

Field evaluation of the CATT/*Trypanosoma brucei gambiense* on blood-impregnated filter papers for diagnosis of human African trypanosomiasis in southern Sudan

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Summary

Most Human African Trypanosomiasis (HAT) control programmes in areas endemic for *Trypanosoma brucei gambiense* rely on a strategy of active mass screening with the Card Agglutination Test for Trypanosomiasis (CATT)/*T. b. gambiense*. We evaluated the performance, stability and reproducibility of the CATT/*T. b. gambiense* on blood-impregnated filter papers (CATT-FP) in Kajo-Keji County, South-Sudan, where some areas are inaccessible to mobile teams. The CATT-FP was performed with a group of 100 people with a positive CATT on whole blood including 17 confirmed HAT patients and the results were compared with the CATT on plasma (CATT-P). The CATT-FP was repeated on impregnated filter papers stored at ambient and refrigerated temperature for 1, 3, 7 and 14 days. Another 82 patients with HAT, including 78 with a positive parasitology, were tested with the CATT-FP and duplicate filter paper samples were sent to a reference laboratory to assess reproducibility. The CATT-FP was positive in 90 of 99 patients with HAT (sensitivity: 91%). It was less sensitive than the CATT-P (mean dilution difference: –2.5). There was no significant loss of sensitivity after storage for up to 14 days both at ambient and cool temperature. Reproducibility of the CATT-FP was found to be excellent (kappa: 0.84). The CATT-FP can therefore be recommended as a screening test for HAT in areas where the use of CATT-P is not possible. Further studies on larger population samples in different endemic foci are still needed before the CATT-FP can be recommended for universal use.

keywords African trypanosomiasis, diagnosis, CATT, filter paper, Sudan

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Introduction

Every year an estimated 300 000 people in sub-Saharan Africa are infected with *Trypanosoma brucei gambiense*, the agent of a disease which is fatal if left untreated. Only 5–10% of the 60 million people at risk are under surveillance (WHO 1998). During the last decades there has been a strong resurgence of Human African Trypanosomiasis (HAT) in several countries such as Angola (Stanghellini & Josenando 2001), Sudan (Moore & Richer 2001) and the Democratic Republic of Congo (Van Nieuwenhove *et al.* 2001). According to these authors and to Ekwanzala *et al.* (1996), the major cause of this resurgence is the collapse of sleeping sickness control programmes in these countries.

Control programmes for HAT rely on mass population screening with subsequent treatment of all infected individuals, in some areas combined with vector control (Stanghellini 1999). The Card Agglutination Test for Trypanosomiasis (CATT/*T. b. gambiense*), a quick and easy to perform, highly sensitive serological test, is the method of choice for both individual and mass screening (Magnus *et al.* 1978). In order to cover a high percentage of the population at risk in a given endemic area, an active control strategy by mobile teams is usually implemented.

In June 2000, Médecins Sans Frontières (MSF) initiated a sleeping sickness control programme in Kajo-Keji County, southern Sudan. It was estimated that about 5% of the 100 000 inhabitants of this county were infected with *T. b. gambiense*. During the first 10 months of the project,

about 13 000 people were passively screened and 900 diagnosed and treated as sleeping sickness cases. In April 2001, active screening activities were started in the county but, because of geographical and security constraints, some

parts of the county remained inaccessible by road to mobile teams. In such a situation, an alternative strategy would be to collect blood samples on filter paper in the field by Community Health Workers. The samples could then be

