OD $_{490}$ absorbances obtained ranged from 0.01 to > 2.0. Positive values were taken as those above X + 2 SD (= 0.397) for pooled readings obtained for the malaria, filariasis and European sera, (Results not shown). The CATT and ELISA results are compared in a 2 x 2 Table (Table 3). Fifty-eight of 91 (63.7%) sera from the field which were field CATT positive (W^+) were positive in the ELISA; while 45 of 58 (77.5%) of the laboratory CATT$^+$ (S^+) sera were positive in the ELISA (Table 3). The concordance between the whole blood CATT results and ELISA results was highly significant (df: 1, X^2 = 19.48, $p < 0.001$). Similarly, the concordance between the CATT results on sera and ELISA results was highly significant (df = 1, X^2 = 23.52, $p < 0.001$), (Table 3).

Table 3: Comparison of field (W) and Serum (S) CATT results with ELISA results on 109 serum samples

	CATT+		CATT-		TOTAL	
	W	S	W	S	W	S
ELISA +	58	45	2	16	60	= 61
ELISA -	33	13	18	35	49	= 48
TOTAL	91	58	20	51	109	= 109

field CATT Vs ELISA, X^2 = 19.48, df = 1, $p < 0.001$
Serum CATT Vs ELISA, X^2 = 23.52, df = 1, $p < 0.001$

Comparison of CATT results on serum and whole blood

Of the 92 sera from field positive persons tested, only 58 (63.0%) were CATT positive. All 17 sera from those who were field CATT negative were CATT negative in the laboratory.

IK levels: Twenty-nine sera comprising 10 Field CATT$^+$ (W^+)/ Serum CATT$^+$ (S^+), 9 W^+/S^- and 10 W^-/S^- were selected and the level of IK in them determined. The ranges of IK titres in the 3 groups of sera were as follows: W+S+: 64 - 1024, W+S-: 256 - 1024, W-S-: 128 - 1024. This means that the IK titres in all the sera were generally high.

Autoantobodies to human RBC: Four groups of sera were screened for autoantibodies to human RBC. Antibody levels (i.e. the OD_{490} absorbances) in the sera of people who had no CATT reactivity (W^-S^-; n = 17), ranged from 0.14 to 0.85 (median = 0.22) as compared to 0.07 to 1.55 (median = 0.34) for those who only reacted on whole blood (W^+S^-; n = 31) and 0.02 to 1.78 (median = 0.30) for those who were CATT positive both on whole blood and sera (W^+S^+; n = 48). The levels in European sera (n = 14) ranged from 0.08 - 0.40 (median = 0.222). The 95% confidence intervals of the medians for the 4 groups were not significantly different ($p > 0.05$).

Complement fixing IC: Most sera from the endemic area agglutinated the indicator human RBC at the 1/2 dilution, indicating adherence of IC to the RBC_5. Twenty-five per cent of the tested sera were still reactive at dilutions above 1/64 (results not shown). Sera with different field and laboratory CATT reactivity profiles had the same overall patterns of agglutination of the indicator cells.

Do "common" trypanosome antigens contribute to antigen reactivity?

None of the absorbed sera had a different CATT reactivity from pre-absorbed sera. A qualitative CATT on the absorbed sera gave the same titre (i.e. 32) for the control and absorbed sera, indicating that the absorptions had no influence on the CATT reactivity of the serum. These results seem to indicate that common trypanosomal antigens do not play an important role in the reactivity of CATT in the Fontem focus.

Discusssion

Of the 709 CATT persons tested between 1985 and 1989, 280 (39.5%) were males and 429 (60.5%) were females. This difference in prevalence between the sexes was significant ($X^2 = 15.91$, $p < 0.001$). This agrees with the findings of others [14].

The prevelance of CATT positivity was significantly associated with age, with higher prevalences in the > 44 year group, indicating that sleeping sickness is an old disease in the Fontem focus. The presence of seropositives in the < 4 year olds either means that they are exposed when their mothers carry them around or that tsetse flies come right to the compounds as shown by others [34].

