

A practical device designed to concentrate cells for cytomorphological studies of biological fluids.

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Key words: Cytocentrifuge, cerebrospinal fluid (CSF), cytomorphology, biological fluids, leukemia.

Abstract. In the present work, a device designed for concentrating cells from biological fluids is described. The instrument consists of a tube in which the inner cavity has a conical shape at one of its ends and a small orifice is found at the bottom, while the tube's exterior maintains its cylindrical shape. The tube is placed inside a second tube that ends on a flat surface on which a glass cover slide is placed. The sample to be studied is placed in the inner tube of the assembled device and spun in a regular clinical centrifuge. Cells are collected on the glass slide, fixed and stained for microscopical studies. The device was tested using 23 samples of cerebrospinal fluid (CSF) from patients with lymphoproliferative diseases. An adequate number of intact cells was recovered for observation, and a precise diagnosis was possible. Cells from three aliquots of each CSF sample were concentrated by this method, and by the more expensive standard commercial cytocentrifuge, with similar results. The device described here provides an easy, efficient and inexpensive method, for the concentration of cells from organic fluids.

Diseño de un instrumento práctico para estudios cito-morfológicos en fluidos biológicos.

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Palabras claves: Citocentrífuga, fluido cerebrospinal, citomorfología, fluidos biológicos, leucemia.

Resumen. En el presente trabajo, se muestra el diseño de un tubo adaptable a la centrifuga clínica, que permite concentrar elementos celulares para estudios morfológicos a partir de fluidos orgánicos como líquido cefalo-

traquideo (LCR), ascítico, pleural, orina etc. El tubo consta de una parte A, cilíndrica, que se hace cónica en su interior y en el exterior conserva la forma, dejando un pequeño orificio de salida. Esta parte se enrosca en un tubo B, más corto que termina en una superficie plana que contiene en su interior una laminilla de vidrio cubreobjeto. El líquido a estudiar se coloca en el tubo ensamblado, se centrifuga en una centrifuga clínica y una vez centrifugado, se retira la laminilla con el centrifugado, se tiñe y se puede observar en el microscopio. Se procesaron 23 muestras de LCR de pacientes con leucemia linfoblástica aguda o con linfoma. En todos los casos, se obtuvieron preparados con número adecuado de células para hacer un diagnóstico morfológico. Las muestras de LCR sometidas al proceso descrito y aquellos donde se utilizó una citocentrifuga comercial, arrojaron resultados similares. En conclusión, el dispositivo presentado, permite sustituir la citocentrifuga en forma eficaz y económica, a la vez que hace accesible el estudio citomorfológico de fluidos orgánicos, a mayor número de pacientes.

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INTRODUCTION

Cytomorphological studies have always been of great interest in medical science since they permit cellular identification and quantification. Most animal tissues offer sufficient numbers of cells to permit morphological study, however, some body fluids have low cell concentrations. Such is the case of cerebrospinal fluid (CSF), and ascitic or pleural effusions.

In many instances, in the early stages of pathological processes, the study of cell morphology in organic fluids can be helpful in reaching a correct diagnosis. However, in order to obtain an adequate cell number, the samples, traditionally, are concentrated by cytocentrifuge, an expensive piece of equipment, not available in most public health centers in underdeveloped countries. The

purpose of this work is to present an unexpensive device, designed to fit any clinical centrifuge, that permits the concentration of cells from most organic fluids.

MATERIAL AND METHOD

The device proposed in this paper is shown in Figures 1, 2 and 3. It consists of two assembled plexiglass cylinders. One cylinder (A) is 110 mm long, with an outer diameter of 20 mm and an inner diameter of 15 mm, becoming conical towards the bottom end, and ending in an orifice of 5 mm in diameter. The other cylinder (B) is 20 mm long, with a 25 mm outer diameter and a 20 mm internal diameter. Cylinder A fits into cylinder B. A 12x12 mm glass coverslip is placed on a 1 mm thick rubber support placed at the internal end of A, under the orifice. The sample to be tested is

