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Heinz bodies interfere with automated reticulocyte counts

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Visual reticulocyte counting has traditionally been considered an economical but time-consuming procedure. Nowadays, automated reticulocyte count is common practice in most hematologic laboratories. The blood sample is automatically stained with a fluorochrome, e.g. thiazole orange or auramine-O, and counted using a flow cytometer. The reticulocyte count is expressed as a percentage and/or in absolute numbers. Additionally, based on the intensity of fluorescence detected, automated reticulocyte counters differentiate low (LFR), middle (MFR) and high fluorescence ratios (HFR), which represent different populations of reticulocytes according to their stage of maturation.[1]

A 20-year-old woman diagnosed as having an unstable hemoglobinopathy, Zürich hemoglobin, presented with fever. She had recently undergone splenectomy. The complete blood counts showed: leukocytes: 4.2×10^9/L, platelets: 121×10^9/L and hemoglobin: 102 g/L, with a mean corpuscular volume of 124 fL. Peripheral blood smears stained with May-Grünwald-Giemsa revealed macrocytosis, mild polychromasia, fine basophilic stippling and a few Howell-Jolly bodies. No pleokaryocytes (hypersegmented neutrophils) were observed; folic acid and vitamin B_{12} were within normal limits. Haptoglobin was low: 20 mg/dL. A very high reticulocyte count was detected by two different automated counters (Figure 1), but only one reported a staining error. A crisis of hemolytic anemia or an error in the automated reticulocyte counts was suspected.

Peripheral blood smears stained with supravital brilliant cresyl blue demonstrated an increased number of reticulocytes (8 % of red blood cells). However, a higher population (26%) exhibited round deeply blue dense inclusions which corresponded to Heinz bodies. Most of these inclusions were located eccentrically in erythrocytes, and a few even inside reticulocytes (Figure 2). Heinz bodies are precipitates of denatured hemoglobin that adhere strongly to the red blood cell membrane. They are frequently seen following splenectomy in unstable hemoglobinopathies[2] such as hemoglobin Zürich, a variant characterized by a β-chain amino acid substitution (β63His→Arg).[2]

Automated reticulocyte counting is usually quicker and more accurate than visual counting. However, this case illustrates how the presence of red blood cells with Heinz bodies, which express higher intrinsic autofluorescence than normal red cells,[3] may interfere with the automated count, giving rise to false high reticulocyte values.

	R2000 (Sysmex)	*Vega Retic (ABX)*	*Visual count*
Red blood cells ($\times10^{12}$/L)	2.90	2.85	
Reticulocytes ($\times10^{12}$/L)	0.62	1.46	
Reticulocytes (%)	21.7	51.4	8
(b) LFR (%)	89.0	79.2	
(c) MFR (%)	10.4	16.8	
(d) HFR (%)	0.6	4.0	
Heinz bodies (%)			26

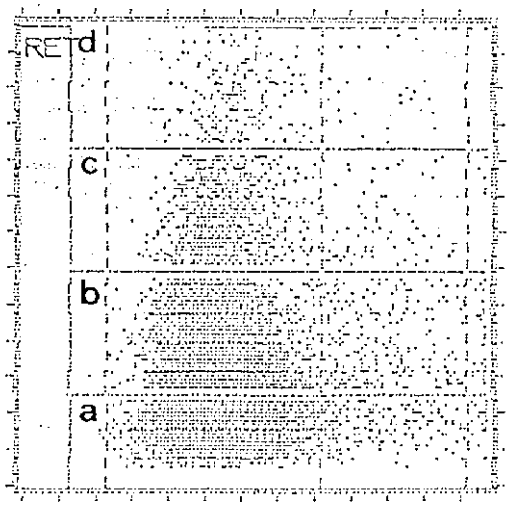

Figure 1. Reticulocyte count provided by two automated counters. Scattergram obtained by flow cytometry technology. The X-axis represents forward scatter and the Y-axis forward fluorescence. Mature red blood cells (a), LFR (b), MFR (c) and HFR (d) areas are shown.

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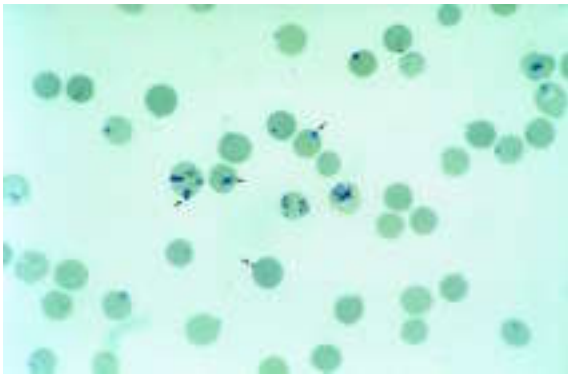

Figure 2. Peripheral blood film showing Heinz bodies (arrows) in erythrocytes as well as in reticulocytes (Brilliant cresyl blue, 1000×).

References

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