



Evaluation of LATEX/*T.b.gambiense* for mass screening of *Trypanosoma brucei gambiense* sleeping sickness in Central Africa

L. Penchenier^{a,*}, P. Grébaut^a, F. Njokou^b, V. Eboo Eyenga^c, P. Büscher^d

^a OCEAC, POB 288, Yaounde, Cameroon

^b University of Yaounde I, Yaounde, Cameroon

^c Ministry of Public Health and Population, Yaounde, Cameroon

^d Institute of Tropical Medicine, Antwerp, Belgium

Received 5 December 2000; received in revised form 25 May 2002; accepted 18 June 2002

Abstract

We compared the Card Agglutination Test for Trypanosomiasis (CATT), which consists of lyophilized bloodstream form trypomastigotes of *Trypanosoma brucei gambiense* (*T.b.g.*) variable antigen type LiTat 1.3, with LATEX/*T.b.g.*, which consists of a lyophilized suspension of latex particles coated with variable surface glycoproteins of *T.b.g.* variable antigen types LiTat 1.3, 1.5 and 1.6. This study was carried out during two mass screening surveys in 1998 in Campo, a sleeping sickness focus in Cameroon, with a low prevalence (0.3%) and in 1999 in Batangafo which belongs to the Central African focus of Ouham which has a higher prevalence (3%). In Campo, we compared the CATT performed on whole blood with the LATEX/*T.b.g.* on diluted blood. In Batangafo, both tests were performed on diluted blood. In all circumstances, the specificity of the LATEX/*T.b.g.* was higher than of CATT. The use of LATEX/*T.b.g.* on diluted blood instead of CATT results in an important decrease of workload and as a consequence, of costs related to parasitological examinations. In the case of Campo the workload was up to 12 times less than when using CATT 1.3 on whole blood and the cost divided by 3. In Batangafo the workload was decreased by nearly 20% with the LATEX/*T.b.g.* Finally, it should be noted that in Batangafo, one of the parasitologically confirmed sleeping sickness patients was negative in CATT and positive in LATEX/*T.b.g.* and that the reading of the test result in LATEX/*T.b.g.* is easier than in CATT.

© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Human African Trypanosomiasis; Central Africa; Screening; Diagnosis; CATT; LATEX; *Trypanosoma brucei gambiense*

1. Introduction

The diagnosis of Human African Trypanosomiasis is based on the detection of the parasite in the host. During mass screening surveys, it is not

* Corresponding author. Present address: Institut de Recherche pour le Développement (ex ORSTOM), 911 avenue Agropolis, 34000 Montpellier Cedex-01, France
E-mail address: lt.penchenier@infonie.fr (L. Penchenier).

possible to perform parasitological examinations on the whole population. That is why, in addition to lymph node palpation and puncture of the adenopathies which have been brought into evidence, the population at risk is screened by serological tests in order to start parasitological detection in the blood only in seropositive individuals.

The serological test currently used for mass screening in *Trypanosoma brucei gambiense* sleeping sickness is the Card Agglutination Test for Trypanosomiasis (CATT; Magnus et al., 1978), which consists of lyophilized bloodstream form trypomastigotes of *Trypanosoma brucei gambiense* variable antigen type LiTat 1.3. Despite its high specificity (Bafort et al., 1986; Zillman and Albiez, 1986; Noireau et al., 1988), the survey team still has to perform parasitological examinations on CATT positive individuals which remain parasitologically negative. In addition, the sensitivity of the CATT has been criticized (Penchenier et al., 1991). It appears that some strains of *T.b.g.* lack the gene coding for LiTat 1.3 (Dukes et al., 1992). Therefore, the development of a latex test which consists of a lyophilized suspension of latex particles coated with a mixture of variable surface antigens of *T.b.g.* variable antigen types LiTat 1.3, 1.5 and 1.6 (Büscher et al., 1999), has incited research groups to compare CATT to LATEX/*T.b.g.* The comparison of both tests, which are distributed by the Laboratory of Serology at the Institute of Tropical Medicine, Antwerp, has been the subject of three studies. The first one in Equatorial Guinea (Simarro et al., 1999), a second one in Cameroon (Penchenier et al., 1998) of which the results are more elaborated below and a third one in Côte d'Ivoire (Jamonneau et al., 2000).

2. Materials and methods

2.1. Sleeping sickness foci

For the comparative evaluation of LATEX/*T.b.g.* and CATT, we chose two sleeping sickness foci:

- The coastal focus in Campo, Cameroon. For more than 50 years now, the observed prevalence is about 0.2–0.3%. We screened the whole population (5793 inhabitants) of that focus dispersed over 30 villages.
- The focus of Batangafo in the Central African Republic, which belongs to the focus of Ouham and which is situated next to the boarder with Tchad. The estimated prevalence lies between 2 and 3%. We have screened five districts of Batangafo of which the estimated population consists of 2827 inhabitants (census of 1998).

