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Acta Tropica 76 (2000) 175–183

ACTA  
TROPICA

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## Preliminary evaluation of LATEX/*T. b. gambiense* and alternative versions of CATT/*T. b. gambiense* for the serodiagnosis of Human African Trypanosomiasis of a population at risk in Côte d'Ivoire: considerations for mass-screening

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Received 16 July 1999; received in revised form 7 March 2000; accepted 23 March 2000

### Abstract

A study was conducted to compare classical card agglutination test for trypanosomiasis (CATT)/*T. b. gambiense* with CATT-EDTA and LATEX/*T. b. gambiense* as alternative field tests for serodiagnosis of Human African Trypanosomiasis. The tests were performed on freshly collected blood in an endemic and a low prevalence area in Côte d'Ivoire. Diagnostic performance of each test was assessed using Quantitative Buffy Coat as the parasitological reference and immune trypanolysis as the serological reference test. According to the parasitological data, CATT-EDTA on 10 µl and LATEX/*T. b. gambiense* on blood diluted 1:4, detecting all confirmed cases with good specificity (respectively 94.6% and 98.1%) yielded better results than the classical CATT did (one false negative and 92.5% specific). However, when immune trypanolysis data and feasibility are taken into account, the classical CATT remains the test of choice for mass screening under the given field conditions. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Human African Trypanosomiasis; Sleeping sickness; *Trypanosoma brucei gambiense*; Diagnosis; CATT; LATEX

*Abbreviations:* EDTA, ethylenediamine tetraacetic acid; PBS, phosphate-buffered saline; VSG, variable surface glycoprotein.

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## 1. Introduction

Sleeping sickness or Human African Trypanosomiasis (HAT) is caused by trypanosomes belonging to the genus *Trypanosoma*. While *Trypanosoma brucei gambiense* is responsible of the chronic form in West and Central Africa, the acute form caused by *Trypanosoma brucei rhodesiense* occurs in East Africa. Despite nearly a century of control activities, HAT still remains a major health problem in sub-Saharan Africa with about 55 million people exposed. It is estimated that the actual number of new cases per year is 300 000 (WHO, 1998). Control of sleeping sickness depends on case detection by means of parasitological examinations of blood, lymph or cerebrospinal fluid. Patients are given treatment only if trypanosomes have been detected in their body fluids. Mass screening of the whole population at risk is currently performed using serological tests in order to select individuals carrying trypanosome specific antibodies, on which parasitological examinations are then carried out. However, in the case of *T. b. gambiense* infection, parasite detection may be difficult, because of often-low parasitemia combined with limited sensitivity of the parasitological techniques. Among available serological tests, the most commonly used in the field is the Card Agglutination Test for Trypanosomiasis or CATT/*T. b. gambiense* (CATT) (Magnus et al., 1978) developed for detection of trypanosome specific antibodies. Bloodstream form trypanosomes of *T. brucei* spp. contain both somatic (common) and variable antigens (Seed, 1974; Le Ray, 1975). Studies on variable antigen type (VAT) repertoires (Van Meirvenne et al., 1975, 1977), revealed that some predominant VATs such as LiTat 1.3 are particularly widespread in *T. b. gambiense*. The CATT reagent consists of a lyophilised suspension of fixed and stained bloodstream form trypanosomes of the LiTat 1.3 VAT. Because of its simplicity and low price, the CATT is a very useful tool for field use.

Despite its good specificity (Bafort et al., 1986; Zillman and Albiez, 1986; Noireau et al., 1987), several studies raised the problem of both sensitivity and positive predictive value of the CAIT

(Noireau et al., 1987; Penchenier et al., 1991). The choice of LiTat 1.3 as CATT antigen has also been questioned because of the observed absence of the LiTat 1.3 gene in four out of ten *T. b. gambiense* stocks isolated at the Fontem focus in Cameroon (Dukes et al., 1992). In the meantime, alternative versions of CATT/*T. b. gambiense* (Pansaerts et al., 1998) and a new serological test, the LATEX/*T. b. gambiense* (LATEX; Büscher et al., 1991, 1999) have been developed in the Laboratory of Serology of the Institute of Tropical Medicine (Antwerp, Belgium).

