Peripheral blood contamination analysis in CSF: Improved method by Flow Cytometry.

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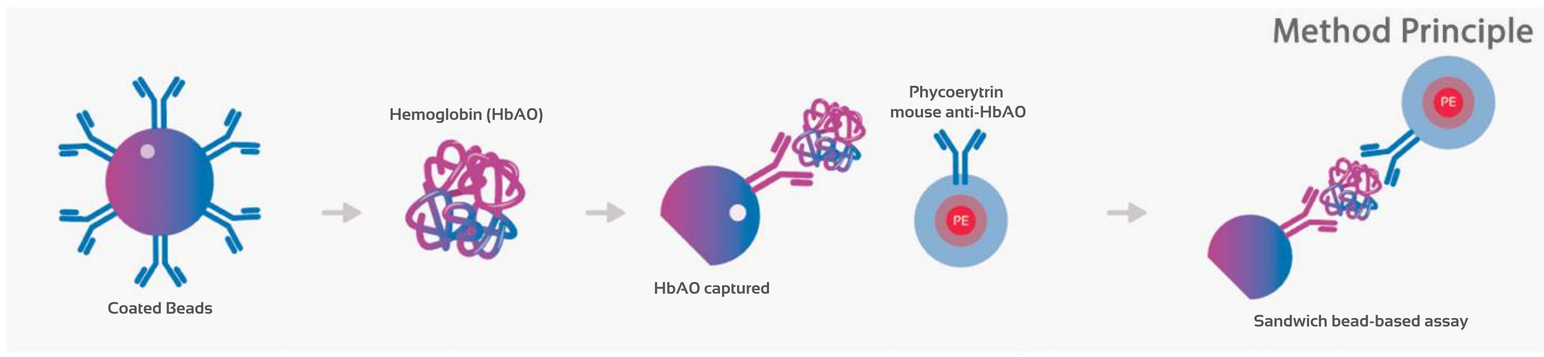
INTRODUCTION

Analysis of cells in cerebrospinal fluid (CSF) is an important clinical procedure for diagnostic and prognostic classification of a wide variety of diseases. The presence of peripheral blood cells, due to peripheral blood contamination, in CSF could complicate the analysis and therefore the diagnosis. Flow cytometry (FCM) is the election method to the CSF analysis in patients with hematological malignancies which suspected tumor infiltration in CSF. Thus, evaluation of CSF peripheral blood contamination should be performed in all cases, especially when there are presences of malignant cells in peripheral blood. The current FCM methods for the determination of contamination in CSF consist of in absolute cell counting of peripheral blood contaminant (e.g. red blood cells and neutrophils), however lack sensitivity enough, particularly in those samples where even visibly undetectable contamination by peripheral blood drastically alter the content of CSF. In this sense, we have developed a new method for evaluating CSF contamination, based on the quantification of total hemoglobin (HbO) as a specific marker of RBCs, which circumvents these drawbacks and helps in the interpretation of diagnostic results.

MATERIAL AND METHODS

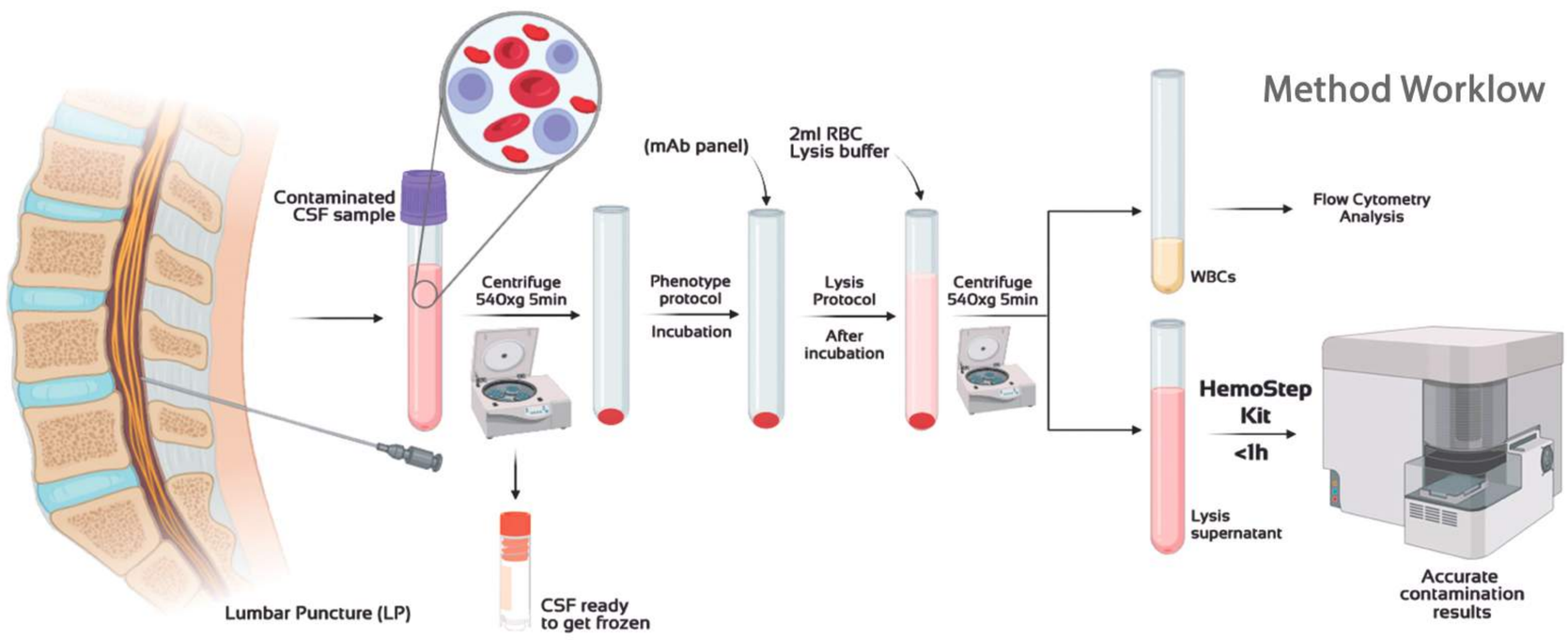
A total of 115 CSF lysis supernatants from patients with infiltrating leukemia's and lymphomas of which 34 showed traumatic punctures, were analyzed by FCM for peripheral blood contamination comparing both classical neutrophil count vs. the new method.