2.2. Study protocol

From each person that was registered (surname, first name, sex, age, origin, village or district...), finger prick blood was collected in two heparinized capillary tubes for the serological screening with CATT and LATEX/*T.b.g.* following the instruction included in the kits.

- In Campo, a focus studied before Batangafo, one these of tubes was used for CATT on whole blood, which is the classical CATT test. The other tube was used for LATEX/*T.b.g.* on diluted blood. In CATT, the test result was given a score from 0 to 4 depending on the absence or intensity of the agglutination (0, negative; 1, doubtful; 2–4, positive). In LATEX/*T.b.g.*, positivity was expressed as the highest blood dilution that gave a positive result (end titre).
- In Batangafo, in order to slim down the study, the protocol was modified to take the results obtained at Campo into consideration. The CATT was first executed on whole blood and in case of positivity was repeated on a two-fold dilution of the blood (up to 1/16). The LATEX/*T.b.g.* was first performed on a 1/4 dilution of the blood and, if positive, on further dilutions (up to 1/16). Positivity was expressed as the highest blood dilution that gave a positive result (end titre).

Parasite detection in the blood was performed on all CATT or LATEX/*T.b.g.* seropositives by Quantitative Buffy Coat (QBC; Bailey and Smith,

1992) and by mini Anion Exchange Centrifugation Technique (mAECT; Lanham and Godfrey, 1970) in Campo and only by QBC in Batangafo. From those individuals that were seropositive without parasitological confirmation in QBC, venous blood was taken and inoculated into kit for in vitro isolation (KIVI of trypanosomes; Aerts et al., 1992).

Each person was screened for the presence of cervical lymph nodes. If positive, a lymph node aspirate was examined independently of the result in CATT or LATEX/*T.b.g.* and of parasite detection in the blood.

In order to verify whether no sleeping sickness patients occurred within the seronegative population, 153 and 80 persons were chosen at random in, respectively, Campo and Batangafo on which QBC was performed. Since it was not possible to perform polymerase chain reaction (PCR; Penchenier et al., 2000) on these persons, we consider those with a negative result in CATT, LATEX/*T.b.g.* and QBC, as true negatives.

All detected sleeping sickness cases were treated in the hospital of Campo or Batangafo by the local medical staff. First stage patients received treatment with Pentamidine; second stage patients received treatment with Melarsoprol according to the national standard protocols of each country.

3. Results

3.1. Participation to the survey

In Campo, we screened 5255 persons or 90.7% (5255/5793) of the total population. In Batangafo, 2078 persons or 73.5% (2078/2827) of the total population presented themselves.

3.2. Serological results

In Campo, 386 of the 5255 examined persons were positive or doubtful in CATT (7.3%) while 141 were positive in LATEX/*T.b.g.* at 1/4 dilution (2.7%). A cross table of the results obtained in both tests is presented in Table 1. All parasitological confirmed patients were positive in CATT and in LATEX/*T.b.g.* at 1/16 dilution.

In Batangafo, 170 of the 2078 examined persons were positive in CATT at 1/4 dilution or higher and 137 were positive in LATEX/*T.b.g.* at the same dilution (Table 2). All the sleeping sickness patients were positive in LATEX/*T.b.g.* at $\geq 1/4$ dilution but one of them was negative in CATT.

3.3. Parasitological results

In the Campo focus, 16 sleeping sickness cases were detected resulting in an observed prevalence of 0.3% (16/5255 \times 100). Fifteen, 14 and eight patients were positive in, respectively, the lymph node aspirate, the QBC and the mAECT. One patient was only positive in mAECT. The sixteenth patient was only confirmed in KIVI. He was strongly positive in CATT (score 4) and in LATEX/*T.b.g.* (1/64). The 153 persons within the seronegative control group were all negative in QBC.

In Batangafo, we detected 58 sleeping sickness with QBC of which 16 were also positive in the lymph node aspirate. One patient was only positive in the lymph node aspirate. KIVI revealed another patient who was positive in CATT (1/8) and in LATEX/*T.b.g.* (1/16) but negative in QBC and lymph node aspirate. A total of 59 patients were detected resulting in an observed prevalence of 2.8% (59/2079 \times 100). The 80 persons within the seronegative control group were all negative in QBC.