In this preliminary study in Côte d'Ivoire, our aim was to compare these new tests with the classical CATT using Quantitative Buffy Coat (QBC; Bailey and Smith, 1992) as parasitological reference test and immune trypanolysis (Van Meirvenne et al., 1995) as serological reference test. We were interested not only in comparing intrinsic properties of each test but also in analysing their feasibility under field conditions. This comparison was carried out in an epidemic area (Sinfra) and a very low endemic area (Bonoufla) of Côte d'Ivoire.

## 2. Materials and methods

### 2.1. Study area and examined population

The present study was conducted in 1996 in two distant areas located in an active focus of HAT in the Western-Central part of Côte d'Ivoire. In Sinfra, the first area, a control program has been carried out since 1995. The overall observed prevalence was around 1.5% (Laveissière, personal communication). During this program, blood was collected on Whatman filter paper no. four from the whole population (more than 50 000 people) to perform MicroCATT which is a microversion of the CATT (Miezan et al., 1991) and which can be used for epidemiological purposes. MicroCATT positive subjects were further examined at the Sinfra laboratory. In Sinfra, the study population consisted of 74 individuals from this control program. Out of them, 21 randomly selected subjects were sampled for serum collection. The second area, Bonoufla, is located near the

Vavoua focus (Laveissière et al., 1986). This area is considered as a very low prevalence area (only two or three trypanosomiasis cases per year passively detected at the Project de Recherches Cliniques sur la Trypanosomiase in Daloa) and was chosen to assess the specificity of the tests. In Bonoufla, the study population consisted of 427 individuals examined in a mass-screening context. From this group, 54 randomly selected subjects were sampled for serum collection.

## 2.2. Parasitological and immune trypanolysis tests

For parasite detection, QBC was systematically performed according to the protocol described by Bailey and Smith (1992).

Immune trypanolysis, according to Van Meirvenne et al. (1995), was performed on the obtained serum samples. The sera were tested at a 1/4 dilution with *T. b. gambiense* VATs LiTat 1.3, 1.5 and 1.6.

## 2.3. Serological field tests

Classical CATT and two alternative CATT versions were performed on freshly collected blood. The agglutination card was rotated at 60 rpm for 5 min.

**Classical CATT:** One drop of reagent reconstituted with PBS + 30 µl of blood (according to the instructions provided with the kit).

**CATTEDTA:** One drop of reagent reconstituted with PBS-EDTA + 30 µl of blood. Active complement, present in freshly collected blood samples, may have an adverse effect on the agglutination reaction of the classical CATT reflected by a prozone phenomenon. This can be avoided by incorporation of EDTA (10 mM) in the buffer (Pansaerts et al., 1998) leading to increased sensitivity.

**CATT EDTA on diluted blood:** One drop of reagent reconstituted with PBS-EDTA + 10 µl of blood + 20 µl of PBS. This version corresponds to the previous one but uses 1/3 diluted blood and is proposed to avoid cross-reactions, thus increasing specificity.

**LATEX/*T. b. gambiense*** was performed according to the instructions provided with the kit. The reagent is composed of semi-purified variable antigens (VATs LiTat 1.3, 1.5 and 1.6) of *T. b. gambiense* bloodstream forms, coupled onto suspended latex particles. As for the CATT, the reagent is lyophilised and has to be reconstituted with 1 ml of PBS. Blood dilutions are prepared in microplates. Then, 20 µl of LATEX reagent is mixed with 20 µl of diluted blood and spread onto a reaction zone (diameter 1.5 cm) with black background (Wellcome card with six reaction zones). The card is rocked on a horizontal rotator at 70 rpm for 5 min. Antibodies present in the blood are revealed by a white macroscopic agglutination.

The test kits (CATT/*T. b. gambiense* and LATEX/*T. b. gambiense*) are available at the Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.

Agglutination scores and corresponding results of the serological field tests are shown in Table 1.

