Ann. Soc. belge Méd. trop.
1973, **53**, 2, 109-112

COLUMN SEPARATION AND MEMBRANE FILTRATION : A ROUTINE METHOD FOR THE DETECTION OF AFRICAN TRYPANOSOMES IN HUMAN BLOOD

by

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Summary — A routine technique is described for the detection of African trypanosomes in human blood. The method is based on the selective anion-exchanger separation of the trypanosomes and their retention on a filter membrane.

KEYWORDS : Sleeping-sickness; Trypanosoma; Anion-exchanger; Membrane filtration.

Salivarian trypanosomes may be selectively separated from host blood by an anion-exchanger method (Lanham, 1968). By varying the ionic strength of the eluating buffer, the system may be adapted to each particular trypanosoma-host combination (Lanham *et al.*, 1970). Apart form its extreme efficiency for the preparation of pure bloodstream from antigens, the column-eluation has proven useful for diagnostic purposes. During experiments of Lanham *et al.* (1970) and Godfrey *et al.* (1971), concerning definitive or suspected cases of human Gambian sleeping sickness, trypanosomes could be demonstrated in the centrifugation deposits of the blood eluates from 9 out of 39 patients in whom the organisms could not be detected either in the gland fluid or in thick blood films.

Recently, (Lanham *et al.*, 1972) a method of column-separation combined with membrane filtration of the eluate has been described. A similar method had previously been introduced in our laboratory and has given satisfactory results. It was thought that a detailed description of our technique might contribute to developing an optimal alternative method.

Column-separation

DEAE-cellulose (*) is equilibrated with the PSG 5:5 buffer system having an ionic strength of 0.181 (Lanham *et al.*, 1970). A slurry layer, 1.5 to 2.0 cm

(*) Type DE-52, Whatman Chromedia.

in heigth is packed into a column with sintered bottom plate (porosity grade 1), and washed with 100 ml of buffer to remove small gel-particles. A column of 3 cm in diameter and 10 cm in heigth is used for fractionating up to 5 ml of blood.

The heparinized blood sample is mixed with its five-fold volume of equilibrated slurry and this mixture, somewhat diluted with PSG-buffer, is applicated onto the column adsorbent. After sedimentation of the sample, elution is achieved by a continuous PSG buffer flow. Most or all trypanosomes are eluted from 5 ml of blood with 100 ml of buffer.

Membrane filtration

The filtration device consists of a pyrex filter holder (*) in connection with a vacuum flask and equipped with a cellulose acetate membrane of pore size 0.45 micron and 25 mm in diameter (**).

Prior to use, the membrane is soaked in distilled water during a few minutes; this prevents folding of the filter during further manipulations.

The whole column-eluate is filtered and the membrane immediately flushed with 20 ml of PSG in order to remove serum proteins. Two ml of a Fuchsin dye solution (***) containing 5 per cent commercial formaline are then allowed to react for 10 minutes; the staining solution is pulled through and the membrane flushed with 10 ml of distilled water. After being dried at 37 °C for 30 minutes, the filter is cleared and mounted with immersion oil (nD = 1,515) or xylene. Oil-mounted membranes may be stored for several months.

A filter area of about 2.5 cm^2 must be examined : this takes about 60 minutes for negative preparations. In positive preparations of course, the first trypanosome may sometimes rapidly be found. Trypanosomes are traced under the ×16 objective; findings are confirmed under the ×40 or ×100 immersion oil objective. The morphological structures are sufficiently differentiated to exclude confusion with other particles.

In order to obtain excellent membrane preparations with neglectable background, prefiltered reagents and clean glassware are used. The sintered bottom plate of the filter apparatus may rapidly be destained with a sodium hypochlorite solution.

Sensitivity of the method

Experiments with artificially subpatent blood and eluates, prepared by adding small numbers of *T. brucei,* indicated that 50 to 100 per cent of the organisms are recovered on the membrane.

(*) Millipore microanalysis filter holder.
(**) Millipore type HAW.
(***) Fuchsin dye solution : To 1 liter of distilled water; add : 60 ml of 5 per cent aqueous phenol, 5 ml of ethylalcohol, 4 ml of glycerol and 125 mg of basic fuchsin; stirr well while heating gently; filter through a membrane as used for trypanosomes. The solution may be stored at room temperature.

Results

A total of 11 blood samples, derived from 6 patients with *T. gambiense* or *T. rhodesiense* have been examined. Some of these blood samples were collected during therapy. The results are summarized in the table. Upon examination of blood samples from individuals, subsequently confirmed to be free of trypanosomiasis, false findings never occurred.

Patient	Infection	Days after biginning therapy	Blood sample volume	Number of trypanosomes on the membrane
F.	*T. gambiense*	0	2 ml	2
D. P.	*T. gambiense*	4	5 ml	several hundreds
L.	*T. rhodesiense*	0	2 ml	several hundreds
		3	1 ml	2
		10	2 ml	1
		12	5 ml	none
V. D. D.	*T. rhodesiense*	0	1 ml	several thousands
		9	3 ml	none
V.	*T. rhodesiense*	1	1 ml	several thousands
		2 weeks	3 ml	none
G.	*T. rhodesiense*	3	3 ml	none

Discussion

According to the fundamental principles of the anion-exchange method, the most complete and rapid elution of scanty trypanosomes, will be obtained when the highest ionic strength and the highest flow rate of buffer, together with a minimal heigth of the adsorbent layer, still compatible with retention of the blood cells, are used. These optimal conditions may be better approximated by premixing the blood sample with adsorbent prior to its application onto the definitive adsorbing layer in the column.

With this technique of sample application, all difficulties due to irregularities in the descending erythrocyte front are completely avoided and a buffer of relatively high ionic strength can be used.

The use of columns with relatively large diameter would equally be advantageous; a diameter of 5 cm might well be optimal for the fractionation of 5 ml blood samples.

Direct filtration of the entire column-eluate and colouration of the trypanosomes in situ would like to guarantee a minor loss of organisms. Instead of giemsa, a simple and rapid staining method with basic fuchsin may alternatively be used. It remains to be verified whether smaller membranes, e.g. having 13 mm in diameter, are equally suitable for the filtration of large eluate volumes.

It is thought that still more simple and efficient methods might be worked out.

Samenvatting — **Kolomafscheiding en membraanfiltratie : een routinemethode voor het opsporen van Afrikaanse trypanosomen in menselijk bloed.**

Een routine techniek voor opsporing van Afrikaanse trypanosomen in menselijk bloed wordt beschreven. De methode steunt op de selektieve afscheiding der trypanosomen met een anionenuitwisselaar en hun inzameling op een filtermembraan.

Résumé — **Séparation sur colonne et filtration sur membrane : une méthode de routine pour la recherche de trypanosomes africains dans du sang humain.**

Une technique de routine est décrite pour la détection de trypanosomes africains dans le sang humain. La méthode est basée sur la séparation sélective des trypanosomes à l'aide d'un échangeur d'anions, et leur concentration par filtration sur membrane.

Acknowledgements — This work has been realised with the financial assistance of the Belgian " Nationaal Fonds voor Kollektief Wetenschappelijk Onderzoek".

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Received for publication on October 25, 1972.

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