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## Adequacy of platelet counting by automated haematology analyzers: An insight on current methodologies

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Madam, in normal haemostasis, the platelets along with blood vessels and coagulation proteins are the key biological components required to arrest bleeding from an injured blood vessel. Platelets are cytoplasmic fragments of megakaryocytes of the bone marrow and have a diameter of 1-3 $\mu$ m with mean platelet volume (MPV) of 2-20 femtoliter (fL). Approximately 70 to 80 % of platelets circulate in the blood, 20 to 30 % are pooled in the spleen. A normal platelet count ranges from 150,000 to 410,000 platelets per microliter of blood.

Platelet count is one of the three important cell lineages that are stated in every complete blood count (CBC) report. In several clinical conditions, important clinical decisions are based on platelet count reported by the laboratory. This is particularly important in patients who are at risk of bleeding where judicious transfusion can save the patient's life.

Both The International Council for Standardization in Haematology (ICSH) and the International Society of Laboratory Haematology (ISLH) recommend flow cytometric enumeration of platelets utilizing monoclonal antibodies directed against platelet antigens as a reference method (IRM).<sup>1</sup> However, owing to its high cost and rather complicated methodology, this method is not routinely available and restricted to a relatively smaller number of laboratories. In under-developed countries like Pakistan with limited health resources, utilization of methods which are more cost-effective and require minimal technical expertise should be utilized. Other methods for platelet estimation in use include 1) microscopy using a haemocytometer, 2) impedance method and 3) fluorescent optical method.

In impedance measurement, cells are made to pass one after the other through a capillary opening. The resistance produced by the passing cell is measured as electronic signal which is directly proportional to the

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size of cell. Whereas in fluorescent optical method, platelet RNA is first stained with a fluorescent dye followed by flow cytometric counting utilizing semi-conductor laser technology. Due to high specificity of fluorescent dye for platelet RNA, accurate and precise estimation of platelets is obtained regardless of the size of platelets or presence of other interfering substances.<sup>2</sup>

Based on differences in principles of two methods, fluorescent optical method is specifically indicated where interferences in impedance method may lead to inaccurate platelet counts. These conditions include presence of: 1) reticulated and large/giant platelets as in Bernard-Soulier syndrome, Gray platelet syndrome, essential thrombocythemia and immune thrombocytopenia 2) fragments of white and red blood cells<sup>2</sup> as in myelodysplastic syndromes and microangiopathic haemolytic anaemias like disseminated intravascular coagulation and thrombotic thrombocytopenic purpura / Haemolytic uremic syndrome (TTP/HUS), 3) significant RBC microcytosis as in severe iron deficiency anaemia and thalassemia syndromes.<sup>3</sup> Other interferences that may produce inaccurate platelet counts by impedance method include micro clots, blood parasites and reagent contamination. Since fluorescent dye in optical method binds specifically to platelet RNA, accurate platelet count is obtained in disorders of platelet granules as well. Good correlation between fluorescent optical method and IRM has been reported for platelet counts up to 30,000 per microliter of blood.<sup>4</sup> Additionally, Coefficient of variation (CV) values of less than 5% for samples with platelet count less than 20,000 per microliter of blood are well documented in the literature.<sup>4,5</sup>

Although, majority of currently available analyzers are equipped with impedance technique, fluorescent optical technique is increasingly being incorporated in newer models. When both the techniques are present in the same analyzer, software can automatically switch between the two based on pre-defined criteria. Thus, in compliance with good laboratory practice, accurate platelet count can be reported 24/7.

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