3.4. Clinical status of the patients

In Campo, all patients were in first stage of the disease (< 5 cells per μ l and no trypanosomes in the cerebrospinal fluid). In Batangafo, 33 patients (55.9%) were in first stage and 26 (44.1%) were in second stage of the disease. All patients received treatment within few days after the parasitological confirmation and the stage determination.

3.5. Sensitivity and specificity of the serological diagnostic tests

The serological results were compared to the parasitological results considering patients with any positive parasitological result as true positive

Table 1

Campo-cross table of the results obtained in CATT on whole blood (score from 0 to 4) and in LATEX/*T.b.g.* on blood dilutions (end titre); () = confirmed sleeping sickness patients

LATEX/ <i>T.b.g.</i>	CATT					Total
	0	1	2	3	4	
< 1/4	4794	69	222	27	2	5114
1/4	60	5	19	6	2	92
1/8	10	0	7	6	1	24
1/16	4	0	2 (1)	3 (1)	1 (1)	10 (3)
1/32	1	0	1	3 (3)	1 (1)	6 (4)
1/64	0	0	0	7 (7)	2 (2)	9 (9)
Total	4869	74	251 (1)	52 (11)	9 (4)	5255 (16)

cases. QBC was carried out on 233 seronegative persons (153 in Campo and 80 in Batangafo) and was negative in all cases. Taking into account these results, for specificity calculation, we consider all persons negative in CATT (whole blood, 0 or end titre < 1/1) and in LATEX/*T.b.g.* (end titre < 1/4) as true negatives.

In Campo, except one person that was negative in CATT but positive in LATEX/*T.b.g.* at 1/4 dilution and which disappeared from the study, all persons positive in at least one serological test (5255–4794 = 461), were examined by QBC. MAECT and KIVI were performed on, respectively, 70 and 31 of them. QBC was also carried out on 153 seronegative controls. The total number of persons on which parasitological examination was carried out is 614 (461+153).

In Batangafo, 11 persons who were positive in CATT or LATEX/*T.b.g.* disappeared from the study. All other persons positive in at least one

serological test (2078–1711 = 367), were examined by QBC and KIVI was carried out on 106 of them. QBC was also carried out on 80 seronegative controls. The total number of persons on which parasitological examination was carried out is 447 (367+80).

The observed sensitivity and specificity of the serological tests are calculated on the basis of the results represented in Tables 3 and 4. In Campo, where CATT was performed on whole blood, a score > 1 was considered positive (312 cases). In Batangafo, where CATT was performed on blood dilutions, an end titre $\geq 1/4$ was considered positive (352 cases). For LATEX/*T.b.g.*, two positive detection limits were retained for calculation of sensitivity and specificity: end titre $\geq 1/4$ and $\geq 1/8$.

Table 5 represents the observed sensitivity and specificity of CATT and LATEX/*T.b.g.* obtained in both foci within this study, compared to the

Table 2

Batangafo-cross-table of the results (end titres) obtained in CATT and LATEX/*T.b.g.* on blood dilutions; () = confirmed sleeping sickness patients

LATEX/ <i>T.b.g.</i>	CATT					Total
	< 1/1	1/1	1/4	1/8	1/16	
< 1/4	1711	163	44	19	4	1941
1/4	8	9	10 (2)	4 (1)	9 (3)	40 (6)
1/8	6 (1)	8	4	11 (4)	14 (10)	43 (15)
1/16	1	2	8 (1)	7 (4)	36 (33)	54 (38)
Total	1726 (1)	182	66 (3)	41 (9)	63 (46)	2078 (59)

Table 3
Observed sensitivity and specificity of CATT in Campo (whole blood) and in Batangafo (blood diluted 1/4)

CATT	Parasitology		
	Positive	Negative	Total
<i>Campo</i> ^a			
Positive	16	296	312
Negative	0	4943	4943
Total	16	5239	5255
<i>Batangafo</i> ^b			
Positive	58	294	352
Negative	1	1725	1726
Total	59	2019	2078

^a Sensitivity, 100%; specificity 94.3%; PPV, 5.1%; NPV, 100%; PPV, positive predictive value; NPV, negative predictive value.

^b Sensitivity, 98.3%; specificity, 85.4%; PPV, 16.5%; NPV, 99.9%; PPV, positive predictive value; NPV, negative predictive value.

results obtained in Equatorial Guinea (Simarro, et al., 1999) and in Côte d'Ivoire (Jamonneau et al., 2000).

4. Discussion

In general survey practice of national control teams, the CATT is performed on whole blood while the LATEX/*T.b.g.* is performed on blood dilutions. Therefore, during the first survey in Campo, the CATT was carried out on whole blood. Only during the second survey in Batangafo, the CATT was also performed on blood dilutions.