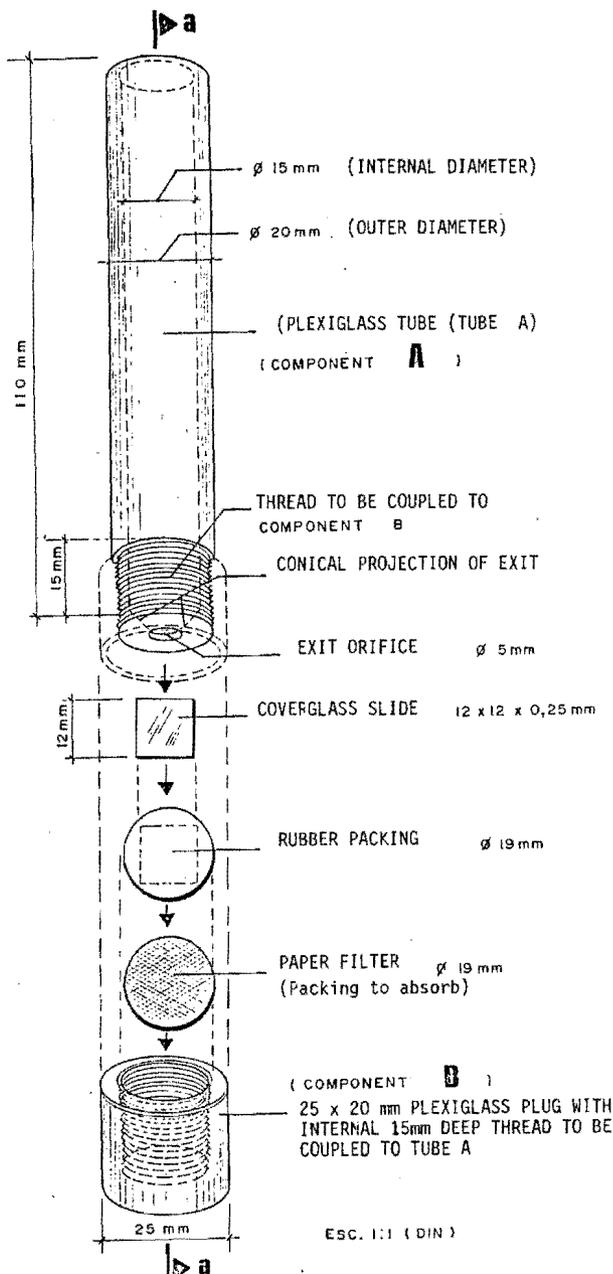


Fig. 1. Diagram of tubes A and B.

placed inside cylinder A of the assembled device (Fig. 2) and spun in a clinical centrifuge. The cells collected on the coverslip are fixed and stained for microscopical studies.

Cell recovering assays

Leukocytes

Leukocytes were obtained from normal whole citrated blood by a Ficoll-Hypaque gradient (1) (Sigma). The number of cells per microliter was first determined in an automated cell counter (DYN 1400).

Serial dilutions of this leukocyte suspension were prepared with normal saline solution. Each dilution

was placed in the device described above and centrifuged.

In order to find the optimal conditions for cell recovery, aliquots of each dilution were spun down at different speeds, for different periods of time.

Twenty three samples of CSF from patients with hematologic malignancies (lymphoblastic leukemias and lymphomas) were processed. Three aliquots of five hundred microliters of each sample were placed in the assembled tube and centrifuged. The recovered cells were fixed, stained with Wright-Giemsa and observed on a Zeiss microscope (x1000). Pretreatment

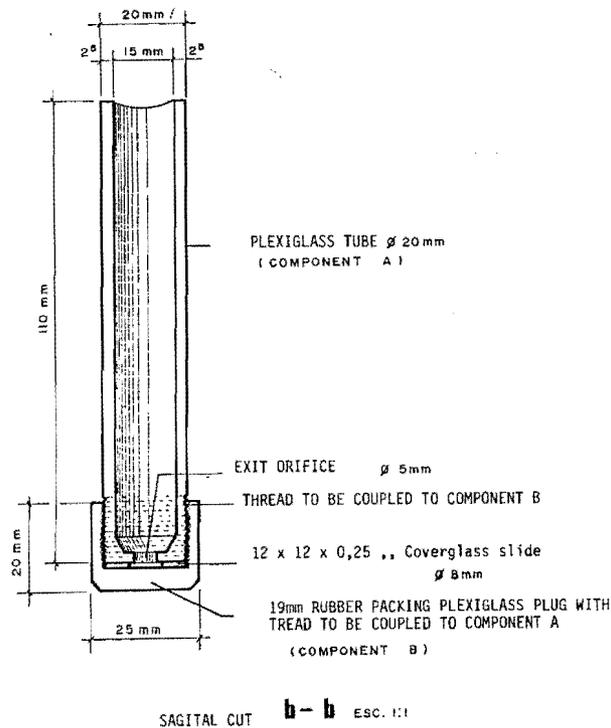


Fig. 2. Assembled device.

