Platelet function tests: buffer induced problems

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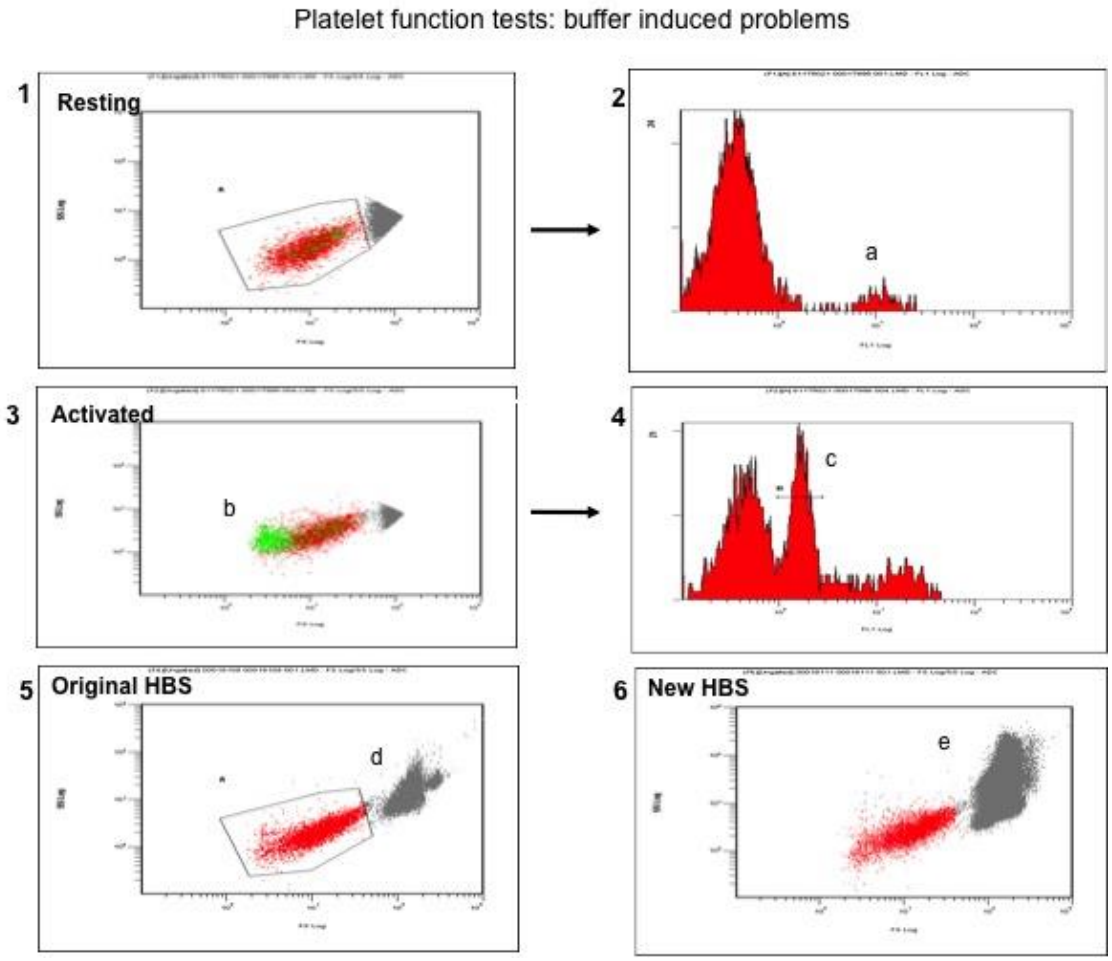

Figures 1 to 6 illustrate a problem induced by a faulty batch of Hepes buffered saline (HBS) used to dilute the whole blood sample. Fig.1 shows the expected forward (FSC)/ side scatter (SSC) plot, with a gate drawn around the platelets, and small portion of the red cells, with higher forward scatter visible through a “live gate”. The histogram in Fig. 2 representing binding of a fluorescently labelled antibody to an activation marker however shows an unexpected positive population (a) in what should have been a completely negative “resting” sample.

When the platelets were activated an unexpected “tail” appeared on the FSC/SSC plot (Fig. 3, area b, in green). Furthermore, the histogram of the activation marker showed an unexpected middle peak (Fig. 4, region c).

Analysis of the FSC/SSC plots without a “live gate” and therefore showing the entire red and white cell populations revealed the source of the problem. The original batch of HBS associated with the unexpected activation had caused “shrinking” of the red cells (Fig. 5, region d) compared with a new batch of HBS (Fig. 6, region e). Such red cell “damage” can result in platelet activation. The data also illustrates the pitfalls of using “live gates” to exclude apparently unwanted events.