## 2.4. Blood and serum collection

Blood was collected by finger-prick in one special QBC tube and in three heparinised microhaematocrit tubes (3 × 60 µl). The blood in these microhaematocrit tubes was transferred to a microplate. The CATT tests were performed in parallel with the LATEX at 1/4 blood dilution in PBS (LATEX 1/4). In case of positivity of the

Table 1  
Agglutination scores and corresponding results of the serological field tests

Tests	Agglutination scores				
CATT	0	+/-	+	++	+++
LATEX	0	1	2	3	4
Results	Negative	Doubtful	Weakly positive	Positive	Strongly positive

Table 2

Number of true positives as detected by the serological field tests within the Sinfra study population

	Classical CATT	CATT EDTA	CATT EDTA on dilution	LATEX 1/4	LATEX 1/8	LATEX 1/16
12 T+ <sup>a</sup>	11	12	12	12	11	8
14 L+ <sup>b</sup>	13	13	11	10	9	3
26 (L+, T+)	24	25	23	22	20	11

<sup>a</sup> T+: positive in parasite detection by means of QBC.<sup>b</sup> L+: positive in immune trypanolysis.

LATEX 1/4, blood dilutions 1/8 and 1/16 were prepared and tested (LATEX 1/8 and LATEX 1/16).

For immune trypanolysis, blood was taken by venepuncture from part of the study population. After coagulation and centrifugation, the serum samples were stored at  $-20^{\circ}\text{C}$  and shipped to the Institute of Tropical Medicine, Antwerp.

### 3. Results

#### 3.1. Seropositivity in the field tests compared to parasite detection

At Sinfra, out of the 74 subjects examined, 12 were parasitologically positive by QBC (16.2%). The number of true positives detected by each serological field test is given in Table 2.

With the exception of LATEX 1/16 (8/12), the field tests detected at least 11 of the 12 parasitologically positive cases. Both CATT EDTA versions detected more cases than the classical CATT (12 vs. 11). LATEX detected less cases in function of increasing dilution of the blood (12 to eight).

#### 3.2. Seropositivity in the field tests compared to the combination of parasite detection and immune trypanolysis

As previously indicated, 21 individuals were randomly sampled for serum collection and immune trypanolysis. This group contained seven parasitologically confirmed cases by QBC, one of whom was negative in immune trypanolysis with all variants. The other sera were positive in immune trypanolysis with at least one variant. Thus,

immune trypanolysis was positive for 14 subjects non-parasitologically confirmed with QBC (L+). Considering immune trypanolysis as 100% specific for antibody presence, seropositivity in the field tests is now compared to both QBC positives (12T+) and non-confirmed immune trypanolysis positives (14L+). Results are shown in Table 2.

With the exception of the classical CATT test, less true positive cases were detected when compared to the previous results (Table 2). This was particularly noticed for CATT EDTA on diluted blood and LATEX tests. CATT EDTA still detected more cases than the classical CATT (25 vs. 24). All LATEX tests detected less true positive cases than the classical CATT.

#### 3.3. Particular results

In general, results obtained with the different serological tests corresponded with each other. However, in some cases from the study population in Sinfra, discordant results were obtained (Table 3). One case (A) was found to be negative by classical CATT but weakly positive by both CATT EDTA versions and by LATEX at the lower dilutions. Cases B and C were strongly positive in almost all CATT versions but only weakly positive in the LATEX tests. On the contrary, case D and E were strongly positive in almost all LATEX tests but weaker in the CATT tests. Case F was negative by immune trypanolysis, weakly positive in the CATT tests and strongly positive in the LATEX tests. All the former cases were parasitologically confirmed by QBC. Case G, non-confirmed by QBC, was positive by immune trypanolysis with only the LiTat 1.5 variant, remained negative in all CATT tests but was positive by LATEX.

### 3.4. Specificity of the serological field tests

At Bonoufla, 427 patients were examined with the serological field tests and QBC. In no case were trypanosomes detected. One person had been treated for trypanosomiasis in the past and was excluded from the study. Among the 426 remaining subjects, 54 were randomly sampled for immune trypanolysis. Out of them, only one was positive. Thus, 425 persons were retained as non-trypanosomiasis cases (T−). The specificity of the serological field tests was calculated on this group. Results are shown in Table 4.

The specificity, calculated as  $100 \times \text{True Negatives} / (\text{True Negatives} + \text{False Positives})$  (Dukes et

al., 1984) of the LATEX tests was higher than that of the CATT tests and increased with dilution of the blood. Among the CATT tests, CATT EDTA on diluted blood was more specific than both the classical CATT and the CATT EDTA.