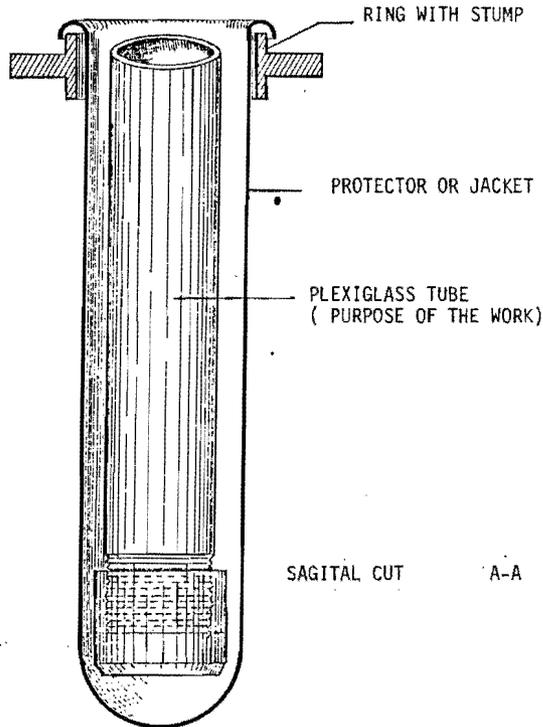
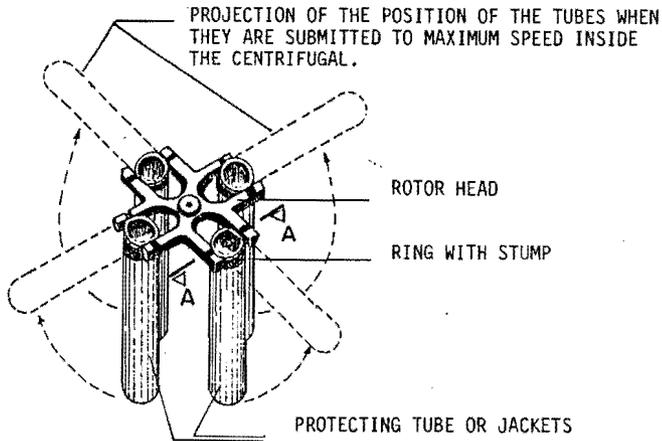


Fig. 3. Position of the device in the clinical centrifuge.

of the coverslips with a 12% bovine albumin solution improved cell adherence.

Cells from 25 consecutive microscopic fields were identified and counted. Aliquots of the above samples were also processed in a commercial cytocentrifuge (Cytospin 2) in order to compare the results with those obtained by our method (2).

Results were expressed as means and standard deviations of the means. Comparisons between groups were made by the Mann-Whitney test. The associations between variables were analysed by linear correlation. Two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

The optimal experimental conditions for leukocyte centrifugation were found to be 180 g during 3 minutes at room temperature. Higher speeds or longer centrifugation times, resulted in the rupture of the plasmatic membrane of neutrophils and monocytes.

Table I shows the means of the cellular counts per microscopic field after centrifugation of CSF using our device.

It is important to note that, even in cases where no cells were found previous to centrifugation, it was possible to observe cells with a well preserved morphology after the fluid was centrifuged in our device (Table I).

The number of cells observed per field was in direct proportion to the number of cells counted before centrifugation (Fig 4 and 5); a good positive correlation between these two variables was obtained ($r: 0.8892$ $p < 0.0001$) (Figure 6).

The preservation of the morphology allowed accurate leukocyte formulas (Fig 7). Similar leukocyte counts were obtained when the sample aliquots were centrifuged using the commercial cytocentrifuge (10.2 4 cells/ per field) or our device (9.5± cells/per field) (Table II).