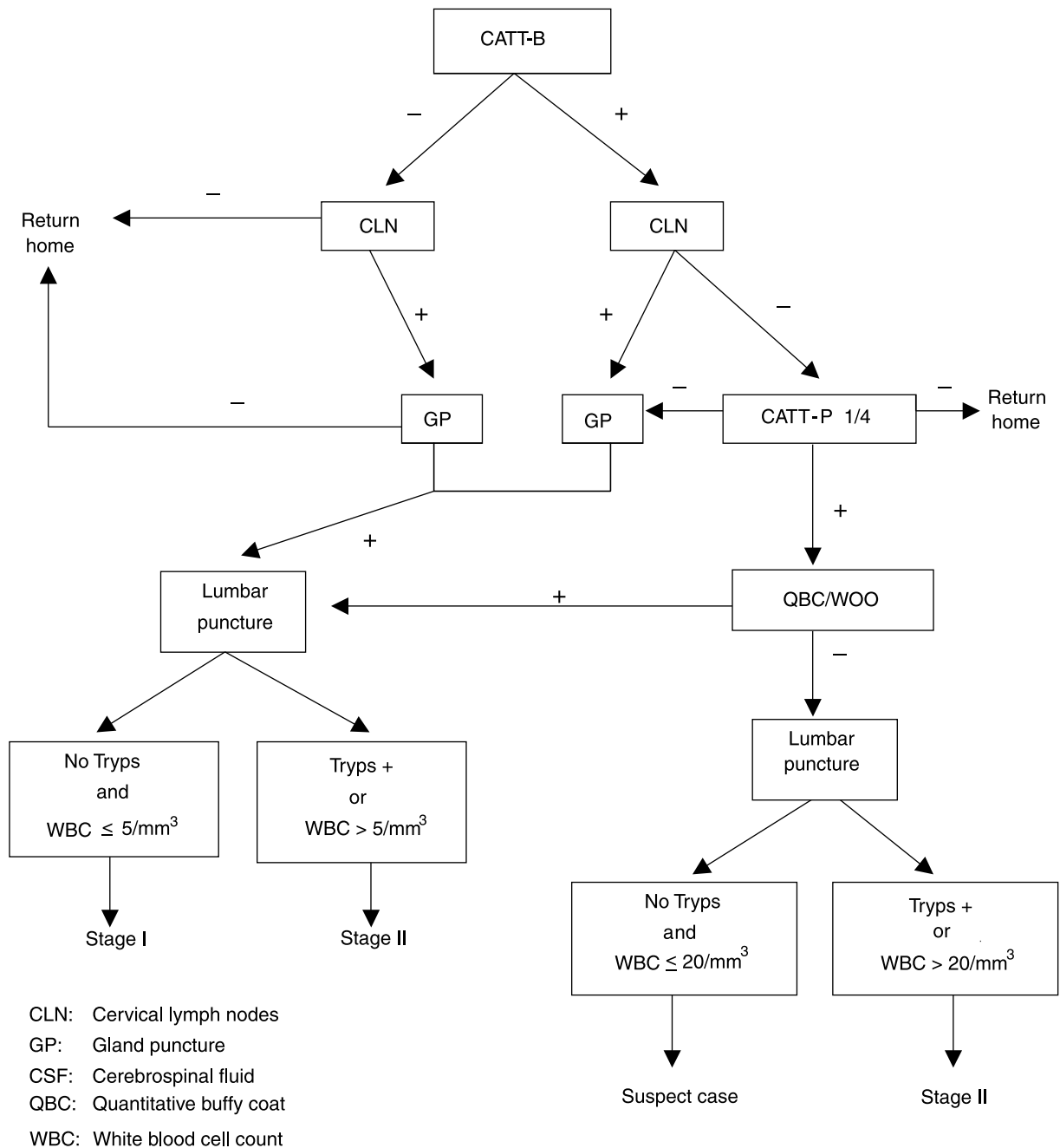


Figure 1 Protocol for diagnosis and stage determination used by the sleeping sickness control programme in Kiri, Southern Sudan.

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brought to the treatment centre for execution of the CATT on eluates prepared from the blood-impregnated filter papers. Seropositive individuals could be referred to the centre for completion of the diagnostic procedures and, if required, treatment.

A first version of the CATT/*T. b. gambiense* performed on blood-impregnated on filter papers (microCATT) has been developed by Miézan *et al.* (1991). These authors showed that among 2083 people tested in Côte d'Ivoire, all 72 parasitologically proven HAT patients were detected by this microtechnique. In a group of 52 parasitologically proven cases of sleeping sickness and 118 uninfected endemic controls in Congo, Noireau *et al.* (1991) found a sensitivity of 94.2% and a specificity of 100% of the CATT on impregnated filter papers. The microCATT, further evaluated in Côte d'Ivoire and the Central African Republic, also revealed high sensitivity and specificity provided the tests were performed on the day of blood sampling. However, when the test was repeated after storage of the blood-impregnated filter papers for 3 and 7 days at ambient temperature, a dramatic decrease in sensitivity was observed (P. Truc, personal communication). Moreover, because of the minute volumes of antigen and test sample used, a major constraint of the microCATT is reading and interpretation of the agglutination patterns (E. Magnus, personal communication).

