A Fluorescent Platelet Count System (PLT-F) could Accurately Replace Manual Verification in Specimens of Thrombocytopenic Cancer Patients

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Abstract
The Sysmex XN-9000 hematology instrument is an automated analyzer that can measure and differentiate red blood cells, white blood cells, and platelets. The instrument uses various technologies to detect platelets, such as electronic impedance (PLT-I) and detection of fluorescent dyes that specifically stain platelets (PLT-F). When platelet accuracy cannot be guaranteed using PLT-I, the count is flagged, and the instrument automatically runs PLT-F. The PLT-F value must be manually verified before reporting the result. Manual platelet count is time-consuming and significantly extends turn-around time. Clinical laboratory test results can be corroborated without manual intervention by a computer-based process known as auto-verification. This proposal aimed to determine whether a non-critical PLT-F value could be auto-verified. Towards this aim, we tested the correlation between the PLT-F value and platelet estimation or manual platelet count method. We found a good correlation between PLT-F and manual platelet counts. We conclude that PLT-F could be auto-verified by the instrument and directly reported to the laboratory information systems (LIS) without being held for technologist review. Enabling the auto-verification of the PLT-F value that meets this criterion can dramatically improve the laboratory’s turn-around time and patient care.

Introduction
Laboratory investigations form a critical part of patient management through accurate diagnostics to administer appropriate treatment. Counting platelets is an essential step in improving the diagnostic procedures and management of hemostasis disorders. The current project centers on platelet counting using the Sysmex XN9000 model. The instrument counts platelets using two different methods:

• PLT-I: Platelet interference is based on the microfluid impedance cytometric techniques to detect the resistance of the particles passing through the orifice. PLT-I is subject to interference from microcytosis and fragmented RBCs, giant platelets, and platelet clumps. Therefore, the impedance method usually yields higher platelet counts.

• PLT-F: Platelet fluorescence is based on fluorescent flow cytometry. After treatment with the fluorescent dyes, the stained particles pass through the side-scattered semiconductor laser first, where the complexity and the granularity of the cell are analyzed. Then the forward-scattered laser measures the cell size. The platelet population, which has a high fluorescence intensity, is differentiated from other particles and cells with low fluorescence intensity.

Methods

• Platelet Estimate: Each specimen had a well-stained smear and was analyzed with a microscope at 100x magnification with immersion oil. The platelet estimates were performed by counting in 10 fields in the smear where the red blood cells are touching but not overlapping. The average number of platelets from ten fields and multiplied by 15 to get a platelet estimate per µL.

• Manual Platelet Count: The platelets are manually counted after red blood cell lysis and platelets disaggregation at 400x magnification under phase microscopy in the 25 small RBC squares on both sides of the hemocytometer. The average platelet count is obtained by averaging the counts from both sides of the chambers.

Results
In this study we used 30 randomly selected blood specimens collected in ethylene-diaminetetraacetic acid (EDTA) tubes at Memorial Sloan Kettering Cancer Center’s Koch Rapid Result Laboratory. Samples were tested within the same day from patients of different ages and genders. All test specimens had platelet results of less than 100 x 10^9 platelets/µL. Concurrently, two laboratory technologists performed platelet estimates and manual platelet count on all the samples in duplicates blindly.

Figure 1: Sysmex XN-9000 Hematology Autoanalyzer at Memorial Sloan Kettering Cancer Center (MSKCC) Koch Rapid Result Laboratory.

• Conventionally hematology analyzers count platelets using a platelet interference (PLT-I) method, which present various limitations and often require manual verification of abnormal counts. An analyzer developed by Sysmex Corporation, Kobe, Japan, performs platelet count using fluorescent flow cytometry (PLT-F), which provides a more accurate count.

• Here we compared two manual methods, manual platelet count and platelet estimate with two results obtained from analyzer PLT-I and PLT-F

• Correlation analysis showed that PLT-F perfectly correlates with the manual platelet count, the most accurate method of platelet quantification.

Figure 2: PLT-F Average Manual Platelet Count Correlation

Figure 3: PLT-F Average Manual Platelet Count Correlation

• The data collected from the completion of all platelet counts generated four scatterplots. The correlation coefficient (r) of 1 indicates a perfect positive linear relationship between variables.

• Non-critical PLT-F can be auto-verified without being held. The PLT-F correlation coefficient to the platelet estimate and manual platelet count has a more significant correlation coefficient (r) than PLT-I.

• Eliminating the unnecessary smear review will improve the turn-around time at the analytical stage, resulting in faster decision-making and patient health management processes and enhancing patient care.

Figure 4: A-D: PLT-I and PLT-F obtained from Sysmex XN-9000, platelet estimate, and manual platelet count performed by laboratory technologists. Average platelet estimate and average manual platelet count calculated by Excel. Scatterplot figures generated by IBM SPSS Statistics - Statistical Analysis Program. Platelet estimate performs first to confirm the Sysmex analyzer; if it doesn’t match, perform manual platelet count act as service for the gold standard for platelet count.

Future Directions

• Considering the feedback from the clinicians about the turn-around time.

• The improved data will be tested with 100 specimens crossing multiple laboratories.

• The results will be compared with different laboratories using the same Sysmex autoanalyzer in MSKCC.

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