Q & A column, 11/14

Editor: Frederick L. Kiechle, MD, PhD

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Submit a Question

Which CBC parameters require correction?

How many NRBCs before WBC count correction?

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Q. When performing a platelet count from a blood sample collected in a sodium citrate tube, the result is multiplied by 1.1 to correct for the volumetric difference in anticoagulant compared to EDTA. Which other CBC parameters, if any, should be similarly corrected?

A. Blood specimens for complete blood count testing are usually collected in a lavender-top tube containing ethylenediaminetetraacetic acid (EDTA) anticoagulant. However, EDTA occasionally induces platelet clumping as an in vitro phenomenon. If platelet clumps are flagged by the hematology analyzer and/or identified on the blood smear, steps should be taken to eliminate the clumps because they can artificially decrease the platelet count, referred to as pseudothrombocytopenia.[1] In addition, platelet clumps may be counted as leukocytes, leading to pseudoleukocytosis. The measured mean platelet volume (MPV) may also be affected.

The first step to resolve platelet clumping is to vortex the specimen for one to two minutes at or near the highest setting, which will be successful in approximately 50 percent of cases.[2] If this does not resolve the clumps, the sample should be recollected in a tube containing a non-EDTA anticoagulant, such as a blue-top sodium citrate tube. The platelet count obtained from the sodium citrate tube must be multiplied by 1.1 to account for the different blood-to-anticoagulant ratio in the citrate tube.

In our laboratory, if vortexing does not resolve platelet clumping, we request the specimen be recollected in both EDTA and citrate tubes. Both tubes are then run on the hematology analyzer and slides are made. If platelet clumps are still present in the EDTA tube, the platelet count and white blood cell count from the citrate tube are multiplied by 1.1 and reported. The MPV from the citrate tube is also reported, but no correction factor is applied because the MPV is not affected by dilution. All other CBC parameters are reported from the EDTA tube.

To report CBC data from a citrate tube only, apply the 1.1 correction factor to all parameters reported per unit volume, which includes the red blood cell count, WBC count, platelet count, hemoglobin, and absolute counts for all cell types in the WBC differential.[3] The hematocrit should also be multiplied by 1.1 or, equivalently, should be calculated using the corrected RBC count (Hct = RBC × MCV/10). The MCH (Hgb/RBC × 10) and MCHC (Hgb/Hct × 100) do not require correction because the 1.1 correction factor cancels out in the numerator and denominator of these calculations. The MCV, RDW, and MPV are not affected by dilution and do not require correction.

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2. Gulati GL, Asselta A, Chen C. Using a vortex to disaggregate platelet clumps. *Laboratory Medicine.* 1997;28:665–667.
3. Perrotta G, Roberts L, Glazier J, Schumacher HR. Use of sodium citrate anticoagulant for routine hematology analysis on the CELL-DYN® 4000: an opportunity to enhance efficiency in the clinical laboratory. *Laboratory Hematology.* 1998;4:156–162.

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Q. What are the regulations for corrective action for nucleated red blood cells? How many NRBCs per high-powered field are required to meet criteria to do the mathematical equation to correct the NRBCs? Also, what is the mathematical equation for correcting NRBCs? Do you have a sample policy on correcting NRBCs and an accompanying equation?

A. Clinical laboratories are required to have a procedure for identifying interfering substances and altering test reports accordingly. CAP checklist requirement HEM.30100 (hematology and coagulation checklist, 2014 edition) specifies that a laboratory must have in place a procedure for detecting and correcting automated white blood cell counts for the presence of NRBCs and megakaryocytes. NRBC values are commonly expressed in proportionate terms as number of NRBCs per 100 WBCs. The corrected WBC count using this NRBC value is equal to the total nucleated cell count multiplied by (100/[100 + NRBCs]). Alternatively, absolute values may be derived simply by multiplying the proportion of each cell type including NRBCs by the total nucleated cell count. Expression of WBC differential results in absolute count units ($\times 10^9$/L) is preferred since values are not subject to the inherent problems interpreting relative percentage values.

There is no universally accepted standard level of NRBCs at which WBC count correction must occur. It is up to the laboratory director to establish acceptable limits in consultation with the medical staff; threshold values that trigger a correction of the WBC count should be no higher than is deemed clinically significant. Though the presence of even one or two NRBCs per 100 WBCs may have significant clinical importance, not correcting the WBC count when NRBCs make up less than five percent of total nucleated cells and reporting a proportionately false high WBC count is not likely to have an impact on clinical decision-making. Some laboratories correct the WBC count for any number of measured NRBCs. The impact of NRBCs on leukocyte differential values should also be considered and corrected because many instruments may classify them erroneously as lymphocytes and report false lymphocytosis or miss reporting significant lymphopenia.

There is a great deal of vendor and model differences in automated CBC instruments that determine reliability and manner in which spurious results are detected and what cell types are potentially interfered with. Users should know the details of their instrument’s performance and limitations. Newer instruments are capable of quantifying one percent or more NRBCs—often below levels reliably detected using microscopic-based methods—and automatically report only the corrected cell counts.

1. de Keijzer MH, van der Meer W. Automated counting of nucleated red blood cells in blood samples of newborns. *Clin Lab Haematol.* 2002;24:243–245.
2. Kwon MJ, Nam MH, Kim SH, et al. Evaluation of the nucleated red blood cell count in neonates using the Beckman Coulter UniCel DxH 800

analyzer. *Int J Lab Hematol.* 2011;33:620–628.
3. Zandecki M, Genevieve F, Gérard J, Godon A. Spurious counts and spurious results on hematology analyzers: white blood cells, red blood cells, hemoglobin, red cell indices, and reticulocytes. In: Kottke-Marchant K, Davis BH, eds. *Laboratory Hematology Practice.* Hoboken, NJ: Wiley-Blackwell/International Society for Laboratory Hematology; 2012:75–95.

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