### 3.5. Feasibility of the serological tests

For assessment of test feasibility, some technical parameters were considered, such as the time needed to perform 1000 tests by a specialised team of ten persons, qualification of the personnel needed and additional necessary material (Table 5). According to the instructions provided with the kits, stability and storage conditions of both

Table 3

Test results obtained for seven particular cases within the study population at Sinfra

Subjects	QBC	Immune trypanolysis	Classical CATT	CATT EDTA	CATT EDTA on diluted blood	LATEX 1/4	LATEX 1/8	LATEX 1/16
A	+	+	−	+	+	2	2	0
B	+	ND <sup>a</sup>	+++	+++	+++	2	0	0
C	+	+	+	+++	+++	2	2	0
D	+	ND	+/-	+/-	+/-	4	4	4
E	+	+	+/-	++	+	4	4	2
F	+	−	++	+	+	4	4	4
G	−	+ <sup>b</sup>	−	−	−	4	3	2

<sup>a</sup> ND, not done.

<sup>b</sup> Positive with only LiTat 1.5 variant.

Table 4

Number of true negatives in the Bonoufla study population and specificity (%) of the serological field tests

	Classical CATT	CATT EDTA	CATT EDTA on dilution	LATEX 1/4	LATEX 1/8	LATEX 1/16
425 T−	393	370	402	417	422	425
Spec (%)	92.5	87.1	94.6	98.1	99.2	100

Table 5

Technical parameters of the serological field tests

Tests	Time to perform 1000 tests (h) <sup>a</sup>	Personnel needed	Additional necessary material
Classical CATT	10	Not qualified	None
CATT EDTA	10	Not qualified	None
CATT EDTA on diluted blood	15	Qualified	Pipette and tips
LATEX	20	Qualified	Pipette, tips and microplate

<sup>a</sup> Estimated time if performed by a specialised team of ten experienced technicians.

the lyophilised and the reconstituted reagents are similar in all test versions.

Due to the need for manipulating pipettes and preparing dilutions of the blood before testing, CATT EDTA on diluted blood and LATEX have to be performed by qualified personnel. Furthermore, more time is needed for testing 1000 individuals with these tests than with the classical CATT. The need for microplates in LATEX renders the tests less feasible than the CATT tests.

#### 4. Discussion

The diagnostic performances of a test we based on sensitivity and specificity. They represent the true intrinsic properties of the test if they are determined from a representative population on which this test could be applied. In a previous study in Cameroon, Penchenier et al. (1998) observed that Latex 1/4 and 1/8 detected all of the 16 parasitologically confirmed sleeping sickness cases. In our study, only 12 parasitologically confirmed cases were included together with 14 immune trypanolysis positive cases. Consequently, extrapolation from our results to mass-screening consideration must be very cautious. Test sensitivities have to be estimated not only from larger studies but also in higher prevalence areas. Conversely, specificities were estimated from a different sample of population (non-preselected, different area) and our results can be considered as obtained from mass-screening under field conditions.

CATT/*T. b. gambiense* is currently used for serological screening of the population at risk for sleeping sickness. The classical test version is a rapid direct agglutination test on whole blood and is particularly suited for application under field conditions. Some test characteristics inherently limit the diagnostic performance of the CATT. The use of undiluted blood may cause non-specific agglutination, lowering specificity, as well as a complement mediated prozone effect, allowing false negative results. The CATT reagent only contains trypanosomes of one variable antigen type, antibodies against which may be absent in trypanosomiasis patients (Dukes et al., 1992). It is

well known that antibody detection tests, using complex antigen preparations, give rise to non-specific cross-reactions. Diallo et al. (1996), working with crude trypanosome antigens observed cross-reactions with other human parasites in ELISA and LATEX agglutination tests. In this respect, non-variant specific epitopes exposed at the surface of the trypanosomes in the CATT reagent could be responsible for the cross-reactivity with sera of pigs, experimentally infected with *Trypanosoma congolense* described by Noireau et al. (1986). In order to avoid such cross-reactions, a LATEX agglutination test, based on a combination of several purified specific antigens, has been developed (Büscher et al., 1991, 1999). In order to avoid the complement mediated prozone effect, addition of EDTA in the reaction mixture has been proposed (Pansaerts et al., 1998).

