AUTOMATED RETICULOCYTE ANALYSIS CORRELATION WITH THE MANUAL METHOD

Thesis

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بسم الله الرحمن الرحيم

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LIST OF ABBREVIATIONS

-BFU ... burst forming unit - erythroid.
-CBC ... complete blood picture.
-CFU - E ... colony forming unit erythroid.
-CFU - S ... colony forming unit - S.
-cmm ... cubic millimeter.
-D ... dalton.
-DIFF ... leucocyte differential analysis.
-DMS ... data management system.
-EDTA ... ethylene diamine tetra acetic acid.
-EEF ... erythroblast enhancing factor.
-E/M ... electron microscope.
-EPO ... erythropoietin.
-fl ... fimtoliter.
-FCM ... flow cytometry.
-Hb ... haemoglobin.
-ID ... identification.
-IL - 3 .... interleukin - 3.
-IL - 6 ... interleukin - 6.
-MCH ... mean corpuscular haemoglobin.
-MCHC ... mean corpuscular haemoglobin concentration.
-MCV ... mean corpuscular volume.
-mg ... milligram.
-mm ... millimeter.

-ml ... milliliter.

-PCV ... packed cell volume.

-PDGF ... platelet derived growth factor.

-RBCs ... red blood cells.

-Ret ... reticulocyte.

-RMI ... reticulocyte maturity index.

-RPI ... reticulocyte production index.

-RNA ... ribonucleic acid.

-ug ... microgram.

-ul ... microliter.

-VCS ... volume, conductivity & Coulter opacity and light scatter.

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Each day approximately 0.8 % of the red blood cells pool needs to be replaced by young erythrocytes produced from the bone marrow (**Berliner et al., 1995**).

Reticulocytes are slightly immature erythrocytes in the final stages of differentiation (**Bessis , 1973**) . These cells are somewhat larger than mature erythrocytes , perhaps 20 % greater in volume (**killman , 1964**).

Reticulocyte count is one of the most valuable , simple , and inexpensive methods for the evaluation and classification of anaemias , as well as in monitoring response of certain anaemias to therapy (**Davis and Biglows , 1990**).

Enumeration of reticulocytes has remained a manual microscopic method for a long time using supravital stains as New methylene blue , brilliant cresyl blue and purified azure B . This method depends on the reaction between these basic dyes and the ribosomal RNA remnants in reticulocytes giving bluish granules or filaments (**Bain , 1995**) .

Then flowcytometry was introduced which was used in reticulocyte enumeration depending on the binding of suitable fluorescent dyes as Thiazole orange to the residual red blood cell -RNA (**Hackney et al., 1989**) .

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Recently , reticulocyte counting can be achieved by Coulter Max - M analyzer which is a quantitative , automated , differential cell counter .The Coulter reticulocyte method combines the established methodology of the New methylene blue procedure with the more standardized and greater precision of flow cytometeric analysis .

This technology allows three dimensional analysis using three independent energy probes --- direct current , radio-frequency and a stable helium - neon laser . This analysis provides simultaneous measurement of the cell size (volume) . internal characteristics (conductivity & Coulter opacity),& surface characteristics (light scatter) .

This technique is easy and fast and its results are presented via a graphic printout (***Buttarello et al ., 1996***) .

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