DISCUSSION

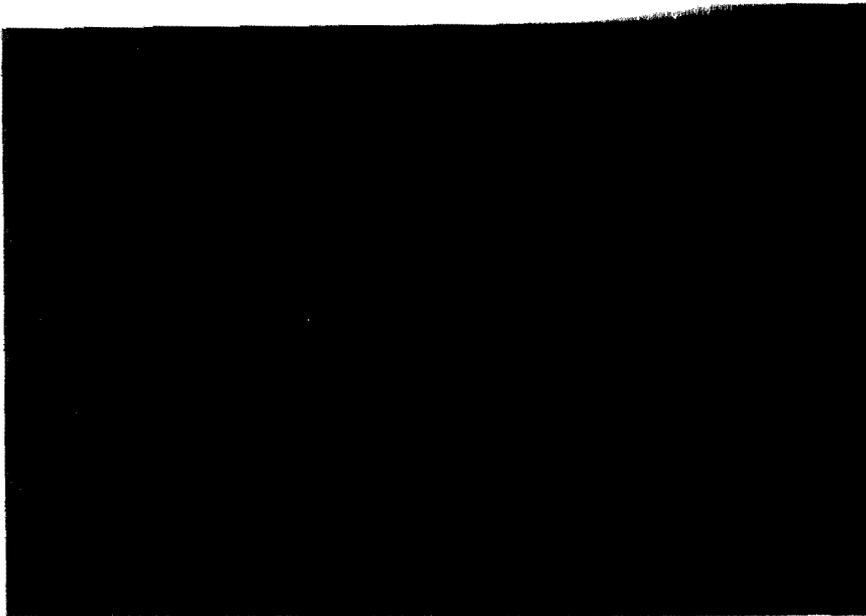
In the present work, a simple

TABLE I
CELLULARITY AND MORPHOLOGIC ASPECTS OF CONCENTRATED
CEREBROSPINAL FLUIDS

N	CELLS/ μ L	CELLS/FIELD	DIFFERENTIAL COUNT %			MORPHOLOGIC INTEGRITY %	
4	0	2 ± 1	L:100			100	
10	50 ± 50	5 ± 3	L:79	N:7	M:6	B:10	94
4	150 ± 50	11 ± 5	L:78	N:7	M:2	B:13	96
3	400 ± 100	42 ± 9	L:65	N:9	M:5	B:21	90
2	600 ± 100	72 ± 23	L:45	N:4	M:3	B:48	94

L: Lymphocytes. N: Neutrophils. M: Monocytes. B: Blasts.

A



B



Fig. 4 Lymphocytes from cerebrospinal fluid, after cellular concentration with the experimental device
A: inicial count: 10 cells/ μ l (x1000)
B: inicial count: 80 cells/ μ l (x1000)

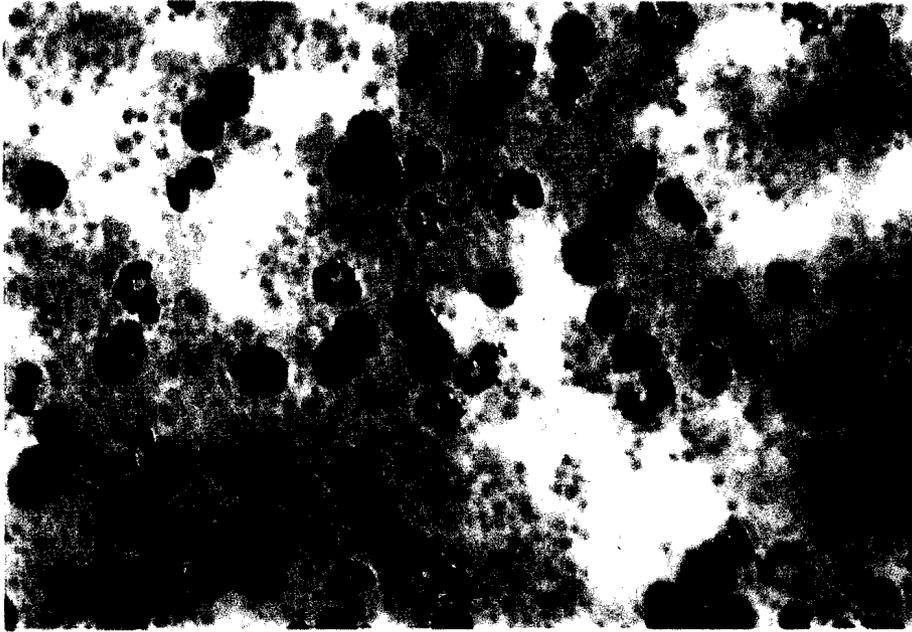


Fig. 5 Neutrophils, platelets and mononuclear cells after cellular concentration with the device. Inicial count: 250 cells/ μ l (x1000)

