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Eliminating or Minimizing the Effects of Cold Agglutinins on the Accuracy of Complete Blood Count Results

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Dear Editor,

I have read the interesting paper by Rim *et al*. [1] regarding the effects of cold agglutinins (CAs) on the accuracy of complete blood count (CBC) results and believe that a number of critical aspects deserve discussion.

Based on a single case of cold agglutinin disease (CAD), the authors propose two optimal sample pre-treatment protocols for eliminating interferences caused by the presence of CAs in the CBC. For this purpose, 16 whole blood dipotassium EDTA (K_2EDTA) samples from the patient were analyzed using four hematological analyzers under different temperature and storage conditions: Sysmex XE-2100 and XN-1000 (Sysmex Corporation, Kobe, Japan), Siemens ADVIA 2120i (Siemens Diagnostics, Tarrytown, NY, USA), and Unicel DxH 800 (Beckman Coulter Inc., Fullerton, CA, USA). Based on their results, the authors propose the following protocols: (i) measuring CBC within one hour if the sample is stored at 37°C after collection and (ii) measuring CBC after incubation at 37°C for one hour if the sample is stored at 4°C for a short period.

The first critical aspect is that neither of these "protocols" is novel or original. Sample collection at 37°C and/or pre-heating before counting was already proposed in 1945 by Finland *et al*. [2] as a method for eliminating CA interferences in cellular counting. Although other methods have been proposed, such as the replacement of plasma with a warm isotonic solution [3], the Finland method is one of the most widely employed to date. More recently, in some hematological analyzers, including Sysmex XE-2100 and XN-1000, the counting in the reticulocyte (RET) channel is sufficient to provide accurate red blood cell (RBC) counts, as well as Wintrobe indices, without thermal pre-treatment of the sample [4-8]. Similar observations have not been described for Unicel DxH 800 and ADIVA 2120i, in which the environmental conditions in the RET channel do not lead to the spontaneous resolution of cold agglutination. Pre-heating at 41°C for one minute has resulted in RBC counts similar to that of the RET channel and that of pre-heating at 37°C for two hours [9].

Apart from the non-originality of the proposed protocols, two other critical aspects should be noted:

(i) The study is based on a single case; this preempts general conclusions and operative suggestions. Clinical features and analytical interferences caused by CA presence in blood depend on many variables such as avidity and the ability to initiate the classic complement pathway. In addition, the thermal amplitude of CA and titer in the blood determine the degree of hemolysis and amplitude of analytical "errors" [10]. Therefore, each CA-containing sample

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can react differently to the pre-analytical treatments, as previously described for 24 out of 48 samples that were not completely corrected even after pre-heating at 37°C for two hours [9].

(ii) The complete reversibility of cold agglutination should be verified in every sample. In fact, regardless of pre-analytical treatment, including short pre-heating at 41°C and counting in the RET channel of Sysmex analyzers, incomplete reversibility of cold agglutination can still be present in samples, in which both the RBC count and the Wintrobe indices have apparently returned to the normal range.

Based on these considerations, only microscopic observation of a slide performed using a sample prewarmed before counting (with the foresight to use a prewarmed glass slide) is useful for excluding (or confirming) the presence of residual RBC agglutination [9].

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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