Simarro et al. (1999) has carried out his study on a population of 343 persons in the M'bini focus (Equatorial Guinea). He compared the CATT on whole blood with the LATEX/*T.b.g.* on blood dilutions (1/4 to 1/32). Like Campo, M'bini is a coastal focus but the latter is situated in a mangrove area unlike the former. The observed prevalence in M'bini is 0.2% and is similar to the observed 0.3% prevalence in Campo, which justifies the comparison between both studies. Within the study of Simarro, the sensitivity and specificity of the LATEX/*T.b.g.* were, respectively, 100 and

Table 4
Observed sensitivity and specificity of LATEX/*T.b.g.* in Campo and in Batangafo

LATEX/ <i>T.b.g.</i>	Parasitology		
	Positive	Negative	Total
<i>Campo</i> ^a			
<i>Positive detection limit 1/4</i>			
Positive	16	125	141
Negative	0	5114	5114
Total	16	5239	5255
<i>Positive detection limit 1/8</i>			
Positive	16	33	49
Negative	0	5206	5206
Total	16	5239	5255
<i>Batangafo</i> ^b			
<i>Positive detection limit 1/4</i>			
Positive	59	78	137
Negative	0	1941	1941
Total	59	2019	2078
<i>Positive detection limit 1/8</i>			
Positive	53	44	97
Negative	6	1975	1981
Total	59	2019	2078

^a Positive detection limit 1/4; sensitivity: 100%, specificity 97.6%; PPV, 11.3%; NPV, 100%; positive detection limit 1/8; sensitivity, 100%; specificity, 99.4%; PPV, 32.6%; NPV, 100%; PPV, positive predictive value; NPV, negative predictive value.

^b Positive detection limit 1/4; sensitivity, 100%; specificity, 96.1%; PPV, 43.1%; NPV, 100%; positive detection limit 1/8; sensitivity, 89.8%; specificity, 97.8%; PPV, 54.6%; NPV, 99.7%; PPV, positive predictive value; NPV, negative predictive value.

99.4% while the sensitivity and specificity of CATT on whole blood were, respectively, 87.5 and 96.7%. In our study, we obtained 100% sensitivity and 97.6% specificity for LATEX/*T.b.g.* and 100 and 94.3% for CATT. The difference between specificities observed in M'bini and Campo is small and may be explained by cross-reactions caused by non-human infective trypanosomes since in Campo, the population lives within villages among many pigs infected with *Trypanosome brucei s.l.* (Penchenier et al., 1999). In M'bini, no pigs infected with trypanosomes were observed and human activities are mainly restricted to fishery.

In Campo, our results show that, when the positive detection limit of CATT on whole blood is set at score 2 and that of LATEX/*T.b.g.* at 1/16, all

Table 5

Observed sensitivity and specificity of the serological tests in Cameroon (Campo), Central African Republic (Batangafo), Equatorial Guinea and Côte d'Ivoire

	Cameroon*	Central African Republic**	Equatorial Guinea*	Côte d'Ivoire*
<i>CATT</i>				
Sensitivity	100	98.3	87.5	Not calculated
Specificity	94.3	85.4	96.7	92.5
<i>Latex/T.b.g.</i>				
Sensitivity	100	100	100	Not calculated
Specificity	97.6	96.1	99.4	98.1

CATT: *, on whole blood; **, on blood diluted 1/4. LATEX/*T.b.g.*: on blood diluted 1/4.

confirmed patients are seropositive and the number of patients to undergo parasitological examinations is greatly reduced by a factor of 12 when screening with LATEX/*T.b.g.* (#25) compared with CATT (#312). With a positive detection limit of 1/8 for LATEX/*T.b.g.*, the workload is reduced by a factor 6 (312/49). Given the same price for both tests, the reduction in cost and workload is considerable when carrying out the LATEX/*T.b.g.* on only one serum dilution (1/8).

In Batangafo, the positive detection limit for LATEX/*T.b.g.* still including all sleeping sickness patients is 1/4. If, as for Campo, a positive detection limit of 1/8 or 1/16 was set, only 53 (89.8%) and 38 (64.4%) cases would have been detected among the 59 sleeping sickness patients. Concerning the CATT on whole blood, one of the patients was negative and would have remained undetected without performing the LATEX/*T.b.g.* All other patients were positive at 1/4 dilution. When setting the positive detection limit of both tests to 1/4 dilution, the number of persons to be examined parasitologically was 170 with CATT and 137 with LATEX/*T.b.g.* thus reducing the work load with a factor 1.2 (170/137) when using the latter test. If, as for Campo, the CATT is performed on whole blood, still missing one sleeping sickness patient, the use of LATEX/*T.b.g.* would have decreased the workload with a factor 2.5 (352/137).

