Q&A column, 11/15

Editor: Frederick L. Kiechle, MD, PhD

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Submit a Question

Q. Is there a recommended procedure for or reference article about checking APTT reagent sensitivities (for the identification of factors VIII and IX) when changing lot numbers and reference range?

A. The activated partial thromboplastin time (APTT) clot-based assay is a global test used to detect factor deficiencies in patients with a bleeding diathesis or as a preoperative screen to ensure normal coagulation laboratory parameters before an invasive procedure. Of note, in patients without a history of hemostatic or thrombotic disorders, the literature suggests that preoperative screening with the prothrombin time (PT) or APTT is of little clinical utility as discussed by the Choosing Wisely campaign.[1] An additional use for the APTT is therapeutic monitoring for unfractionated heparin, yet this is being replaced by anti-Xa assay in many institutions.

Fig. 1a

Normal pooled plasma (µL)	Factor deficient plasma (µL)	Predicted factor activity (%)
500	0	100
450	50	90
400	100	80
350	150	70
300	200	60
250	250	50
200	300	40
150	350	30
100	400	20
50	450	10
0	500	0

Sample protocol for preparation of plasma with factor activities ranging from zero percent to 100 percent.

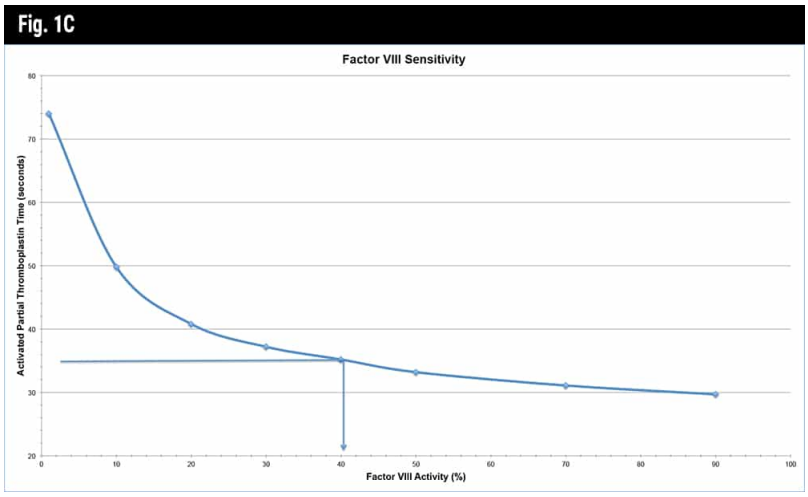
Fig. 1b

Percent activity (%)	APTT (seconds)
90	29.7
70	31.1
50	33.2
40	35.2
30	37.2
20	40.8
10	49.8
0	74.0
Normal APTT reference range	25.3 to 34.5 seconds

Tabulated results of diluted normal pooled plasma with factor deficient plasma yielding percent factor VIII activity and corresponding APTT. The normal reference range of the APTT (mean ± 2 SD) is provided.

It is desirable to have APTT systems that are sensitive to factor levels in the 30 percent to 40 percent range.[2] Having a system in which APTT (or PT) begins to prolong when factor levels are higher than 40 percent is likely to provide little clinical utility and may initiate unnecessary laboratory workups. Conversely, laboratories would like to detect mild factor deficiency (for example, mild hemophilia A) in patients at risk for bleeding during high-risk hemostatic challenges such as neurosurgery. In this scenario, an APTT sensitivity of 20 percent for factor VIII activity would be unacceptably low. Ultimately, there is a balance between being adequately sensitive to factor deficiencies that cause bleeding (factors VIII, IX, and XI) without unnecessarily detecting mild factor deficiencies that do not cause bleeding (factor XII, prekallikrein, high-molecular-weight kininogen).

The Clinical and Laboratory Standards Institute provides guidance for determining the APTT sensitivity to clotting factor deficiencies in its H47-A2 publication.[3] The CLSI recommends performing APTT (or PT) on samples with a range of single factor activity (e.g. zero percent to approximately 100 percent). The dilutions are prepared by mixing assayed normal pooled plasma with assayed factor deficient plasma (**Fig. 1a**). The factor activity and corresponding APTT results for each dilution are tabulated (**Fig. 1b**) and then graphically represented with the APTT values (in seconds) on the y-axis and the percent activity for the factor on the x-axis (**Fig. 1c**) using log-log graph paper. This procedure does not require that the factor activity be measured; rather, the APTT is plotted against the predicted factor activity after mixing with factor deficient plasma. The upper limit of the laboratory's APTT reference interval (mean PTT ±2 SD) is drawn on the graph, and this is the estimate of the sensitivity for that assay reagent-instrument system. The CLSI clearly states, "This value should be considered an estimate and should not be considered absolute, because of variables in the materials used."



It is important to confirm the adequacy of the normal pooled plasma. Plasma should be pooled from at least 20 donors[4] to ensure sufficient factor concentrations (near 100 IU/dL or %), and this information is provided by the commercial manufacturer.[3] Lawrie, et al., found that the CLSI-recommended procedure is misleading.[5] In particular, they found that using different normal pooled plasmas and factor deficient plasmas resulted in varying factor activities for a certain system. In addition, when performing thrombin generation tests (TGT), they discovered the potential to generate thrombin was not completely dependent on the level of component clotting factors. From these data, they suggest lyophilized deficient plasmas may have procoagulant factors exerting an effect on the TGT or the test systems described in the CLSI guidelines.

Thus, the authors recommend that factor sensitivity "should either be determined by the reagent manufacturers for specific instrument/reagent systems or by individual laboratories using well-characterized samples from

patients with inherited coagulation deficiencies. We would suggest testing a minimum of 20 deficient samples with potencies evenly distributed in the range 10–50% for each of the intrinsic coagulation factors (factors VIII, IX, XI and XII).” This recommended procedure will be difficult for most laboratories due to limited access to such factor deficient samples.

Whether performing the APTT sensitivity studies per the CLSI recommendations or by using samples from factor deficient patients, either method can be problematic for smaller laboratories. Many routine coagulation laboratories do not have factor activity assays available in their labs. The CLSI does state that the APTT sensitivity may be obtained from manufacturers or from published studies.[3] Many experts in coagulation laboratories find the CLSI procedure helpful to estimate sensitivity, to assess the adequacy of the upper limit of the reference interval,[2] and to assist in the interpretation of APTT mixing studies. Notably, the CAP does not require that laboratories assess the sensitivity of their PT or APTT reagents with reagent lot changes, but it is good laboratory practice to understand the assay’s performance or, at least, be aware of information provided by the manufacturer.

1. Choosing Wisely, an Initiative of the ABIM Foundation. www.choosingwisely.org/societies/american-society-for-clinical-pathology.
2. Higgins R, Olson J. [Q&A column.] cap today. January 2015, page 55.
3. Clinical and Laboratory Standards Institute. One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline—Second Edition (H47-A2). May 30, 2008.
4. College of American Pathologists. Hematology and Coagulation Checklist. HEM. 37991. July 28, 2015.
5. Lawrie AS, Kitchen S, Efthymiou M, Mackie IJ, Machin SJ. Determination of APTT factor sensitivity—the misguiding guideline. *Int J Lab Hematol.* 2013;35(6):652–657.

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