Recently, a modified version of the CATT/*T. b. gambiense* designed for testing eluates from blood-impregnated filter papers (CATT-FP) has been developed in the Department of Parasitology of the Prince Leopold Institute of Tropical Medicine (ITMA) in Antwerp and showed promising results. Here we describe the first field evaluation of the CATT-FP performed in the laboratory of the sleeping sickness treatment centre (SSTC) of Kajo-Keji County in southern Sudan. In this study, we evaluated the sensitivity of the CATT-FP and compared it with the CATT on plasma (CATT-P). We also studied the stability and the reproducibility of the CATT-FP.

Materials and methods

The study was conducted in January and May 2001 at the Kiri SSTC, an MSF-Switzerland-run 60-bed hospital located in Kajo-Keji County, southern Sudan. Patients with either trypanosomes in the blood (quantitative buffy coat (QBC) or Woo test), lymph or cerebrospinal fluid (CSF) or patients with a positive CATT on plasma (titre $\geq 1/4$) and a CSF white blood cell (WBC) count of $>20/\text{mm}^3$ were considered as HAT cases and treated according to stage of illness (Figure 1). Individuals with a positive CATT on plasma (titre $\geq 1/4$), with no

trypanosomes in the blood, lymph or CSF and a CSF WBC count of $\leq 20/\text{mm}^3$ were considered as suspect cases and controlled again at 3, 6 and 12 months. HAT was excluded in individuals with negative microscopic examinations if the CATT on whole blood and on plasma (titre = $1/4$) was negative. The study was divided into two phases.

Phase 1

In the first 2 weeks of January 2001, all people found to be positive with CATT on whole blood (CATT-B) were included in the study after informed consent was given. Blood was collected for routine diagnosis and, for the purpose of this study, an extra 4.5 ml of heparinized blood was taken. A total of nine filter papers (Whatman no. 4) were immediately impregnated until the blood spot reached a diameter of 3 cm on both sides of the filter paper. The remaining blood was centrifuged and the plasma was stored in a refrigerator. The filter papers were allowed to dry at room temperature without exposure to direct sunlight for a minimum of 1 h. The CATT-FP was performed on the same day (day 0) from one of the blood-impregnated filter papers. All remaining filter papers were packed in sealed plastic bags and silica gel was added as a desiccant. A set of four filter papers was kept at ambient temperature in the laboratory at 25–34 °C while another set of four filter papers was stored in the refrigerator at 2–10 °C. These two sets of filter papers were tested with the CATT-FP on days 1, 3, 7 and 14. In order to compare the results obtained with CATT-P with those of CATT-FP, twofold plasma dilutions were prepared, tested and end-titres determined. Thirty people with a negative CATT-B, all selected from the hospital staff, were entered as controls.

Table 1 Sensitivity of the CATT-FP on day 0 for 100 individuals with a positive CATT on whole blood

CATT-FP end-titre	No trypanosomes detected (N)	Parasitologically proven cases (N)	Sensitivity (%)	95% CI (%)
Negative	48	–		
1/1	16	2	100	(80–100)
1/2	8	–	88	(62–95)
1/4	3	9	88	(62–95)
1/8	4	4	35	(15–56)
1/16	1	2	12	(2–31)
1/32	3	–	0	(0–80)

Phase 2

In May 2001, on day 0, all patients with HAT diagnosed were tested by CATT-FP and the CATT-P at the Kiri SSTC laboratory. To assess test reproducibility, a duplicate impregnated filter paper was kept at ambient temperature in sealed plastic bags with Silicagel and sent to the Department of Parasitology of ITMA where the CATT-FP was repeated 4–8 weeks later.

Preparation of blood eluates

Blood proteins were eluted from the filter papers in flat bottom microplates (Greiner, 96 wells) as follows: 150 µl of CATT buffer were pipetted into a well of a microplate and nine discs of 6 mm diameter, punched out of the dried blood spot on the filter paper, placed into the buffer. The microplate was covered in order to avoid evaporation, incubated at ambient temperature for 1 h and gently shaken for 15 s after 15, 30, 45 and 60 min, respectively.

