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Evaluation of an EDTA version of CATT/*Trypanosoma brucei gambiense* for serological screening of human blood samples

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Abstract

CATT/*Trypanosoma brucei gambiense*, a direct card agglutination test designed for field surveys on human African trypanosomosis, is currently used with freshly collected heparinized blood samples. When testing serum samples, it has been observed earlier that, at lower sample dilutions, a complement-mediated inhibition phenomenon may cause false negative test results. This can be avoided by adding an anticomplementary agent such as di-sodium ethylenediaminetetraacetate dihydrate (EDTA) to the reaction. As the sensitivity of the blood assay might be improved in the same way, this possibility has been examined under both laboratory and field conditions, by adding EDTA to the test buffer or, as an anticoagulant, to the blood samples. The CATT-EDTA versions proved up to 7% more sensitive but also 1–2% less specific than the current test. CATT buffer supplemented with EDTA remained stable for at least 2 years at +45 °C.

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

1. Introduction

In field surveys on gambiense sleeping sickness, a direct card agglutination test, CATT/*Trypanosoma brucei* (*T.b.*) *gambiense* (Magnus et al., 1978), is currently used as a serological screening test for detection of anti-trypanosome antibodies. The freeze dried CATT-antigen consists of DEAE-column purified, formaldehyde-fixed, Coomassie Blue stained clone trypanosomes of *T.b. gambiense* Variable Antigen Type LiTat 1.3 (Van Meirvenne et al., 1995). Usually the test is performed on a drop (about 25 μl) of undiluted

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fresh blood, collected in heparinized capillary tubes. Under these conditions, the test sensitivity might be adversely affected by a complement-mediated inhibition phenomenon, similar to the one observed with *Brucella* and *Leptospira* direct agglutination tests (Cho and Ingram, 1971; Malkin, 1984) as well as with immune precipitation reactions (Schifferli et al., 1982; O'Sullivan et al., 1988; Baatrup, 1989).

For CATT/*T.b. gambiense* with serum samples, a prozone phenomenon has already been reported by Pansaerts et al. (1998). The same authors observed that this prozone can be avoided by adding the Ca^{2+}/Mg^{2+} chelating agent di-sodium ethylenediaminetetraacetate dihydrate (EDTA) to the reaction mixture.

In the present study, the effect of adding EDTA to the CATT/*T.b. gambiense* with fresh, undiluted human blood samples has been examined. Different test versions of CATT/*T.b. gambiense* have been evaluated both in the laboratory and during sleeping sickness field surveys in Uganda, the République Démocratique du Congo, Guinea Equatorial and Côte d'Ivoire. EDTA was either incorporated into the test buffer or used as an anticoagulant for blood sampling.

2. Materials and methods

2.1. Blood samples

Four groups of human blood samples were included in the study. Group 1 was of mixed ethnic origin. Groups 2–4 were collected during field surveys on human African trypanosomosis. Data on age and sex of the donors were not available. Details are given in Table 1.

Group 1 consisted of venous blood samples collected in Monovette syringes (Sarstedt) containing EDTA (1.5 mg/ml blood) as anticoagulant, from 358 individuals at the clinic of the Institute of Tropical Medicine in Antwerp. This is a randomly collected, non trypanosomosis control group.

Group 2 consisted of blood samples collected in heparin-coated capillary tubes from a total of 1969 individuals examined during field surveys in Northern Uganda ($n = 202$), Guinea Equatorial ($n = 329$) and the République Démocratique du Congo ($n = 1438$). This group included a total of 115 parasitologically positive trypanosomosis (T +) patients.

Group 3 consisted of paired blood samples. One series was collected in heparin-coated and simultaneously, another series in EDTA-coated capillary tubes, from 239 individuals examined during a field survey in Côte d'Ivoire. This group included 12 T + patients.

