Author's Accepted Manuscript

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PII: S2352-5517(16)30042-7 DOI: http://dx.doi.org/10.1016/j.plabm.2017.05.001 Reference: PLABM61

To appear in: Practical Laboratory Medicine

Received date: 8 November 2016 Revised date: 21 April 2017 Accepted date: 1 May 2017

Cite this article as: Masako Kaido, Yuri Takagi, Mari Kono, Fumie Nakazawa, Shiori Yamamoto, Atsushi Wada and Takashi Morikawa, Investigation of morphological changes for the discrimination of nucleated red blood cells an other leukocytes in Sysmex XN hematology analyzer scattergrams using transmission electron microscopy, *Practical Laboratory Medicine* http://dx.doi.org/10.1016/j.plabm.2017.05.001

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ACCEPTED MANUSCRIPT

Investigation of morphological changes for the discrimination of nucleated red blood cells and other leukocytes in Sysmex XN hematology analyzer scattergrams using transmission electron microscopy

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Abstract

Background: The WNR channel of the XN-Series automated hematology analyzer (Sysmex) counts white blood cells (WBCs) and simultaneously performs a differential counting of basophils and nucleated red blood cells (NRBCs). The detection process involves exposing the cells to WNR-specific reagents containing an acidic detergent and a fluorescent dye and measuring the intensity of the forward scattered light (FSC) and side fluorescence light (SFL).

Method: We treated isolated peripheral WBCs and NRBCs with specific reagents and assessed the morphological changes in NRBCs and each leukocyte type using transmission electron microscopy (TEM).

Results: The results from a flow cytometer (FCM) showed that, after exposure to the reagents, basophils appeared on the highest FSC and SFL areas compared to other leukocytes on the WNR scattergram. Owing to the hemolysis of reticulocytes and erythrocytes, NRBCs that survived the reagent treatment could be distinguished by their lower intensity than those of the other leukocytes on the WNR scattergram. We investigated the significance of the relationship between the TEM and FCM results after the reagent treatment.

Conclusion: We confirmed that the WNR channel differentiates the blood cells on the WNR scattergram based on differences in the amount of residual cytoplasm and nucleic acids.

Key words: automated hematology analyzer, flow cytometry, transmission electron microscopy, leukocytes, NRBCs, scatter light intensity, fluorescent intensity

Highlights

•The WNR channel of the XN-Series counts WBCs and simultaneously performs a differential count of basophils and NRBCs.

•Basophils had the highest scattered light and fluorescent intensities after WNR reagent treatment on

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