# Q&A column, 9/17

**Editor: Frederick L. Kiechle, MD, PhD**

Submit your pathology-related question for reply by appropriate medical consultants. CAP TODAY will make every effort to answer all relevant questions. However, those questions that are not of general interest may not receive a reply. For your question to be considered, you must include your name and address; this information will be omitted if your question is published in CAP TODAY.

Submit a Question

***Q. I received a sample with very high hemoglobin grossly. When I ran the sample on the Cell-Dyn Ruby, it was unable to calculate the parameters related to Hgb. I diluted the EDTA blood and ran the test again. In this scenario, should I multiply all the indices and Hgb-related parameters with the dilution factor? Which parameters should I multiply with the dilution factor?***

**A.** The vast majority of automated hematology analyzers, including the Abbott Cell-Dyn Ruby system, determine Hgb (hemoglobin concentration) by first lysing the red cells of a known volume of input sample, diluting the lysate in an oxidant-containing solution, and inferring the hemoglobin concentration by absorptiometry relative to known standards. In contrast, the RBC parameter (or red blood count) is a directly computed count of the number of red cells in a known dilution of input sample; likewise, the MCV (or mean corpuscular volume) is calculated from the histogram of red cell sizes, which are determined by passing an aliquot of the input sample through a flow cytometer. The remaining red cell parameters including Hct (hematocrit), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), and RDW (red cell distribution width) are typically calculated from these empirically determined results (see *Wintrobe's Clinical Hematology* for the equations defining these values).

Thus, if an input blood sample must first be diluted prior to testing (in cases, for example, of erythrocytosis in which neat-tested samples result in analyzer errors or unexpected results), only *some* of the complete blood count parameters need be adjusted by the dilution factor. Notably, Hgb and RBC will need to be adjusted as both of these quantities will have been reduced by the process of dilution. Since the distribution of red cell size, which is used to determine MCV and RDW, is not altered by dilution of the sample prior to analysis, these parameters are *not* subject to adjustment. However, since Hct is calculated using the RBC (diluted) and MCV (not diluted) values, this parameter must also be adjusted for dilution. Finally, the astute reader will also note that for MCH and MCHC, the dilution factors to adjust both Hgb and RBC will cancel out in their calculations, so these parameters do not need to be adjusted.

It should be noted that laboratories are advised to fully validate their protocols, including those that may necessitate manual sample dilution prior to automated analyses. One easy way to do this in the above context would be to perform a serial dilution process. Linearity of results in such a dilution experiment serves as a means of reassurance that the analysis workflow in the context of sample predilution is valid.


1. Smock KJ, Perkins SL. Examination of the blood and bone marrow. In: Greer JP, Arber DA, Glader B, et al., eds. *Wintrobe's Clinical Hematology.* 13th ed. Philadelphia, Pa.: Wolters Kluwer Health; 2014.
2. Cell-Dyn Ruby [operator's manual]. Abbott Park, Ill.: Abbott Laboratories; 2006.



*Etienne Mahe, MD, MSc, Pathologist, Division of Hematology Department of Pathology and Laboratory Medicine, University of Calgary, Alberta, Canada*

*Member, Hematology/Clinical Microscopy Resource Committee*

[hr]

***Q. How is the heparin therapeutic range established and verified? How many patient samples are needed to establish the range?***

**A.** The anti-factor Xa assay is designed to measure plasma heparin (unfractionated heparin [UH] and low-molecular-weight heparin [LMWH]) levels and to monitor anticoagulant therapy.

Therapeutic ranges of heparin are as follows: LMWH: 0.5–1.2 IU/mL; UH: 0.3–0.7 IU/mL. Prophylactic ranges of heparin are as follows: LMWH: 0.2–0.5 IU/mL; UH: 0.1–0.4 IU/mL.
If APTT is used to measure UH, an anti-Xa assay should be used as a reference method, and citrated plasma samples from a minimum of 40 patients should be used to establish ranges of APTT.

Olson JD. How to validate heparin sensitivity of the aPTT. CAP TODAY. 2004;18(10):72–78.

*Dong Chen, MD, PhD, Pathologist Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minn.*
*Chair, Coagulation Resource Committee*

[hr]

***Q. Is there a regulation that addresses the submission of proficiency testing results with regard to faxing and electronic reporting and the location of testing and reporting?***

**A.** Laboratories have different options for submitting proficiency testing results, such as faxing or electronic submission. They need to follow instructions from the PT provider on how to submit the results. CAP checklist requirements COM.01800 and COM.01900 and CLIA regulation 42 CFR 493.801(b) address the potential problems relating to proficiency testing interlaboratory communication and referral of testing. Laboratories must ensure that the testing is performed at the location of the laboratory for which the proficiency testing was ordered and that the results are reported from that same laboratory location (i.e. same CLIA number/CAP number). The laboratory cannot send the samples or the results to another laboratory prior to the deadline for submission of the results to the PT provider.

*Lyn Wielgos, MT(ASCP), Checklist Editor, CAP Accreditation Programs*
*College of American Pathologists Northfield, Ill.*
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*Dr. Kiechle is a consultant, clinical pathology, Cooper City, Fla. Use the reader service card to submit your inquiries, or address them to Sherrie Rice, CAP TODAY, 325 Waukegan Road, Northfield, IL 60093; srice@cap.org. Those questions that are of general interest will be answered.*