CATT positive prevalence varied significantly with the year of screening, with the highest of 5.24% recorded in 1985 (Table 1). This might reflect lack of adequate training for those who started performing the test when it was introduced in 1985 although it is possible that subsequent treatment of CATT positive patients led to a dramatic decline in CATT positive prevalence after 1985.

Seventeen of the 77 CATT positive suspects (22%) were later confirmed as trypanosomiasis patients in 1989 while 38 of 116 CATT$^+$ (32.7%) were confirmed in 1988. Confirmation is usually by thick blood films or gland pucture, both very insensitive parasitological methods. Probably more cases would have been confirmed if more sensitive parasitological methods like the minature-anion exchange centrifugation technique (m-AECT) were used[23].

Field and Laboratory CATT$^+$ results usually fail to agree [12,14]. Sixty-three per cent of the field CATT$^+$ results were confirmed on serum in this study. Further, we showed the presence of IK, autoantibodies to RBC and complement binding ICs in some of the sera from Fontem, but they do not seem to contribute to the differences between field and laboratory CATT results.

In this study, 3 of 26 parasitologically proven trypanosomiasis patients had CATT positive results on whole blood which could not be confirmed on serum. In a previous study in Mamfe (Cameroon) up to 9 of 16 confirmed patients had whole blood CATT positive results which could not be confirmed on serum[12]. In the light of this, we recommend that in trypanosomiasis foci like Fontem whe e the idea CATT antigen is not yet available, im unological suspects should be identified using th CATT on

whole blood, not serum or plasma.

We have shown here that reactivity in the Fontem focus seems to be specific for trypanosome antibodies. However, we failed to abrogate CATT reactivity by absorption with procyclics from two *T.b. gambiense* stocks isolated from the Fontem focus and with formalin fixed *T.b. gambiense* bloodstream trypanosomes from the Fontem focus. This is probably an indication that reactions with "common" trypanosome antigens are not important in CATT reactivity in the Fontem focus.

In the absence of cross-reactions with other parasitoses and with "common" trypanosome antigens, CATT reactions in the Fontem focus are most probably attributable to non-specific cross-reactions with other VATs. This is especially so since, although none of the sera studied from the Fontem focus lysed trypanosomes with the LiTat 1.3 VAT, 40% of them lysed 5 other VATs (LiTat 1.1, 1.4, 1.5, 1.6 and 1.8)[13]. This is conceivable, as different combinations of several antigenic determinants are exposed at the surface of live trypanosomes of each individual VAT[35]. Cross-reactive VATs would contain both similar and different surface exposed epitopes[36], the degree of cross- reactivity depending on the number of similar epitopes expressed by the two VATs. Since some sera from the Fontem focus react in the CATT with the LiTat 1.3 VAT but fail to react with it in trypanolytic tests[13], it is possible that different sets of exposed epitopes on the trypanosome surface mediate these two reactions.

In conclusion the results presented here suggest that: (a) IK, anti-RBC antibodies and complement fixing ICs bound to RBCs do not contribute to differences between results of CATT on whole blood and serum; (b) the CATT positive reactions in the Fontem focus are VAT specific since "common" trypanosome antigens and other parasitoses have no significant contribution to the reaction and (c) the LiTat 1.3 VAT is not an ideal for antigen for mass screening in the Fontem focus. Problems of CATT performance in this focus will remain until an ideal VAT expressed by the trypanosome in the focus is identified and incorporated into the test.

Acknowledgements

We are grateful to the mobile team of the Department of Preventive medicine (Fontem autonoumous sector), who helped in the survey work. We thank Dr. P. Dukes for supplying us with procyclics of *T.b. gambiense* isolated from Fontem and Mr. J. M. Katende who gave us fixed MiTat 1.2 trypanosomes.