Group 4 consisted of paired blood samples. One series was collected in heparin-coated and simultaneously, another series in EDTA-coated capillary tubes, from 100 individuals examined during a field survey in Guinea Equatorial. This group included 11 T + patients.

All T + patients of Groups 2–4 had been classified as 'new cases'. Trypanosomes had been detected by current parasitological methods (WHO, 1983), i.e. microscopic examination of lymph node aspirate, blood (wet blood film, micro-Haematocrit Centrifugation Technique (m-HCT), mini-Anion Exchange Centrifugation Technique (m-AECT)) or cerebrospinal fluid.

Individuals of Groups 2–4 classified as putative trypanosomosis negative (T −), showed no clini-

Table 1
History of the blood samples of Groups 1–4

	Ethnic origin	Collected in	Endemic focus	Year of sampling	Number of samples
Group 1	Mixed	Belgium	–	1998	358
Group 2	African	Uganda	Adjumani	1995	202
		Guinea Eq.	Mbini	1996	329
		R.D. Congo	Bandundu	1997	1438
Group 3	African	Côte d'Ivoire	Aboisso	1997	239
Group 4	African	Guinea Eq.	Bata, Mbini, Luba, Kogo	1997	100

cal signs of sleeping sickness and tested negative in trypanosome detection assays.

2.2. CATT procedure

All blood samples were tested immediately after collection. Tests were done in a blinded manner, without knowledge of the parasitological status of the test persons.

Two different antigen preparations were used, (i) the reference 'PBS-version', obtained by reconstitution of the freeze-dried antigen reagent with 2.5 ml CATT-buffer (PBS, pH 7.2) per vial; and (ii) the experimental 'EDTA-version', involving reconstitution of the reagent with 2.5 ml CATT-buffer containing 10 mM EDTA (Janssen Chimica). In both test versions, 25 μl of the undiluted blood samples (one drop from the capillary tube) and about 50 μl of CATT-reagent (one drop from the antigen dropping vial) were dispensed onto the reaction areas of a plastified test card. The mixtures were spread out by means of a stirring rod and rocked on a Card Test Rotator at 60 rpm for 5 min. The agglutination patterns were scored as '0' (negative), '1' (weakly positive), '2' and '3' (positive) or '4' (strongly positive). Based on previous studies, the cut-off was defined as '1', i.e. samples with agglutination patterns of '1' were considered positive.

2.3. Stability of the EDTA buffer

Stability of the EDTA buffer was assessed in the laboratory by comparative titration of a set of positive and negative reference sera, using, respectively, fresh PBS or fresh and stored (+ 45 °C, 2 years) EDTA buffer for serum dilutions (1:2–1:128) and for reconstitution of the CATT reagent.

2.4. Statistical methods

Test sensitivity and specificity estimates with exact confidence intervals (C.I. 95%) were calculated by standard methods (Clopper and Pearson, 1934) and values compared using the McNemar's χ^2-test at 1 degree of freedom with continuity correction (Pagano and Gauvreau, 2000).

Table 2
Results of CATT-PBS and CATT-EDTA obtained with blood samples of Group 2 (field surveys) collected in heparin-coated capillary tubes

	CATT-PBS+	CATT-PBS−
T+ (n = 115)[a]		
CATT-EDTA+	104	8
CATT-EDTA−	0	3
T− (n = 1854)[b]		
CATT-EDTA+	64	21
CATT-EDTA−	0	1769

T+, parasitologically confirmed trypanosomosis.

[a] Sensitivity (95% C.I.), CATT-PBS = 90.4% (83.5–95.1%); CATT-EDTA = 97.4% (92.6–99.5%). McNemar's $\chi^2 = 6.13$; $P = 0.01$.

[b] Specificity (95% C.I.), CATT-PBS = 96.5% (95.6–97.3%); CATT-EDTA = 95.4% (94.4–96.3%). McNemar's $\chi^2 = 19.05$; $P = < 0.001$.

