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A new in vitro test for human serum resistance of *Trypanosoma (T.) brucei*

Short communication

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It has been known since Laveran (1902) that normal human serum has a trypanocidal effect on *Trypanosoma b. brucei*. After various attempts to identify the cytotoxic factor(s) (Aaronovitch and Terry 1972; Hawking et al., 1973; Hawking, 1979; Van Meirvenne et al., 1973), Rifkin (1978) found a high density lipoprotein in normal human blood and serum with trypanocidal activity.

The trypanocidal activity of normal human blood or serum has been used by Rickman and Robson (1970, 1972) who developed a simple indirect test to determine the subspecific identity of *Trypanosoma (T.) brucei*. This test, known as the blood-incubation infectivity test (BIIT) includes incubation of trypanosomes in human blood or serum in vitro at 37° C and subsequent inoculation of the suspension into susceptible laboratory animals. *T. b. rhodesiense* and *T. b. gambiense* are relatively resistant to the cytotoxic effect of the normal human blood or serum whereas *T. b. brucei* is neutralized.

Brun et al. (1981) described an in vitro culture system for bloodstream forms of different subspecies of the subgenus *Trypanosoma (T.) brucei* which enabled the continuous growth of *T. b. rhodesiense* and *T. b. gambiense* in the presence of normal human serum. In this system, stocks of *T. b. brucei* were rapidly lysed whereas they grew in inactivated rabbit serum. This in vitro culture system was used to test the human serum resistance of several stocks of *Trypanosoma (T.) brucei*.

The in vitro test system consists of a feeder layer of fibroblast-like cells derived from embryos of *Microtus montanus*, with HEPES-buffered Minimum Essential Medium, with Earle's Salts (EMEM), supplemented with 15% normal human serum in the test wells and 15% inactivated rabbit serum in the control wells (Brun et al., 1981).

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Table 1 shows the stocks and clones derived from primary isolates of *Trypanosoma (T.) brucei* which were tested. The history of these isolates, except for STIB 703 and STIB 710, is given elsewhere (Geigy et al., 1973a, 1975; Brun et al., 1981). STIB 703 was isolated from a patient in Southern Tanzania in 1982 and stabilated after one mouse passage. STIB 710 was isolated from a patient in Daloa (Ivory Coast) in 1982 by the direct transfer of blood parasites into culture.

The initiation and maintenance of the bloodstream form cultures was carried out according to Brun et al. (1981). Parasites were taken from the first raising parasitaemia of mice or *Microtus montanus* (TH-3/78 [031]) infected with trypanosomes from stabilates. Almost confluent feeder layers in 2-cm^2 wells were inoculated with 10^5 trypanosomes/well. The test wells contained 15% normal human serum and the control wells 15% inactivated rabbit serum in EMEM. The tests were carried out at least 3 times and the cultures were maintained for at least 10 days with daily medium replacement.

Table 1 shows the test results of 12 different stocks and 4 corresponding clones. STIB 247, STIB 246, STIB 348 (clone of 246) and EATRO 1856 were consistently neutralized by the action of normal human serum. All the other stocks and clones showed continuous growth in the presence of human serum. Stabilate STIB 349 (clone of STIB 324) did not infect one volunteer (Geigy et al., 1975). However, the in vitro test was positive although human serum resistance was only present in a few parasites.

Initially, some stocks (e.g. STIB 229, 347, 350, 324 and STIB 349) contained only a minority of human serum resistant forms whereas most trypanosomes were lysed within a few hours in the presence of human serum. Especially the clones contained only very few resistant forms as indicated by the initial disappearance of the trypanosomes in the culture with human serum and the reappearance of a resistant population after 4–6 days.

The sensitivity of the test was analyzed by adding a single human serum resistant trypanosome of STIB 389 to 10^5 trypanosomes of STIB 247 (well A). As controls 10^5 trypanosomes of STIB 247 (well B) and a single trypanosome of STIB 389 (well C) were inoculated into culture wells. All three wells (A, B and C) contained 15% human serum. Well A showed a small growing parasite population after one week which resembled in its growth characteristic the clone growing in well C. The 10^5 trypanosomes in well B were all lysed within a few hours and no trypanosomes could be detected after 2 weeks. These results show that the test is able to detect single human serum resistant parasites among high numbers of human serum sensitive parasites.

This new test allows the detailed analysis of an eventual fluctuation of human serum resistance in a stock or primary isolate during long term in vitro cultivation. The stability of human serum resistance of different primary isolates before and after tsetse fly transmission is currently being investigated.

Table 1. Human-serum resistance and sensitivity of *Trypanosoma (T.) brucei* stocks and clones

Isolated from	Place, Country	Year	Stabilate No.	Continuous growth in vitro with		Man-tested[1]
				human serum	inact. rabbit serum	
Hartebeest	Serengeti, Tanzania	1970	EATRO 1856	−	+	ND
Hartebeest	Serengeti, Tanzania	1970	EATRO 1873	+	+	+
Hartebeest	Serengeti, Tanzania	1971	STIB 229	+	+	ND
Hartebeest	Serengeti, Tanzania	1971	STIB 347*	+	+	ND
Hartebeest	Serengeti, Tanzania	1971	STIB 246	−	+	ND
Hartebeest	Serengeti, Tanzania	1971	STIB 348*	−	+	−
Hartebeest	Serengeti, Tanzania	1971	STIB 247	−	+	ND
Lion	Serengeti, Tanzania	1971	STIB 241	+	+	+
Lion	Serengeti, Tanzania	1971	STIB 350*	+	+	+
Hyaena	Serengeti, Tanzania	1971	STIB 324	+	+	+
Hyaena	Serengeti, Tanzania	1971	STIB 349*	+	+	−
Man	Homa Bay, Kenyia	1973	STIB 365	+	+	
Man	Northern Tanzania	1980	STIB 389	+	+	
Man	Southern Tanzania	1982	STIB 703	+	+	
Man	Ivory Coast	1978	TH-3/78 (031)	+	+	
Man	Ivory Coast	1982	STIB 710	+	+	

* Clone of the above isolate; [1] see Geigy et al. (1973b, 1975); ND = Not done

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