CATT on filter paper eluate

One vial of CATT antigen was reconstituted with 1.25 ml of CATT buffer (double concentration compared with the usual CATT procedure). Using an automatic pipette, 30 µl of the eluate were put into a circle of a CATT test card, 25 µl of the CATT antigen (half the volume compared with the usual CATT procedure) were added and mixed with the eluate by means of a stirring rod. The test card was rocked for 5 min at 60 r.p.m. on a flat bed rotator (ITMAS type B2). Agglutination patterns were scored as negative (–), doubtful (+/–), or positive (+, ++ and +++). Samples with a doubtful agglutination score were considered positive. If the CATT-FP with undiluted eluate was positive, serial twofold dilutions of the eluate (1/2 to 1/32) in CATT buffer were prepared, tested and end-titres determined.

CATT on plasma samples

One vial of CATT-antigen was reconstituted with 2.5 ml of CATT buffer. Serial twofold dilutions (from undiluted eluate up to 1/32) were prepared in CATT buffer. Twenty-five microlitres of each dilution and one drop (about 50 µl) of antigen suspension were dispensed onto the reaction areas of a test card. The mixtures were spread out by means of a stirring rod and allowed to react on the card test rotator at 60 r.p.m. for 5 min. Agglutination patterns were scored as described for CATT-FP.

Statistical analysis

To test the diagnostic performance of the CATT-FP, we calculated its sensitivity to detect HAT cases for each dilution, as well as the corresponding 95% confidence intervals. The threshold was chosen for the highest test sensitivity. The stability of the filter paper eluates was tested by comparing changes in the sensitivity of detecting parasitologically proven HAT cases over time and under different storage conditions (ambient *vs.* low temperature). Differences were tested using the chi-square test. As a measure of agreement, we used the Spearman correlation coefficient because the dilution titres were not normally distributed. Reproducibility of the test was assessed by comparing the test visual interpretation (–, ±, +, ++ or +++) done in the field (Kiri SSTC) with the same interpretation done in a reference laboratory centre (Department of Parasitology, ITMA) and the mean dilution difference. We calculated Cohen's Kappa coefficient for negative (–) *vs.* positive results (±, +, ++ or +++)). All statistical tests were two-tailed, with a significance level of 0.05.

Results

Sensitivity

Of 404 people screened, 100 tested CATT-B positive during the first phase of the study, including 17 (17%) patients diagnosed with HAT: six stage 1 patients (35%) and 11 stage 2 patients (65%). All 17 HAT cases were parasitologically proven and had a positive CATT-FP. A rapid loss in sensitivity occurred with progressive dilution of the eluate (Table 1). During the second phase of the study, 82 patients were diagnosed with HAT. Infection was parasitologically confirmed in 78 patients while four individuals had more than 20 WBC/mm³ in the CSF and a positive CATT-P (dilution ≥1/4). Thirty-two patients were in stage 1 and 50 were in stage 2. The CATT-FP was positive in 73 of the 82 patients. The test results were unclear (+/–) for six patients, 1+ in 16 patients, 2+ in 31 patients and 3+ in 20 patients. Overall, 90 of the 99 patients diagnosed with HAT had a CATT-FP positive when undiluted eluates were tested. Therefore, the sensitivity of the CATT-FP was 91%. When only the 95 parasitologically proven HAT patients were considered, the sensitivity of the CATT-FP was 94%.

In the 83 people with no parasitological evidence of HAT, 60 were CATT-P positive at a dilution of 1/4 or higher and were considered as suspect cases. Follow-up at 3 or 6 months intervals was available for only eight of these 60 suspect cases, and in three of them trypanosomes were found during follow-up. Interestingly these three

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patients, with initial CATT-P end-titres of 1/16 (two patients) and 1/32 (one patient), initially tested CATT-FP positive (end-titres = 1/2).

Agreement between tests

The results of the end-dilution of CATT-FP and CATT-P are presented in Table 2. Both tests were significantly correlated (Spearman correlation coefficient: 0.70, $P < 0.001$). When compared with the CATT-P, the CATT-FP was less sensitive with a mean difference of -2.51 dilution. All 30 people with negative CATT-B entered as controls had negative CATT-FP.