The results obtained in this study, where classical CATT was compared to CATT-EDTA and LATEX, show that the improvement of the diagnostic performance of serological tests for sleeping sickness is not obvious. Compared to parasitological results (QBC), the addition of EDTA in the CATT indeed prevents a prozone effect, exemplified by subjects A, C and E in Table 3, thus allowing the detection of all 12 cases, even when using only 10 µl of blood. The use of more than one variable antigen in the LATEX seems also to increase the number of true positive cases detected, provided the sample is not too diluted. Subjects D and E, doubtful by classical CATT, reacted strongly in LATEX. This was probably due to low titers of anti-LiTat 1.3 antibodies but high titers of anti-LiTat 1.5 and/or anti-LiTat 1.6 antibodies in these samples. However, by mixing different antigens in the same reagent, the reactivity of each individual antigen in the final mixture is reduced. This may lead to false negative results as exemplified in subjects B and C. These samples apparently contained high titers of anti-LiTat 1.3 antibodies but not of anti-LiTat 1.5 and/or anti-LiTat 1.6 antibodies, resulting in high reactivity with CATT but only weak or no reactivity with LATEX.

Knowing that the sensitivity of QBC is not 100% (Truc et al., 1998) it seemed interesting to include a serological reference test in the study.

As shown by Van Meirvenne et al. (1995), immune trypanolysis is 100% specific and thus immune trypanolysis positive individuals can be considered as being or having been infected with *T. b. gambiense*. When the capacity to detect true positive cases is compared with positivity in QBC and/or immune trypanolysis, only the CATT EDTA on undiluted blood scored better than the classical CATT version. None of the CATT versions or the LATEX versions detected all the immune trypanolysis positive individuals. This could be explained by the high amount of antibodies needed to yield a positive result in the rapid agglutination assays. On the other hand, as exemplified by subject F who was negative by immune trypanolysis, both CATT (whole trypanosomes) and LATEX (purified VSGs) reagents contain epitopes other than the strictly variant specific ones which may react with sleeping sickness patient's blood. Subject G, positive by LATEX and by immune trypanolysis with LiTat 1.5 only but negative in all CATT versions (i.e. lacking anti-LiTat 1.3 antibodies), demonstrates the advantage of using more than one antigen. However, the overall reactivity of this antigen mixture in LATEX is too low to detect all immune trypanolysis positives. The fewer positive cases detected by the LATEX as compared to the CATT versions, can also be partly explained by the preselection of the study population at Sinfra by microCATT, thus introducing a bias towards anti-LiTat 1.3 positivity for the parasitologically confirmed individuals.

The specificity of antibody detection tests depends on the specificity of the incorporated antigen. Complex antigens, as present in CATT and to a lesser extent in LATEX, usually bear cross-reacting epitopes that may react with non-specific antibodies at low blood dilutions. This phenomenon is illustrated by the results obtained in this study. By adding EDTA to CATT and testing undiluted blood, prozone was avoided and more true positive cases were detected but specificity decreased. Dilution of the blood sample increased the specificity of both the CATT-EDTA and the LATEX versions. All the LATEX versions were more specific than the CATT versions due to the use of purified VSG antigens. However, even

purified VSGs still bear some non-specific epitopes limiting the specificity of the test at low blood dilution.

The ultimate diagnostic performance of a serological test in a given situation is determined by its sensitivity and specificity and by the prevalence of the infection (Gerstman, 1998). Sensitivity and specificity usually are inversely related, in other words, a very sensitive test may yield many false positives (e.g. CATT-EDTA on whole blood) and a very specific test may yield many false negatives (e.g. LATEX 1/16). Considering the parasitologically confirmed group and the negative population, both CATT-EDTA on 10 µl blood and LATEX 1/4 allowed the detection of all cases with acceptable specificity (respectively, 94.6 and 98.1%) and performed better than the classical CATT. In this way, if we consider the WHO definition of sleeping sickness cases (i.e. confirmation of the infection with a parasitological test), the LATEX performed on 1/4 diluted blood seems to be the most efficient. Its good specificity will avoid time-consuming parasitological examination of false positive cases. However, considering the immune trypanolysis positive individuals as possible sleeping sickness patients, the best overall diagnostic performance was obtained with the classical CATT with 92.5% specificity and detecting 24 (T + , L + ) out of 26. According to the expected prevalence of the infection, it is up to the user to decide whether a test with high positive predictive value or a high negative predictive value is needed.

