

LASER SCANNING CYTOMETRY (LSC) FOR DETERMINATION OF A DIFFERENTIAL BLOOD PICTURE WITH MINIMAL SAMPLE VOLUME

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BACKGROUND

In clinical diagnosis it is often important to obtain as much information as possible out of a minimal specimen volume. Determination of a differential blood picture is essential for most diagnostics. By standard automatic analyzers 500 µl of blood is required for determining a differential blood picture (DBP). In some cases this is a substantial volume for the critically ill patients. Therefore, aim of this study was to develop a method to determine a DBP with substantially reduced specimen volume. This is enabled by a microchamber, which is analyzed cytometrically by the LSC (CompuCyte, Cambridge, MA). With this method only 10 µl EDTA blood is necessary to generate a DBP.

MATERIAL AND METHODS

Blood is mixed with aliquots of DRAQ5 (a DNA dye that penetrates the membrane of vital cells) and an antibody mixture (CD14, CD45). 20 µl of this cell suspension was filled into a Neubauer counting chamber. Due to the defined volume of the chamber it is possible to determine the cell count per volume. DRAQ5 allows the differentiation of nucleated white blood cells and non nucleated erythrocytes. Therefore, trigger was set on DRAQ5 signal to count leukocytes. Different leukocyte subsets could be distinguished due to the used antibodies. For erythrocyte counting cell suspension was diluted another 150x. 20 µl of this dilution was analyzed in the same way with trigger on FSC signal.

RESULTS

This method allows the massive reduction of blood sample volume for determination of a DBP (10 µl instead of 500µl). Comparison with routine laboratory analysis (CELL-DYN 4000, Abbott Laboratories, Abbott Park, IL) showed good correlation ($r^2=0.96$). Also the intra-assay variance of the results obtained with the LSC was less than 7% for all parameters.

CONCLUSION

With this method only 2% of standard sample volume is needed to generate a DBP. In patients with low blood volume such as neonates and in critically ill infants every effort has to be taken to reduce the blood volume needed for diagnostics. Therefore, this method should be established in paediatric cardiology for routine diagnostics and cost reduction.