Single centrifugation of cerebrospinal fluid in a sealed pasteur pipette for simple, rapid and sensitive detection of trypanosomes.

T.W. Miézan[1], H.A. Meda[2], F. Doua[1], N.N. Djè[1], V. Lejon[3] and P. Büscher[3]*

[1] Projet de Recherches Cliniques sur la Trypanosomiase, BP 1425, Daloa, Côte d'Ivoire.

[2] OCCGE-Institut Pierre Richet, 01 BP 1500, Bouaké 01, Côte d'Ivoire.

[3] Institute of Tropical Medicine, Dep. of Parasitology, Nationalestraat 155, B-2000, Antwerpen, Belgium.

* Corresponding author

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Treatment of human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* requires distinction between haemo-lymphatic and meningo-encephalitic stages. The presence of trypanosomes in the cerebrospinal fluid (CSF) is not only an evidence for diagnosis, but also indicates central nervous system invasion (WHO, 1998).

When their number in CSF is high, trypanosomes are detectable during the cell count. In case of low trypanosome number, the detection is facilitated by centrifugation of CSF and microscopic examination of the sediment (LAVERAN & MESNIL, 1912). CATTAND *et al.* (1988) improved the sensitivity of trypanosome detection by introducing a double centrifugation (DC) technique: after the first centrifugation, the sediment is taken up in micro-haematocrit tubes which are centrifuged and examined for trypanosomes at the bottom of the tube. The DC technique is not widely practised due to the number of

manipulations and the need of two centrifuges. Here we describe a modified single centrifugation (MSC) technique as an alternative to DC, which is compared with other WHO therapeutic parameters (WHO, 1998).

The data were collected at the Projet de Recherches Cliniques sur la Trypanosomiase Daloa, Côte d'Ivoire. For routine diagnosis and stage determination of HAT, eight ml of CSF were obtained from 52 CATT-positive persons by lumbar puncture. According to WHO (1998), the following examinations were performed: parasite detection, cell count and protein concentration. Trypanosomes were searched for in blood by the mini-Anion Exchange Centrifugation Technique (mAECT), Quantitative Buffy Coat and Kit for In Vitro Isolation, in lymph by examination of the lymph node aspirate. Presence of trypanosomes in CSF was examined by DC on 6 ml of CSF and in parallel by MSC. For MSC, a sealed pasteur pipette was prepared as for mAECT (LUMSDEN *et al.*, 1979). The pipette was filled with 2 ml of CSF and centrifuged (10 minutes, 2000 rpm). Using the mAECT viewing-chamber, the sediment in the bottom of the tube was microscopically examined for the presence of trypanosomes. The cell number in CSF was counted using a Malassez counting chamber and the CSF protein concentration was assayed with the Coomassie brilliant blue method. The different methods were compared using Mc Nemar χ^2 for $\alpha = 0.05$.

Combining all techniques, trypanosomes were detected in 42 patients (80.8%). According to WHO criteria (WHO, 1998), 35 of these patients (83.3%) were in the meningo-encephalitic stage. Trypanosomes were detected in 34 CSF samples; the DC and MSC techniques were positive in respectively 28 and 33 patients (table 1). MSC detected trypanosomes in 6 samples negative with DC (with 6, 8, 10, 20, 194 and 338 cells/μl in CSF), whereas DC detected trypanosomes in only one MSC negative sample

(with 726 cells/µl). Although MSC appeared more sensitive, the difference of sensitivity between MSC and DC was not statistically significant ($\chi^2 = 2.29$; $p > 0.05$). There was no significant difference between the MSC technique and the white cell count ($\chi^2 = 0.50$; $p > 0.05$) or total protein concentration in CSF ($\chi^2 = 0.57$; $p > 0.05$) as criteria for stage determination.

We conclude that MSC is rapid, simple and sensitive for diagnosis and stage determination of HAT. The test can be performed in 10 minutes. Trypanosomes can be detected in the bottom of the sealed pasteur pipette, avoiding the need to transfer the sediment on a slide. MSC is less elaborated than DC since only one centrifugation step is needed and is cheaper since only a sealed pipette and one centrifuge are necessary.

MSC is also recommended for post-treatment follow-up.

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Table 1: Comparison of MSC with DC, CSF cell count and protein concentration.

Other techniques	MSC		Total
	negative	positive	
DC			
Negative	8	6	14
Positive	1	27	28
Total	9	33	42
CSF cell count			
$\leq$ 5 cells/mm^3	7	0	7
> 5 cells/mm^3	2	33	35
Total	9	33	42
CSF protein content			
$\leq$ 370 mg/l	7	5	12
> 370 mg/l	2	28	30
Total	9	33	42