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Short Report

A kit for *in vitro* isolation of trypanosomes in the field: first trial with sleeping sickness patients in the Congo Republic

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Low parasitaemias in patients infected with *Trypanosoma brucei gambiense*, with its limited infectivity to rodents, result in poor diagnosis and isolation (DUKES *et al.*, 1991). Procyclic forms grow readily in culture medium (e.g., BRUTSAERT & HENRARD, 1936; CUNNINGHAM, 1977), but the transformation of low numbers of bloodstream forms from rodent blood to the procyclic phase is difficult (BIENEN *et al.*, 1980; TRUC, 1991). DUKES *et al.* (1989) achieved such transformation using the natural cycle in the vector by feeding infected blood to susceptible laboratory-reared tsetse flies. However, in the field the usual approaches require either inoculation of rodents, which is unreliable, or deep-freezing of patients' blood.

To solve these problems, we have developed a kit for *in vitro* isolation of trypanosomes (KIVI). It allowed direct introduction of patients' blood into culture medium, with the subsequent transformation to, and multiplication of, procyclic trypanosomes. The medium (GLSH-DCA) comprised glucose, lactalbumin, serum and haemoglobin (LE RAY, 1975) diluted with an equal volume of Hanks's solution (HANKS & WALLACE, 1949; LE RAY, *et al.*, 1970) and complemented with 3 mM *cis*-aconitate (final concentration) according to BRUN & SCHÖNENBERGER (1981). Ten ml of blood were drawn into either a syringe containing 0.5 ml of 5% Liquoïde® Roche (sodium polyanetholesulphonate) anticomplementary anticoagulant (LE RAY *et al.*, 1970), or a Monovette® (Sarstedt) syringe containing heparin (lithium salt). The blood mixture was then dispensed equally into 2 vials (R1), each containing 10 ml of GLSH-DCA; one also contained a supplement of antibiotics (penicillin, 5000 iu/ml; gentamycin, 200 µg/ml; 5-fluorocytosine, 50 µg/ml). The vials were mixed by gentle manual agitation and kept at room temperature. In the initial laboratory tests, a minimum concentration of $2{\cdot}5\times10^4$ trypanosomes per ml could be transformed and cultured.

Results from 10 patients sampled on 2 separate occasions (1989, 1990) in the Bouenza focus, Republic of the

Table. Trypanosome isolation by KIVI and by rat inoculation from ten sleeping sickness patients in the Congo Republic

Patient	CATT[a]	LN[b]	MHC[c]	Rat[d]	Anticoagulant[e]	KIVI R1[f]	KIVI R2[g]	Stock no.
November 1989								
Minja	+	NS	+	+41	Li	27–36	20→4	ITMAP 2202
Balpa	+	NS	+	NEG	He+Li	24–36	20→4	ITMAP 2203
Silou	+	T+	++	+38	He+Li	31–36	20→16	ITMAP 2204
Bissi	+	T+	+	NEG	Li	27–36	20→4	ITMAP 2205
Pave	+	NS	+	NEG	Li	NEG	20→NEG	–
April 1990								
Dicar	+	T+	+	NEG	Li	18–25	15→5	ITMAP 2208
					He	NEG	ND	
Koa	NEG	T+	++	+26	Li	10–54	10→3	ITMAP 2209
					He	NEG	ND	
Bousa	+	T+	++	NEG	Li	15–54	15→3	ITMAP 2210
					He	NEG	ND	
Houm	+	NEG	+++	ND	Li	NEG	13→NEG	–
					He	NEG	13→NEG	
Babi	+	T+	+	NEG	Li	NEG	14→NEG	–
					He	NEG	ND	

[a]Card agglutination test for trypanosomes (MAGNUS *et al.*, 1978).
[b]Lymph nodes: NS, not swollen; T+, trypanosomes seen in lymph fluid.
[c]Microhaematocrit centrifugation (WOO, 1970): +, 1–5; ++, 6–15; +++, >15 trypanosomes.
[d]Rat inoculation: time (days) to positive by thin blood film examination; NEG: negative by wet smear over 2 months; ND, not done.
[e]Li: Liquoïde (see text); He: heparin
[f]R1: first and last days of patency in initial culture following inoculation. NEG: negative.
[g]R2: age in days of R1 when subinoculation into R2 was performed; arrow indicates day R2 became positive. NEG: negative.

Congo, are presented in the Table; at the same time, 1 ml of blood from each patient was inoculated into a rat. The inoculated KIVIs were sent or brought back to Europe, where 2 to 4 weeks after the initial inoculation, they were examined. Subinoculation (R2) was performed into blood-agar (TOBIE, 1949) and Cunningham's medium (CUNNINGHAM, 1977). R1 and R2 vials were kept under observation for one month.

Of the 10 sleeping sickness patients with low-grade parasitaemias, 7 provided a positive culture in KIVI whereas only 3 were infective to rats. Isoenzyme characterization for 24 loci (TRUC, 1991) showed that all the isolates belonged to classical *T. b. gambiense*. Our results also confirm the low infectivity of *T. b. gambiense* in Central Africa to rodents.

In this preliminary study, KIVI was more effective than rat inoculation in isolating human parasites. Liquoïde (Roche) was confirmed to be the best anticoagulant (WEINMAN, 1960). The operational value of KIVI in field work was demonstrated by the long period during which it sustained the growth and viability of procyclic trypanosomes (25–54 d; average 40 d). Work is now in progress to improve the KIVI and test it in other areas of Africa, and to evaluate its diagnostic value for hosts with subpatent infections.

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