It appears that LATEX/*T.b.g.*, both in Campo and in Batangafo, allows a considerable decrease in the number of parasitological examinations to be performed on seropositive cases. This observation corresponds with results obtained by [Jamon-](#)

[neau et al. \(2000\)](#) on a population of 425 non-infected persons in Côte d'Ivoire. They observed 92.5 and 98.1% specificity of, respectively, CATT on whole blood and LATEX/*T.b.g.* on 1/4 blood dilution. In addition, the reading of the agglutination in LATEX/*T.b.g.* is easier than in CATT.

Acknowledgements

This study received financial support from the Fond d'Aide à la Coopération du Ministère français des Affaires Etrangères, Secrétariat d'Etat à la Coopération et à la Francophonie. Our thanks go to the OCEAC team in Yaounde and the personnel of the hospital in Campo as well as the team of the national trypanosomiasis control programme in the Central African Republic.

References

- Aerts, D., Truc, P., Penchenier, L., Claes, Y., Le Ray, D., 1992. A kit for in vitro isolation of trypanosomes in the field: first trial with sleeping sickness patients in the Congo Republic. *Trans. R. Soc. Trop. Med. Hyg.* 86, 394–395.
- Bafort, J.M., Schutte, C.H.J., Gathiram, V., 1986. Specificity of Trestryp CATT card agglutination test in a non-sleeping sickness area of Africa. *South Afr. Med. J.* 69, 541–542.
- Bailey, J.W., Smith, D.H., 1992. The use of acridine orange QBC technique in the diagnosis of African trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.* 86, 630.
- 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.

- 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) in *Trypanosoma brucei gambiense* stocks from Cameroon. *Acta Trop.* 51, 123–134.
- Jamonneau, V., Truc, P., Garcia, A., Magnus, E., Büscher, P., 2000. 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. Consideration for mass-screening. *Acta Trop.* 76, 175–183.
- Lanham, S.M., Godfrey, D.G., 1970. Isolation of salivaria trypanosomes from man and other mammals using DEAE-cellulose. *Exp. Parasitol.* 28, 521–534.
- Magnus, E., Vervoort, T., Van Mervenne, N., 1978. A card agglutination test with stained trypanosomes (CATT) for serological diagnosis of *Trypanosoma b. gambiense* trypanosomiasis. *Ann. Soc. belge Méd. Trop.* 58, 169–176.
- Noireau, F., Lemesre, J.L., Nzoukoudi, M.Y., Louembet, M.T., Gouteux, J.P., Frézil, J.L., 1988. 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.* 82, 237–240.
- Penchenier, L., Jannin, J., Moulia-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 masse de la trypanosomiase humaine à *Trypanosoma brucei gambiense*. *Ann. Soc. belge Méd. Trop.* 71, 221–228.
- Penchenier, L., Grébaut, P., Ebo'o Eyenga, V., Bodo, J.M., Njiokou, F., Binzouli, P., Simarro, P., Soula, G., Herder, S., Laveissière, C., 1998. Le foyer de trypanosomiase humaine de Campo (Cameroun) en 1998. Aspects épidémiologiques, état de l'endémie et comparaison des CATT 13 et CATT LATEX/*T.b.gambiense* dans le dépistage de masse. *Bull. liais. Doc. OCEAC* 31, 8–19.
- Penchenier, L., Grébaut, P., Ebo'o Eyenga, V., Bodo, J.M., Njiokou, F., Binzouli, J.J., Simarro, S., Soula, G., Herder, S., Laveissière, C., 1999. Le foyer de trypanosomiase humaine de Campo (Cameroun). Historique et situation de l'endémie en 1998. *Bull. Soc. Path. exot.* 92, 185–190.
- Penchenier, L., Simo, G., Grébaut, P., Nkinin, S., Laveissière, C., Herder, S., 2000. Diagnosis of the human trypanosomiasis *Trypanosoma brucei gambiense* in central Africa by the polymerase chain reaction. *Trans. R. Soc. Trop. Med. Hyg.* 94, 392–394.
- Simarro, P.P., Franco, J.R., Ndongo, P., 1999. Field evaluation of several serological screening tests for sleeping sickness (*T. b. gambiense*). *Bull. liais. Doc. OCEAC* 32, 28–33.
- Zillman, U., Albiez, E.J., 1986. The Testtryp CATT (Card Agglutination Test for Trypanosomiasis): a field study on gambiense sleeping sickness in Liberia. *Trop. Med. Parasitol.* 37, 390–392.