References

1. Magnus E, Vervoort T, Van Meirvenne N. A card agglutination test with stained trypanosomes (CATT) for serological diagnosis of *T.b. gambiense* trypanosomiasis. Ann. Soc. belge. Med. Trop. 1978; 58: 169-176.
2. Bafort JM, Schutte CHJ, Gathiran V. Specificity of the Testryp CATT and agglutination test in a non-sleeping sickness area of Africa. South Afr. Med. J. 1986; 69: 541-542.
3. Noireau F, Gouteux JP, Duteurtre JP. Valeur diagnostique du test d'agglutination sur carte (Testryp CATT) dans le depistage de masse de la trypanosomiase humaine au Congo. Bull. Soc. Path. Exot. 1987; 80: 797-803.
4. Noireau F, Lemestre JL, Nzoukoudi MY *et al.* Serodiagnosis of sleeping sickness in the Republic of the Congo: Comparison of indirect immunofluorescent antibody test and card agglutination test. Trans. R. Soc. Trop. Med. Hyg. 1988; 82: 237- 240.
5. Van Nieuwenhove S, Declercq J. Mass serodiagnosis and treatment of serological positives as a control strategy in Trypanosomiasis gambiensis. In: Crooy PJ eds. Symposium on the diagnosis of African Sleeping sickness due to T. gambiense Smith Kline-Rit (Antwerp) 1983: 71-75.
6. Pepin J, Guern C, Mercier D *et al.* Utilisation du Testryp^{R} CATT pour le depistage de la trypanosomiase à Nioki, Zaire. Ann. Soc. belge. Med. Trop. 1986; 66: 213-224.
7. Zillmann U, Albiez EJ. The Testryp^{R} CATT (Card Agglutination test for Trypanosomiasis): A field study on gambiense sleeping sickness in Liberia. Tropenmed. Parasitol. 1986; 37: 390-392.
8. World Health Organisation. Epidemiology and control of African Trypanosomiasis. Report of an Expert Committee. Technical Report series 739, 1986. World Health Organisation, Geneva.
9. Magnus E, Le Ray D, Van Meirvenne N. Variable antigen repertores of *Trypanosoma (T.) brucei.* In Crooy PJ, ed. Symposium on the diagnosis of African sleeping sickness due to *T.*

gambiense. Smith Kline-RIT (Antwerp) 1983: 39-44.