3. Results

3.1. Group 1 (non trypanosomosis controls)

The 358 fresh EDTA blood samples were tested with the reference CATT-PBS version. Eight samples were positive, thus yielding an observed test specificity of 97.8% (95% CI, 95.6–99.0%; results not shown).

3.2. Group 2 (field surveys)

The 1969 heparinized blood samples of this group were comparatively tested with both the PBS-version and the EDTA-version of CATT. The results are shown in Table 2. With CATT-EDTA, the observed overall sensitivity [97.4% (95% CI, 92.6–99.5%)] increased by 7.0% compared with CATT-PBS [90.4% (95% CI, 83.5–95.1%)]. However, the specificity of this test version [95.4% (95% CI, 94.4–96.3%)] decreased by 1.1% compared with CATT-PBS [96.5% (95% CI, 95.6–97.3%)]. The CATT-EDTA was significantly more sensitive (McNemar's $\chi^2 = 6.13$; $P = 0.01$) and significantly less specific (McNemar's $\chi^2 = 19.05$; $P = < 0.001$) than the CATT-PBS.

3.3. Group 3 (field survey)

The blood samples of Group 3, collected in both heparin- and EDTA-coated capillary tubes, were simultaneously tested with CATT-PBS (Table 3). With the EDTA samples, the observed test specificity [88.5% (95% CI, 83.7–92.4%)] decreased by 1.8% compared with the heparin samples [90.3% (95% CI, 85.7–93.8%)]. However, there was no significant difference in specificity between the two test versions (McNemar's $\chi^2 = 2.25$; $P = 0.13$). Both test versions detected all 12 trypanosome infected patients [sensitivity = 100% (95% CI, 73.5–100%)].

3.4. Group 4 (field survey)

The 100 blood samples of Group 4, collected in heparin-coated capillary tubes were tested with CATT-EDTA and, simultaneously, those collected in EDTA-coated capillary tubes were tested with CATT-PBS. Results are shown in Table 4. Eleven patients with parasitologically confirmed trypanosome infection and 89 putative T− individuals were tested. The combination of EDTA-coated capillary tubes with CATT-PBS [sensitivity = 90.9% (95% CI, 58.7–99.8%)] rendered the test 9.1% more sensitive than heparin-coated capillary tubes tested with CATT-EDTA [sensitivity = 81.8% (95% CI, 48.2–97.7%)]. This difference however was not significant (McNemar's $\chi^2 = 0$; $P = 1.00$). When EDTA-coated capillary tubes were tested with CATT-PBS, specificity [95.5% (95% CI, 88.9–98.8%)] was 2.3% lower than that of heparin-coated capillaries tested with CATT-EDTA [97.8% (95% CI, 92.1–99.7%)]. Again, this difference was not significant (McNemar's $\chi^2 = 0.50$; $P = 0.48$).

Table 3
Results of CATT-PBS obtained with blood samples of Group 3 (field survey) collected in heparin-coated (Hep cap) or EDTA-coated (EDTA cap) capillary tubes

	Hep cap+	Hep cap−
T+ (n = 12)[a]		
EDTA cap+	12	0
EDTA cap−	0	0
T− (n = 227)[b]		
EDTA cap+	22	4
EDTA cap−	0	201

T+, parasitologically confirmed trypanosomosis.
[a] Sensitivity (95% C.I.), Hep cap = 100% (73.5–100%); EDTA cap = 100% (73.5–100%).
[b] Specificity (95% C.I.), Hep cap = 90.3% (85.7–93.8%); EDTA cap = 88.5% (83.7–92.4%). McNemar's $\chi^2 = 2.25$; $P = 0.13$.