In terms of mass screening in the field, other parameters such as feasibility and price should be taken into account when evaluating serological tests. The price of CATT, CATT-EDTA and LATEX is the same. Stability of all the reagents and storage conditions are compatible with their use in the field. All tests need only 5 min reaction time. Problems arise when blood dilutions have to be prepared as for CATT-EDTA on 10 µl (on the card) or for LATEX (in microplates). Extra accessory materials such as pipettes and microplates are needed and specially trained personnel are required, unlike the classical CATT test. In addition, all extra manipulations will reduce the number of persons that can be tested per day. Thus

both classical CATT and CATT-EDTA on undiluted blood allowed the screening of twice as many people per day as the LATEX tests. For the LATEX protocol, the agglutination cards should be adapted (ten instead of six reaction zones with a diameter of 1.5 cm instead of 2.8 cm) and special attention should be paid to prevent the LATEX-blood mixture drying out before the end of the test. Recently, new agglutination cards have been designed and protocols are being tested to avoid the need of blood dilutions in LATEX.

Since the reference tests (immune trypanolyse and QBC) are not 100% sensitive, the observed results of the agglutination tests in this study should be considered as maximal concerning true positive detection capacity and as minimal concerning specificity. Further studies on a large, unbiased collection of samples originating from different endemic regions with high and low disease prevalence are needed to obtain conclusive evidence on the diagnostic performance of the different serological field tests.

## Acknowledgements

We would like to express our sincere thanks to the team of Institut Pierre Richet/OCCGE at Bouaké (Côte d'Ivoire) for their excellent technical help. This work was supported by the Institut de Recherche pour le Développement, by Fonds d'Aide à la Coopération, Ministère Français des Affaires Etrangères, Secrétariat d'Etat à la Coopération et à la Francophonie and by the International Livestock Research Institute, Nairobi, Kenya.

## References

- Bafort, J.M., Schutte, C.H.J., Gathiram, V., 1986. Specificity of the Testryp CATT card agglutination test in a non-sleeping-sickness area of Africa. *S. Afr. Med. J.* 69, 541–542.
- Bailey, J.W., Smith, D.H., 1992. The use of the acridine orange QBC technique in the diagnosis of African trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.* 86, 630.
- Büscher, P., Draelants, E., Magnus, E., Vervoort, T., Van Meirvenne, N., 1991. An experimental latex agglutination test for antibody detection in human African trypanosomiasis. *Ann. Soc. Belg. Méd. Trop.* 71, 267–273.
- Büscher, P., Lejon, V., Magnus, E., Van Meirvenne, N., 1999. Improved latex agglutination test for detection of antibodies in serum and cerebrospinal fluid of *Trypanosoma brucei gambiense* infected patients. *Acta Trop.* 73, 11–20.
- Diallo, P.B., Truc, P., Méda, H.A., Kamenan, A., 1996. Diagnostic sérologique de la Trypanosomiase Humaine Africaine à *Trypanosoma brucei gambiense*: 1 — obtention et utilisation d'antigènes bruts dans les tests ELISA et d'agglutination au latex. *Bull. Soc. Pathol. Exot.* 89, 262–268.
- Dukes, P., Rickman, L.R., Killick-Kendrick, R., Kakoma, I., Wurapa, F.K., de Raadt, P., Morrow, R., 1984. A field comparison of seven diagnostic techniques for Human African Trypanosomiasis in the Luangwa Valley, Zambia. *Tropenmed. Parasit.* 35, 141–147.
- Dukes, P., Gibson, W.C., Gashumba, J.K., Hudson, K.M., Bromidge, T.J., Kaukus, A., Asonganyi, T., Magnus, E., 1992. Absence of the LiTat 1.3 (CATT antigen) gene in *Trypanosoma brucei gambiense* stocks Cameroon. *Acta Trop.* 51, 123–124.
- Gerstman, B.B., 1998. Measures of association and potential impact. In: Gerstman, B.B. (Ed.), *Epidemiology Kept Simple: An Introduction to Classic and Modern Epidemiology*. Wiley-Liss, New York, pp. 120–138.
- Laveissière, C., Hervouët, J.P., Mérouze, F., Cattand, P., 1986. La campagne pilote de lutte contre la trypanosomiase humaine dans le foyer de Vavoua (Côte d'Ivoire). 4. Bilan de la campagne: les prospections médicales et la participation de la population. *Cah. ORSTOM Sér. Ent. Méd. Parasitol.* 24, 111–120.
- Le Ray, D., 1975. Structures antigéniques de *Trypanosoma brucei brucei* (Protozoa, Kinetoplastida). Analyse immunoelectrophorétique et étude comparative. *Ann. Soc. Belg. Méd. Trop.* 55, 129–311.
- Magnus, E., Vervoort, T., Van Meirvenne, N., 1978. A card-agglutination test with stained trypanosomes (CATT) for the serological diagnosis of *T. gambiense* trypanosomiasis. *Ann. Soc. Belg. Méd. Trop.* 59, 169–176.
- Miezan, T., Doua, F., Cattand, P., De Raadt, P., 1991. Evaluation du Testryp CATT appliqué au sang prélevé sur papier filtre et au sang dilué, dans le foyer de trypanosomiase à *Trypanosoma brucei gambiense* en Côte d'Ivoire. *Bull. World Health Organ.* 69, 603–606.
- Noireau, F., Gouteux, J.P., Frézil, J.L., 1986. Sensibilité du test d'agglutination sur carte (Testryp CATT) dans les infections porcines à *Trypanosoma (Nannomonas) congolense* en République du Congo. *Ann. Soc. Belg. Méd. Trop.* 66, 63–68.
- Noireau, F., Gouteux, J.P., Duteurtre, J.P., 1987. Valeur diagnostique du test d'agglutination sur carte (Testryp CATT) dans le dépistage de masse de la trypanosomiase humaine au Congo. *Bull. Soc. Pathol. Exot.* 80, 797–803.
- Pansaerts, R., Van Meirvenne, N., Magnus, E., Verhelst, L., 1998. Increased sensitivity of the card agglutination test CATT/*Trypanosoma brucei gambiense* by inhibition of complement. *Acta Trop.* 70, 349–354.



