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Increased sensitivity of the card agglutination test CATT/*Trypanosoma brucei gambiense* by inhibition of complement

R. Pansaerts, N. Van Meirvenne, E. Magnus *, L. Verhelst

Institute of Tropical Medicine, Nationalestraat 155, B2000 Antwerp, Belgium

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Abstract

CATT/*Trypanosoma brucei* (*T.b.*) *gambiense* is an antibody detection test currently used in field surveys on Gambian sleeping sickness. The screening test is usually performed on a drop of freshly collected heparinized blood, followed by a more specific confirmation test on diluted blood, plasma or serum. This approach may be biased by the occurrence of a complement-mediated prozone phenomenon causing lower test sensitivity at lower sample dilutions. A simple remedy is by addition of a Ca^{2+} chelating agent such as EDTA.

Keywords: Human African trypanosomiasis; *Trypanosoma brucei gambiense*; Diagnosis; CATT; Prozone; Complement

1. Introduction

The card agglutination test CATT/*Trypanosoma brucei* (*T.b.*) *gambiense* (Magnus et al., 1978) is a rapid antibody detection assay currently used in several African countries to facilitate field surveys on Gambian sleeping sickness.

* Corresponding author. Tel.: +32 3 2476368; fax: +32 3 2476373; e-mail: emagnus@itg.be

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The freeze-dried antigenic reagent consists of formaldehyde-fixed, Coomassie blue-stained bloodstream-form trypanosomes of variable antigen type LiTat 1.3 (Van Meirvenne et al., 1995). Prior to use the reagent is resuspended in phosphate-buffered saline (PBS, pH 7.2). The test is performed on disposable cards with 10 circular (18 mm) reaction areas. Each area is loaded with about 25 μl of test sample and a drop (45–50 μl) of reagent. Both components are mixed with a stirring rod and agitated on a standardized rotator (ITMAS version, 60 rpm) for 5 min. The macroscopic agglutination patterns, ranging from negative to strongly positive, are scored by matching with reference photographs.

During field surveys, the entire population at risk is first screened by the easiest version of CATT, i.e. by testing a drop of freshly collected heparinized blood. Blood test-positive individuals are then submitted to a more specific second test on a 1:4 or 1:8 dilution of blood, plasma or serum. The laboratory observations reported here cast some doubt on the efficacy of the former screening protocol. Full range titrations of CATT-positive animal and human serum samples that have been either freshly collected or rapidly frozen at −70°C sometimes reveal a strong prozone phenomenon characterized by weaker agglutination scores at lower dilutions of test sample. This has never been observed upon testing hundreds of serum samples that have been stored under less stringent conditions. Such prozone might adversely affect the sensitivity of CATT screening tests.

A selection of laboratory data exemplifying the prozone phenomenon, a tentative explanation of the mechanism pointing to complement activation and a simple remedy are presented.

2. Materials and methods

2.1. Serum samples

Only a few demonstrative serum samples are considered here.

The first series is a selection of seven CATT-positive serum samples which had been frozen at −70°C shortly after collection. Four samples (H1–4) are of human origin. H1 was obtained from a laboratory worker 14 days after accidental infection with *T.b. gambiense* bloodstream-form trypanosomes of variable antigen type (VAT) LiTat 1.3. Successful drug treatment with eflornithine (DFMO) had been started on day 6 after infection. H2 was obtained from a yet untreated patient about 2 months after contracting a *T.b. gambiense* infection in Congo–Kinshasa. H3 and H4 are from two patients in Ivory Coast without parasitological evidence of past or present trypanosomiasis. Three samples (R1–3) are from rabbits. R1 is an immunization antiserum obtained by repeated i.v. injection of formaldehyde-fixed LiTat 1.3 trypanosomes. R2 and R3 are LiTat 1.3 infection sera obtained on day 27.

The second series consists of 16 freeze-dried samples from human patients with parasitologically confirmed *T.b. gambiense* infection. They are random representatives of a collection of hundreds of similar samples, all CATT-positive and showing no prozone upon full range titration of two-fold serial dilutions.

2.2. Test procedures

All tests were run with an ordinary freeze-dried CATT reagent from one and the same batch, following the usual standard test protocol, except for the modifications stated hereafter. Two-fold dilutions of the serum samples in PBS were submitted to a full range titration using three different test protocols, designated (A), (B) and (C). (A) ordinary test protocol, freeze-dried CATT reagent reconstituted with PBS; (B) as (A), but freeze-dried CATT reagent reconstituted with PBS-EDTA, pH 7.2 (10 mM ethylenediaminetetraacetic acid disodium salt dihydrate, Janssen Chimica); (C) as (A), but serum samples previously heat inactivated at 56°C for 30 min. For recording the agglutination results, the following scores were adopted: negative (−), weakly positive (±), positive (+), strongly positive (+ +) and very strongly positive (+ + +).

3. Results

The results obtained with serum samples of group 1 are summarized in Table 1. In the ordinary test version A, serum samples R1,2,3 and H1,2 display a prozone phenomenon at dilutions 1/2 and/or 1/1. More importantly, four undiluted samples are completely non-reactive. In test versions (B) and (C), prozone is no longer observed and there is some improvement of the agglutination scores in general, even for serum samples H3 and 4. However, the end-titers obtained by different methods are roughly the same.