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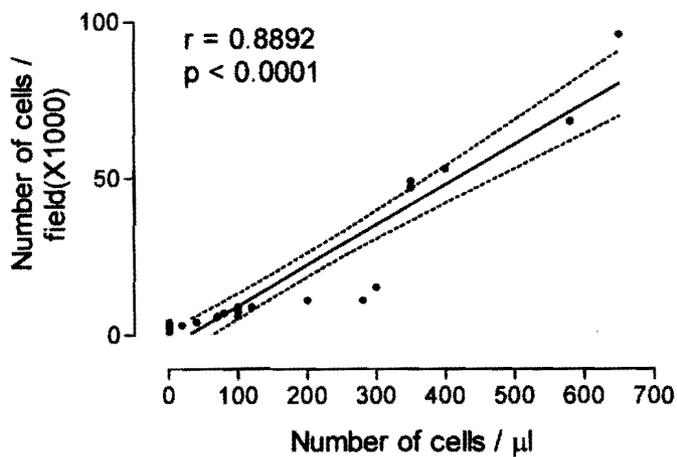


Fig. 6 Correlation between number of cells found in the samples without centrifugation (cell/ μ l) and samples after centrifugation (cells/microscopy field).

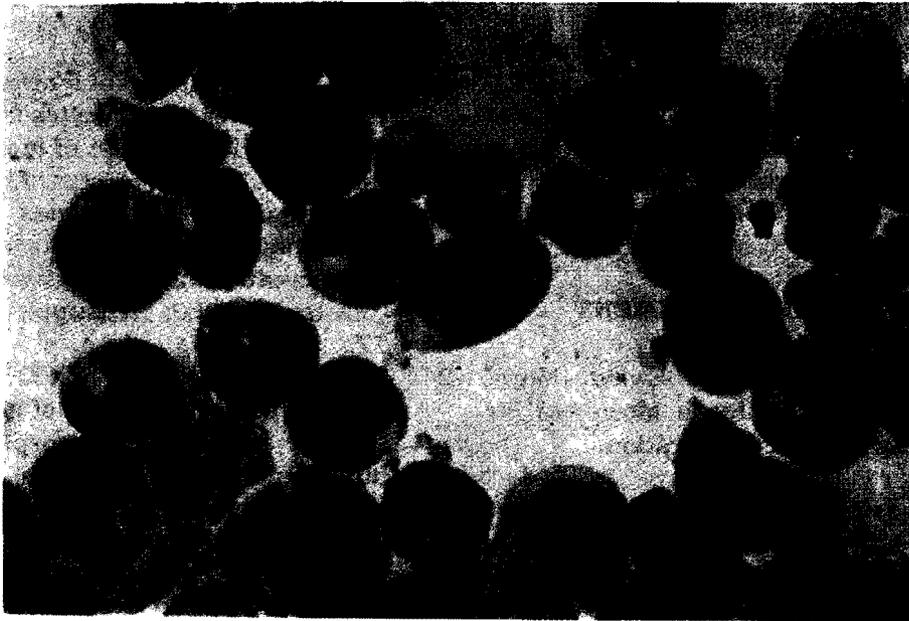


Fig. 7. Well preserved cellular morphology from cerebrospinal fluid sample. Initial count: 200 cells / μ L (x1600).

TABLE II
RELATIONSHIP BETWEEN THE NUMBER OF CELLS/FIELD AND THE CELLULAR CONCENTRATION OF THE SAMPLES (CELLS/ML)

	Sample (Cells/mL)		
	3×10^5	1×10^5	0.5×10^5
Cytocentrifuge (Cells/field)	9.0 ± 0.58 (5)	6.3 ± 0.46 (5)	3.6 ± 0.84 (5)
Device (Cells/field)	8.5 ± 0.82 (5)	5.8 ± 0.58 (5)	3.5 ± 0.54 (5)
p value	NS	NS	NS

* Cells counted at x 1000

device for the concentration of cells from body fluids is described. It can efficiently substitute the commercial cytocentrifuge in the study of low cell concentration samples. A good preservation of the cellular integrity, which is essential for the diagnosis of malignant, infectious or infiltrative

processes, was obtained in samples centrifuged using our device. The instrument can be used for cell studies of most organic fluids, such as pleural, ascitic, and sinovial effusions, urine, etc., and it can be fitted into any clinical centrifuge by a simple change of the buckets.

In conclusion, the use of the device described in the present work, offers an accesible and reliable method for clinical laboratories that can not afford a cytocentrifuge, allowing a rapid and accurate diagnosis of infiltrative or infectious processes in organic fluids.

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