- Penchenier, L., Jannin, J., Mouliia-Pelat, J.P., Elfassi de la baume, F., Fadat, G., Chanfreau, B., Eozenou, P., 1991. Le problème de l'interprétation du CATT dans le dépistage de la trypanosomiase humaine à *Trypanosoma brucei gambiense*. Ann. Soc. Belg. Méd. Trop. 71, 221–228.
- Penchenier, L., Grébaut, P., Bodo, J.M., Ebo'o Eyenga, V., Njiokou, F., Simo, S.G., Nkinin, S., Ndong Asumu, P., Simarro, P., Herder, S., Soula, G., 1998. Le foyer de trypanosomiase humaine de Campo (Cameroun) en 1998. Aspects épidémiologiques, état de l'endémie et comparaison des CATT 1.3 et CATT Latex dans le dépistage de masse. Bull. Liais. Doc. OCEAC 31, 8–19.
- Seed, J.R., 1974. Antigens and antigenic: variability of the African trypanosomes. J. Protozool. 21, 639–646.
- Truc, P., Jamonneau, V., N'Guessan, P., Diallo, P.B., Garcia, A., 1998. Parasitological diagnosis of African trypanosomiasis: a comparison of the QBC<sup>®</sup> and miniature anion-exchange centrifugation techniques. Trans. R. Soc. Trop. Med. Hyg. 92, 288–289.
- Van Meirvenne, N., Janssens, P.G., Magnus, E., 1975. Antigenic variation in syringue passaged population of *Trypanosoma (Trypanozoon) brucei*. 1. Rationalization of the experimental approach. Ann. Soc. Belg. Méd. Trop. 55, 1–23.
- Van Meirvenne, N., Magnus, E., Vervoort, T., 1977. Comparisons of variable antigenic types produced by trypanosome strains of the subgenus *Trypanozoon*. Ann. Soc. Belg. Méd. Trop. 57, 409–423.
- Van Meirvenne, N., Magnus, E., Büscher, P., 1995. Evaluation of variant specific trypanolysis tests for serodiagnosis of human infections with *Trypanosoma brucei gambiense*. Acta Trop. 60, 189–199.
- WHO, 1998. Control and surveillance of African trypanosomiasis. Report of a WHO Expert Committee. WHO Technical Report Series 881.
- Zillman, U., Albiez, E.J., 1986. The Testryp CATT (Card Agglutination Test for Trypanosomiasis): a field study on gambiense sleeping sickness in Liberia. Trop. Med. Parasitol. 37, 390–392.