Table 4
Results of CATT-PBS and CATT-EDTA obtained with blood samples of Group 4 (field survey) collected in EDTA-coated (EDTA cap) and heparin-coated (Hep cap) capillary tubes

		EDTA cap	
		CATT-PBS+	CATT-PBS−
T+ (n = 11)[a]			
Hep cap	CATT-EDTA+	9	0
	CATT-EDTA−	1	1
T− (n = 89)[b]			
Hep cap	CATT-EDTA+	2	0
	CATT-EDTA−	2	85

T+, parasitologically confirmed trypanosomosis.
[a] Sensitivity (95% C.I.), CATT-PBS = 90.9% (58.7–99.8%); CATT-EDTA = 81.8% (48.2–97.7%). McNemar's $\chi^2 = 0$; $P = 1.00$.
[b] Specificity (95% C.I.), CATT-PBS = 95.5% (88.9–98.8%); CATT-EDTA = 97.8% (92.1–99.7%). McNemar's $\chi^2 = 0.50$; $P = 0.48$.

3.5. Stability of EDTA-buffer

The end titres of 4 T+ and 3 T− reference serum samples obtained in association with the fresh and stored EDTA buffers were similar (with only a single difference of one dilution) to those with fresh PBS (Table 5). Both fresh and stored EDTA buffer abolished the prozone phenomenon of the T+ sera to the same extent.

Table 5
CATT end titres (reciprocal) of four positive (T+) and three negative (T−) reference serum samples using respectively fresh PBS or fresh and stored (+45 °C, 2 years) EDTA buffer for serum dilutions and for reconstitution of the CATT antigen

Reference serum	CATT-PBS	CATT-EDTA	
	PBS buffer fresh	EDTA buffer fresh	EDTA buffer 2 years/+45 °C
1 (T+)	32 (4)	32 (0)	32 (0)
2 (T+)	32 (2)	32 (0)	32 (0)
3 (T+)	>64 (2)	>64 (0)	>64 (0)
4 (T+)	32 (2)	32 (0)	16 (0)
5 (T−)	0	0	0
6 (T−)	0	0	0
7 (T−)	0	0	0

Between brackets, highest serum dilution still showing prozone effect. (0) = no prozone.

4. Discussion

In Group 2, incorporation of EDTA as an additive to the buffer component into the CATT–*T.b. gambiense* test for screening of blood samples collected in heparin-coated capillary tubes, led to an increase in test sensitivity of up to 7%. However, in Groups 2 and 3, the specificity of CATT-EDTA was 1–2% lower compared with the classical CATT-PBS. Only in Group 2 significant differences in sensitivity ($P = 0.01$) and in specificity ($P = < 0.001$) between the two test versions were observed.

The positive CATT-results obtained with the putative T− individuals of Groups 1–4 could be explained by the fact that trypanosomes may have been missed due to the limited sensitivity of the parasitological tests used, by cross-reactions with antibodies against other parasites or by unknown aspecific reactions. As a consequence of the limited sensitivity of trypanosome detection tests, the given percentages of test specificity should be considered as minimal values.

At first sight, the system using EDTA-coated capillaries seems quite attractive. In Group 4 it yielded the highest sensitivity, albeit on a small number of samples. From a practical point of view however, field use of EDTA capillary tubes is not indicated. Test results might be influenced by the variable concentration of EDTA in the blood sample, depending on the volume of blood taken up into the capillary tube. This does not apply to the heparinized capillary tubes as heparin has no influence on complement activity. Moreover, during sampling in the field, a problem of blood coagulation in the capillary tubes was more frequently observed with EDTA-coated than with heparin-coated capillary tubes.

The test version with EDTA incorporated into the CATT-buffer seems easier to standardize. It was evaluated in four different settings on a relatively large number of samples in Groups 2 and 4 (126 T+ and 1943 T−) and yielded satisfactory results. Stability experiments suggest that CATT-EDTA buffer can be stored for at least 2 years at 45 °C, making it suitable for field use.

In conclusion, depending on the expected prevalence rate of the infection and on the positive predictive value pursued, CATT-screening on undiluted blood can be done either with the classical, more specific, CATT-PBS or with the more sensitive CATT-EDTA, using blood collected in heparin-coated capillary tubes.

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