The new method is based on a sandwich bead-based assay that enable the HbO quantification and correlated with the degree of contamination in RBC/µl.



RESULTS

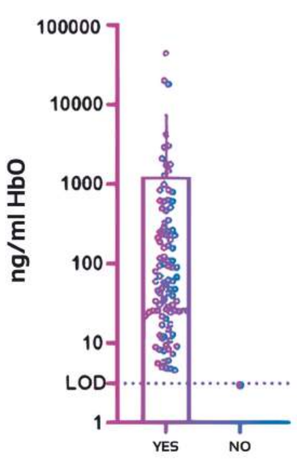
1. Improved method by flow cytometry



Method Worklow

The developed method allows the determination CSF peripheral blood contamination in a <1h protocol circumventing the problems arising from CSF cytotoxicity and minimizes sample usage, which is particularly important in paucicellular samples, by using as sample the supernatant of RBC lysis that is usually discarded.

2. Quantification of visibly undetectable contaminations

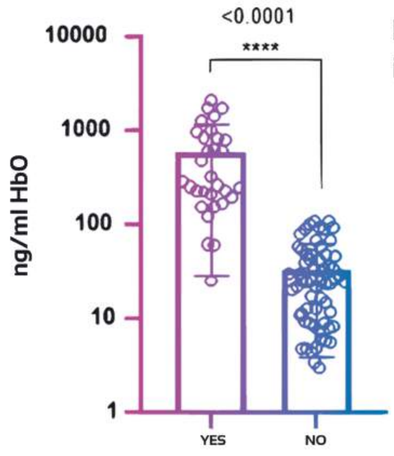


High analytical sensitivity
- LoD: [3,15 ng/ml]

The analytical sensitivity of the kit allows it to detect contaminations lower than 1 RBC/µl.

Expected values of contamination in [RBC/µl] **(n= 115).**

3. Correct classification between traumatic and non-traumatic LP samples with great accuracy



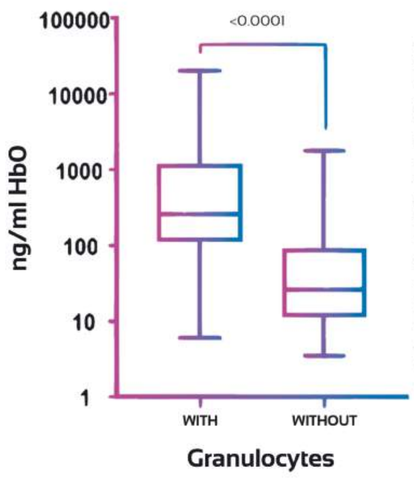
Expected values of contamination in [HbO ng/ml] in traumatic lumbar Puncture. (n= 34).

Better performance than traditional methods

	Kit HbO	Granulocyte count	p
Traumatic LP	34/34 (100%)	25/34 (70%)	<0.001

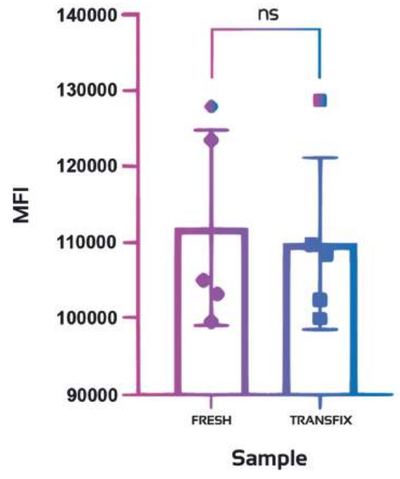
Neutrophil absolute counting method detected contamination in 70% of the samples identified as traumatic punctures, whil the new method detected peripheral blood contamination in 100% of these samples.

4. Agreement between methods



Significant differences between [Hb ng/ml] and in samples contaminated by the presence of granulocytes and non-contaminated samples. In parallel it has been shown a direct linear correlation (r=0.9), between [ng/ml Hb] and n° granulocyte.

5. Compatible with stabilized samples



Comparative between same sample stabilized with Transfix (1:20) and without stabilization (fresh).

CONCLUSIONS

New method:
- Improves very significantly the sensitivity of cell count for the detection of peripheral blood contamination in CSF samples helping to interpret diagnostic results.
- Circumvent the problems derived from CSF in vitro cytotoxicity and minimize sample usage.
- Compatible with samples stabilized with TransFix.
- Easy and quick protocol, compatible with conventional cytometers.

Patented method. Manufactured by Immunostep under USAL license.
"Methods and kits for the detection of cancer infiltration of the central nervous system" / EP2551673B1; US9746472B2

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