Instrument-dependent interference of Howell-Jolly bodies in reticulocyte enumeration
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To the Editor,

Reticulocyte enumeration in peripheral blood is an important diagnostic procedure in the evaluation of erythropoiesis in bone marrow. Traditionally, reticulocytes are counted after staining with basic dyes such as Brilliant Cresyl Blue (BCB) or new methylene blue (NMB), which stain the RNA containing reticulofilamentous material in vitally stained unfixed preparation of cells on a glass slide [1]. In subsequent microscopic evaluation, reticulocytes can be discriminated from mature red blood cells by an intracellular deep blue precipitate. Nowadays, state-of-the-art hematology automatic counters replace the labor intensive microscopic evaluation of reticulocytes with quick and reliable enumeration of reticulocytes in whole blood samples [2]. The technique is based on specific dyes, which mostly are polynucleotide-specific, thus binding DNA as well as RNA [2]. Here, we describe a recently observed case where two out of three state-of-the-art hematology analyzers displayed a strong discrepancy between the methods used (Table 1). While Beckman Coulter LH 750 automated counter produced a reticulocyte percentage resembling the BCB staining and manual counting (1.1%, absolute 49 × 10^9/L, reference 30–120 × 10^9/L) both Sysmex XE-5000 and especially CELL-DYN Sapphire counted falsely elevated reticulocytes (94 × 10^9/L and 377 × 10^9/L, respectively, reference 30–120 × 10^9/L).

The interference of nucleic acid containing structures present in erythrocytes in the correct determination of reticulocytes has been described for automatic hematological cell counters. For example, the presence of Pappenheimer bodies, basophilic stippling and Heinz bodies were reported to result in falsely elevated reticulocyte numbers [3]. Malaria parasites in erythrocytes, although they are usually faintly stained, caused spurious elevated reticulocyte counts using CELL-DYN 4000 automatic systems [4]. Also, the presence of Howell-Jolly bodies interferes with the automated reticulocyte
counting using Coulter Epics Profile II flow cytometer, resulting in falsely elevated levels [5]. In our evaluation, counting with Beckman Coulter LH 750 generated reticulocyte numbers comparable to microscopic examination after supravital BCB staining, while CELL-DYN Sapphire and – to a lesser extent – Sysmex XE-5000 counted falsely elevated numbers of reticulocytes. This discrepancy might be explained by the analyzer-specific staining, detection and gating strategies used. Beckman Coulter LH 750 stains blood cells with new methylene blue, a colorimetric dye comparable to BCB, and discriminates reticulocytes from other cells by volume, conductivity, and laser light scatter; the latter signal is proportional to the residual RNA within the red cell. This staining is specific for RNA remnants in reticulocytes [2]. In contrast, Sysmex XE-5000 and CELL-DYN Sapphire instruments use fluorescent dyes, staining polynucleotides within cells. CELL-DYN Sapphire combines a cyanine dye (Sybr II) and narrow angle light scattering to separate reticulocytes from platelets, RBC and nucleated cells [2, 6]. Sysmex XE-5000 uses similar techniques, with polymethylene dye, and forward light scattering differentiating platelets and RBC/reticulocytes based on cell volume. The variation between these two analyzers in reticulocyte concentration possibly results from a combination of different dyes with different subgating strategies.

Overall, the use of polynucleotide-specific fluorescent dye in state-of-the-art hematological analyzers such as Sysmex XE-5000 and CELL-DYN Sapphire can cause pseudoreticolocytosis in the presence of Howell-Jolly bodies or other nucleic acid remnants. This is not observed using an RNA-specific dye in Beckman Coulter LH750 instruments. Therefore, the reticulocyte numbers obtained with automatic hematological counters can be spuriously elevated and need to be interpreted with caution in the presence of nucleic acid containing inclusions. This case underlines that improvement of some automatic hematology analyzers with respect to the detection of reticulocytes is necessary and achievable, thereby contributing to a better standardization of automated reticulocyte enumeration.

Conflict of interest statement

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