Since complement activation was thought to be responsible for the prozone phenomenon, some additional experiments were undertaken with serum samples R1,2,3 and H1,2. Partial heat inactivation at 50°C for 20 min, which solely blocks the alternative and not the classical pathway of complement activation, did not abolish the prozone. Substitution of EDTA by 10 mM EGTA, a Ca^{2+} but not Mg^{2+} chelating agent currently used to selectively inhibit the classical pathway of complement, proved equally effective.

For control purposes, the 16 serum samples of group two were also submitted to a full range titration with the three test versions. As seen in Fig. 1, the respective end-titers were rather similar, though several serum samples showed a drop in titer after heat inactivation.

4. Discussion

The present observations strongly suggest that the sensitivity of CATT/*T.b. gambiense* may be adversely affected by a prozone mechanism involving the classical pathway of complement activation. Since complement is a rather unstable enzyme system, the phenomenon is hardly observed with serum samples that have not rapidly been stored at ultralow temperatures. It might be relatively prominent and lead to false-negative results, however, when testing freshly collected undiluted

Table 1
CATT titration results obtained with sera of group 1: agglutination scores at different serum dilutions

Serum	Test protocol	1/1	1/2	1/4	1/8	1/16	1/32	1/64	End titer^{-1}
R1	A	−	±	++	+++	++	++	+	64
	B	+++	+++	+++	+++	+++	++	+	64
	C	+++	+++	+++	+++	+++	++	+	64
R2	A	−	+	++	++	++	+	−	32
	B	++	+++	++	++	++	+	−	32
	C	++	++	++	++	++	±	−	32
R3	A	−	−	+	+	+	−	−	16
	B	++	++	++	++	+	±	−	32
	C	++	++	+	+	−	−	−	8
H1	A	−	+	+	+	+	−	−	16
	B	++	++	++	++	++	+	−	32
	C	++	++	++	++	+	+	−	32
H2	A	±	+	++	++	+	±	−	32
	B	++	++	++	++	+	±	−	32
	C	+	++	+	+	±	−	−	16
H3	A	++	+	−	−	−	−	−	2
	B	+++	++	±	−	−	−	−	4
	C	+++	++	±	−	−	−	−	4
H4	A	+	+	+	±	−	−	−	8
	B	++	++	++	+	−	−	−	8
	C	++	++	++	+	−	−	−	8

(A) Ordinary test version; (B) test version with EDTA; (C) test version with heat-inactivated sera; R1–3, rabbit sera; H1–4, human sera.

or weakly diluted blood or serum. This remains to be critically investigated during field surveys. Heat inactivation of the test serum or simply the addition of a Ca^{2+} chelating (anticoagulating and anticomplementary) agent such as EDTA, seems to provide an efficient remedy.

Analogous complement-mediated inhibition phenomena have been reported for *Brucella* and *Leptospira* direct agglutination tests on sheep and goat sera (Cho and Ingram, 1971; Malkin, 1984) and for immune precipitation reactions (Schifferli et al., 1982; O'Sullivan et al., 1988; Baatrup, 1989).

An improved version of CATT could possibly be obtained by incorporation of EDTA in the freeze-dried reagent or in the reconstitution buffer, or simply by the use of EDTA-coated sampling capillary tubes. Ongoing field trials have given quite encouraging preliminary results. EDTA indeed significantly improves the test sensitivity, be it at the expense of a minor decrease in specificity.

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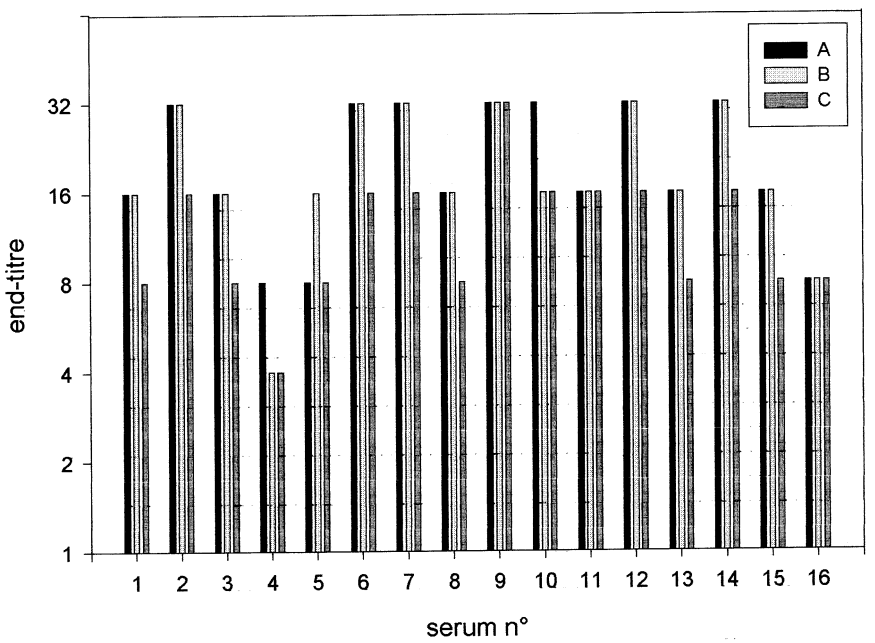

Fig. 1. Reciprocal end-titers of 16 human serum samples of group 2 obtained in CATT versions (A), (B) and (C).

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