10. Vervoort T, Magnus E, Van Meirvenne N. Serological tests for sleeping sickness: importance of antigen selection. In Crooy PJ. ed. Symposium on the diagnosis of African sleeping sickness due to *T. gambiense*. Smith Kline-RIT (Antwerp) 1983: 47-50.
11. Asonganyi T. Performance of the card agglutination test for trypanosomiasis (CATT): Trends and perspectives. Ann. Univ. Sci. Santé 1985; 2: 81-86.
12. Asonganyi T, Hengy C, Louis JP *et al.* Renaissance of an old sleeping sickness focus in Mamfe, Cameroon: epidemiological, immunological and parasitological findings. Report of 20th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, Mombassa, 10-14 April 1989, in Press.
13. Vervoort T, Dukes P. A new *Trypanosoma brucei gambiense* serodeme in South-West Cameroon? Report of the 20th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, Mombassa, 10-14 April 1989, in Press.
14. Edeghere H, Olise PO, Olatunde DS. Human African trypanosomiasis (sleeping sickness): New endemic foci in Bendel State, Nigeria. Trop. Med. Parasitol. 1989; 40: 16-20.
15. Kazyumba G, Berney M, Brighouse G *et al.* Expression of the B cell repertoire and autoantibodies in human African trypanosomiasis. Clin. Exp. Immunol. 1986; 65: 10-18.
16. Ingram DG, Soltys MA. Immunity in trypanosomiasis. IV. Immuno-conglutinin in animals infected with *Trypanosoma brucei*. Parasitology 1960; 50: 231-239.
17. Wolga JI, Daniel - Ribeiro C, Gaillat J *et al.* Autoanticorps dans les trypanosomiases humaines Africaines. Anticorps antimuscles lisse au cours d'une maladie à *Trypanosoma gambiense*. Bull. Soc. Path. Exot. 1981; 74: 676- 684.
18. Medof ME, Oger JJF. Competition for immune complexes by red cells in human blood. J. Clin. Lab. Invest. 1982; 7: 7-11.
19. Virella G, Shuler CW, Sherwood T. Non-complement dependent absorption of soluble immune complexes to human red cells. Clin. Exp. Immunol. 1983; 54: 448-454.
20. Euozan JP, Brengues J. La trypanosomiase humaine dans le foyer de Fontem — Cameroon: Deuxieme enquete entomologique. *Doc ronéo* 1982; Rapport 7.74 ENT. OCEAC - ORSTOM.
21. Mondet B, Berl D, Barbazan P *et al.* Etude sur les glossines vectrice de la trypanosomiase humaine dans les foyers de Fontem et de la plaine des Mbos (Cameroun). 1. Etudes preliminaires dans les foyers de Fontem. Doc. roneo 1982. Rapport 2/82/Ent. Med. Centre Pasteur du Cameroun.
22. Asonganyi T, Suh S, Tetuh MD. Prevalence of domestic animal trypanosomiasis in the Fontem sleeping sickness focus, Cameroon. Rev. Elev. Med. Vet. Pays. Trop. 1990; 43: 69-74.
23. Lumsden WHR, Kimber CD, Dukes P *et al.* Field diagnosis of sleeping sickness in the Ivory Coast; Comparison of the miniature anion-exchange centrifugation technique with other parasitological methods. Trans. R. Soc. Trop. Med. Hyg. 1981; 75; 242-245.
24. Katende JM, Musoke AJ, Nantulya VM *et al.* A new method for fixation and preservation of trypanosomal antigens for use in indirect immunofluorescence antibody test for diagnosis of bovine trypanosomiasis. Trop. Med. Parasitol. 1987; 33: 41-44.
25. Dukes P, Kaukas A, Hudson KM *et al.* A new method for isolating *Trypanosoma brucei gambiense* from sleeping sickness patients. Trans. R. Soc. Trop. Med. Hyg. 1989; 83: 636-639.
26. Grab DJ, Bwayo JJ. Isopycnic isolation of African trypanosomes on percoll gradients formed in situ. Acta Tropica 1982; 39: 363-366.
27. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilising the principle of protein dye binding. Analyt. Biochem. 1976; 72: 248- 254.
28. Asonganyi T, Lando G, Ngu JL. Serum antibodies against human brain myelin proteins in Gambian trypanosomiasis. Ann. Soc. belge. Med. Trop. 1989; 69: 213-221.
29. Evans WH. Preparation and characterisation of mammalian plasma membranes. In: Work TS, Work E, eds. Laboratory Techniques in Biochemistry and molecular Biology, Vol. 7, part 1. Elsevier/North Holland, Amsterdam, 1978.
30. Hautenen A, Koistinin V, Penttinen K *et al.* Use of ELISA for measuring immunoconglutinin. J. Immunol. Meth. 1978; 21: 335-340.
31. Lachmann PJ. Sedimentation pattern technique for measuring conglutination: Its application to

demonstrating immunoconglutinins to C'4. Immunology 1966; 11: 263-271.

32. Inada Y, Kamiyama M, Kanemitsu T *et al.* Detection of circulating immune complexes: A new application of immune adherence hemagglutination. Ann. Immunol. 1981; 132C: 181-190.
33. Moulian - Pelat JP, Spiegel A, Baudon D *et al.* Interet en épidemiologie de la representation graphique du test de Tukey. Med. Trop. 1989; 49: 285-288.
34. Gouteaux JP, Eouzan JP, Noireau F. Modification du comportement de *Glossina palpalis palpalis* lié à l'epizootie de peste porcine de 1982 dans un village Cangolais. Acta Tropica 1987; 44: 333-337.
35. Magnus E, Vervoort T, Van Meirvenne N. Serological cross-reactions among trypanosome variable antigen isotypes of the subgenus Trypanozoon. Ann. Soc. belge. Med. Trop. 1982; 62: 25-29.
36. Barbet AF, Davis WC, McGuire TC. Cross neutralization of two different trypanosome populations derived from a single organism. Nature 1983; 300: 453-456.

(Accepted 28 March, 1991)