Stability

The sensitivity of CATT-FP was not significantly affected by the different storage conditions (Table 3). CATT-FP

Table 2 Agreement between CATT-FP and CATT-P in 100 individuals

CATT-FP end-titre (N)	CATT-P end-titre (N)					
	1/1	1/2	1/4	1/8	1/16	1/32
Negative	3	18	17	7	1	2
1/1	–	2	6	4	3	3
1/2	–	–	1	2	4	1
1/4	–	–	–	1	1	10
1/8	–	–	1	–	–	7
1/16	–	–	–	–	1	2
1/32	–	–	–	3	–	–

Spearman correlation coefficient: 0.7, $P < 0.001$.

Table 3 Results of the CATT-FP (dilution 1/1–1/32) on blood-impregnated filter papers stored at ambient or low temperature during 1, 3, 7 and 14 days

CATT-FP end-titre	Stored at room temperature				Stored in refrigerator			
	Day 1	Day 3	Day 7	Day 14*	Day 1	Day 3	Day 7	Day 14
Negative	57	50	62	60	53	51	52	56
1/1 (undiluted)	13	18	8	16	18	17	20	17
1/2	10	12	15	10	10	11	8	10
1/4	8	12	9	6	9	10	9	5
1/8	6	5	4	6	6	7	9	11
1/16	4	3	2	1	4	3	1	1
1/32	2	–	–	–	–	1	1	–
Threshold 1/1								
Sensitivity† (%)	88	100	94	100	88	100	100	94
P-value‡ (<i>vs.</i> day 0)	0.14	1.0	0.31	1.0	0.14	1.0	1.0	0.31

* One missing data. † Number of confirmed HAT patients with a positive CATT-FP (dilution $\geq 1/1$) on day X divided by number of confirmed HAT patients. ‡ Chi-square test.

retains its sensitivity in all conditions, even when stored for 14 days at room temperature. We observed only a slight decrease in sensitivity after 1 day, in both storage conditions.

Reproducibility

When performed in the reference laboratory at ITMA, the CATT-FP was positive in 70 of 82 patients (sensitivity: 85%) compared with 73 of 82 patients (sensitivity: 89%) when performed in the field (Table 4). Reproducibility was excellent when a threshold for any positive or doubtful CATT-FP was used (Kappa coefficient: 0.84).

Discussion

The combined results of the two phases of this study show that 90 of 99 patients diagnosed with *T. b. gambiense* infection tested positive in CATT-FP with undiluted eluate performed on day 0 (sensitivity: 91%). When only the 95 parasitologically proven HAT patients were considered, the sensitivity of the CATT-FP increased to 94%. Diluting

Table 4 Concordance between CATT-FP performed in the field (Kiri, South Sudan) and in the reference laboratory (ITMA – Belgium) on 82 patients with African trypanosomiasis

		ITMA		
		Negative	Doubtful	Positive
Field	Negative	9	0	0
	Doubtful	3	1	2
	Positive	0	2	65

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the eluate decreased the sensitivity of the CATT-FP. Stability of the blood impregnated filter papers was excellent when stored at ambient temperature (25–34 °C) or in the refrigerator (2–10 °C) for up to 2 weeks. Data on humidity levels were not available.

Previous studies using microCATT (Miézan *et al.* 1991; Noireau *et al.* 1991) already demonstrated a very good sensitivity of the CATT when performed on day 0 from blood impregnated filter paper. The major constraint for widespread use of the microCATT was the rapid decrease in sensitivity when filter papers were stored for several days as recently observed by Truc *et al.* (personal communication). In addition, the use of minute volumes of antigen and test sample make the reading and interpretation of agglutination difficult. In our study, the excellent stability of the blood-impregnated filter papers could be because of the strict dry storage condition of the filter papers.

However, the lack of a gold standard for the diagnosis of HAT, related to the limited sensitivity of the standard trypanosome detection techniques, impairs the evaluation of the true performance of all serodiagnostic tests, including that of the CATT-FP. Consequently, the sensitivity of the CATT-FP might have been overestimated in the present study. Interestingly though, the three serological suspects who became parasitologically positive during the 12-month follow-up had an initial positive CATT-FP. The use of statistical methods such as the Latent Class Analysis, correcting for the absence of gold standard, proved to be useful in evaluating the performance of diagnostic tests for visceral leishmaniasis (Boelaert *et al.* 1999) and could be used in future diagnostic studies on HAT.

Reproducibility of the CATT-FP was also found to be excellent (κ : 0.84) despite the fact that up to 8 weeks elapsed between the collection of filter papers in Sudan and their testing in the reference laboratory in Antwerp. Three patients were found negative in Antwerp but gave doubtful readings, interpreted as positive, in Kiri. The few observed discrepant results could be because of slightly different techniques of elution of the filter papers (three filter paper discs and 50 μ l of CATT buffer in Antwerp *vs.* nine filter paper discs and 150 μ l of CATT buffer in Kiri) or different interpretations of agglutination patterns by laboratory technicians.

When the end titres obtained with CATT-FP were compared with those of CATT-P, there was a mean difference in end titre of 2.5 dilution. The result of the CATT-FP on undiluted eluate thus corresponds with a mean CATT-P end-dilution between 1/4 and 1/8. A similar concordance was found by Noireau *et al.* (1991) in Congo.

The design of our study did not allow for an adequate evaluation of specificity and positive predictive value (PPV)

of the CATT-FP. In the future it would be interesting to conduct a cohort study on parasitologically negative and CATT-FP positive individuals to identify relevant risk factors associated with disease during follow-up. The PPV of a positive CATT-FP in individuals with a high pretest probability could be sufficiently high to allow for treatment of unconfirmed seropositives, as discussed by Simarro *et al.* (1999) for Angolese individuals with a CATT-P >1/8 in an area of high disease prevalence. Treating parasitologically unconfirmed highly suspect patients with pentamidine could be particularly beneficial in regions like Kajo-Keji County, where follow-up of suspect patients is rarely possible.

The CATT-FP can be used for screening population for HAT when the use of more sensitive tests such as the CATT-B is not possible. Before it can be recommended for routine use, its positive and negative predictive values should be evaluated on larger population samples and in different endemic foci and their values should be sufficiently high compared with the CATT-B or other diagnostic assays.

References

- Boelaert M, El Safi S, Goetghebeur E, Gomes-Pereira S, Le Ray D & Van der Stuyft P (1999) Latent class analysis permits unbiased estimates of the validity of DAT for the diagnosis of visceral leishmaniasis. *Tropical Medicine and International Health* **4**, 395–401.
- Ekwanzala M, Pépin J, Khonde N, Molisho S, Bruneel H & De Wals P (1996) In the heart of darkness: sleeping sickness in Zaire. *Lancet* **348**, 1427–1430.
- Magnus E, Vervoort T & Van Meirvenne N (1978) A card-agglutination test with stained trypanosomes (C.A.T.T.) for the serological diagnosis of *T.b.gambiense* trypanosomiasis. *Annales de la Société Belge de Médecine Tropicale* **58**, 169–176.
- Miézan 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. *Bulletin de l'Organisation Mondiale de la Santé* **69**, 603–606.
- Moore A & Richer M (2001) Re-emergence of epidemic sleeping sickness in southern Sudan. *Tropical Medicine and International Health* **6**, 342–347.
- Noireau F, Force-Barge P & Cattand P (1991) Evaluation of Testryp CATT applied to samples of dried blood for the diagnosis of sleeping sickness. *Bulletin of the World Health Organization* **69**, 607–608.
- Simarro PP, Ruiz JA, Franco JR & Josenando T (1999) Attitude towards CATT-positive individuals without parasitological confirmation in the African Trypanosomiasis (*T.B. gambiense*) focus of Quiçama (Angola). *Tropical Medicine and International Health* **4**, 858–961.

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Stanghellini A (1999) Prophylactic strategies in human African Trypanosomiasis. In: *Progress in Human African Trypanosomiasis, Sleeping Sickness* (eds M. Dumas, B. Bouteille, A. Buguet). Springer-Verlag, Berlin, pp. 301-313.

Stanghellini A & Josenando T (2001) The situation of sleeping sickness in Angola: a calamity. *Tropical Medicine and International Health* 6, 330-334.

Van Nieuwenhove S, Kande Betu-Ku-Mesu V, Mansinsa Diabakana P, Declercq J & Miaka Mia Bilenge C (2001) Sleeping sickness resurgence in the DRC: the last decade. *Tropical Medicine and International Health* 6, 335-341.

World Health Organization (1998) Control and surveillance of African trypanosomiasis. *World Health Organisation Technical Report Series* 881.