

FORUM

Should multiple factor dilutions be performed for all patient coagulation factor assays? Let the debate begin!

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Abstract

Laboratory assessment of blood coagulation factors may be undertaken for various reasons, including investigating the possibility of hemophilia or unexpected prolongation in routine coagulation assays (eg, prothrombin time, activated partial thromboplastin time). Several guidelines recommend performing multiple dilutions (usually 2-3) on all patient test samples to evaluate “parallelism” as a guide to the presence of potential “inhibitors,” be they factor inhibitors, lupus anticoagulant, or related to the presence of anticoagulant therapy. The current Forum argues against mandating investigation of parallelism (or multiple dilutions) for all samples destined for testing, instead suggesting that a more targeted approach will likely provide better clinical utility and use of laboratory resources.

KEYWORDS

activated partial thromboplastin time, anticoagulants, blood coagulation factors, inhibitors, lupus anticoagulant

Essentials

- Current Clinical and Laboratory Standards Institute and College of American Pathologists guidelines recommend multiple dilutions for all patient factor assays.
- This can identify “nonparallelism,” which may suggest the presence of an “inhibitor.”
- This Forum argues against this general approach.
- A more focused approach targeting additional testing of abnormal factor results is preferred.

Deficiencies in coagulation factors can lead to bleeding, as commonly seen in congenital deficiencies of factors VIII and IX (hemophilia A and B, respectively). In turn, laboratory assessment of coagulation factors may be undertaken to investigate the possibility of hemophilia.^{1,2} Factor

assays may be alternatively undertaken to investigate unexpected prolongations in routine coagulation assays, including prothrombin time (PT) and activated partial thromboplastin time (aPTT).^{3,4} Factor assays may also be used to monitor replacement therapy in hemophilia.^{1,2}

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The most common method of assessing factor levels is the one-stage clotting assay.¹ Here, the ability of a patient sample to correct a prolonged clotting assay containing a specific factor-deficient plasma (ie, the factor of interest) is assessed relative to a known calibrator plasma reference. The clotting assays represent modifications of either aPTT (eg, factors VIII, IX, XI, and XII) or PT (eg, factors II, V, VII, and X). The number of dilutions undertaken for the plasma standard (ie, the reference calibration curve) varies from laboratory to laboratory or instrument to instrument, according to local preference or expert or manufacturer recommendations, but is usually set between three and seven. Likewise, the number of dilutions undertaken for the patient test plasma (ie, as read off the calibration curve) also varies from laboratory to laboratory or according to local preference or expert or manufacturer recommendations.

One guidance document from the Clinical and Laboratory Standards Institute (CLSI)⁵ recommends at least two dilutions for *all* patient samples, and the College of American Pathologists (CAP) recommends three dilutions.⁶ The premise for multiple patient dilutions therein is to identify potential nonparallelism, which may occur in the presence of “inhibitors,” and that may otherwise lead to false low factor levels. Obviously, such requirements increase test costs and testing complexity but should be undertaken if clinically useful. The aim of the current Forum piece, however, is to raise questions regarding the continued relevance of such recommendations in contemporary times. CLSI guidance documents are followed by laboratories worldwide but particularly in the United States, and CAP guidance is a mandatory requirement for accreditation to CAP accreditation standard, again particularly relevant for US laboratories.

The concept of nonparallelism in the CLSI document⁵ does not solely align to the possibility of lupus anticoagulants (LAs) but also to potential anticoagulant effects (eg, heparin or lepirudin) and factor inhibitors. The premise is that should nonparallelism be observed, then factor activity results may be influenced by these “interfering substances” and should be regarded as incorrect. The CLSI document⁵ was last reaffirmed in 2020 and cites several past papers in support of the recommendation,⁷⁻⁹ the latest published in 2010. The CAP checklist⁵ also cites references^{5,10} to support multiple dilutions/assessment of parallelism, one being a 1995 book chapter,¹⁰ and the other being the CLSI document,⁵ thereby creating a circular argument. Thus, cited CLSI/CAP documents represent dated information, which may no longer be contemporarily relevant.

Another more recent guidance, written on behalf of the British Society for Haematology, Haemostasis and Thrombosis Task Force,¹¹ and in part updating a 2013 guidance from the British Committee for Standards in Haematology,¹² also recommends (for one-stage factor assays) performance of multiple dilutions (“at least three”) for patient test samples to demonstrate parallelism (which may also be lost in patients with inhibitors), further suggesting that agreement of <20% deviation from each other be considered linear or parallel. The 2020 guidance¹¹ cites the CAP checklist⁶ in support of this recommendation, thereby creating more circular arguments that may no longer reflect contemporary relevance.

It is not our intention here to propose that assessment of parallelism never has value in laboratory diagnostics, but rather to argue against “universal” recommendations to assess parallelism as the default position. We will admit our bias here and disclose that routine parallelism assessments are certainly not the default position in our laboratory. Also, the prompt for writing this Forum article was a reviewer comment in relation to a separate peer-reviewed publication of ours that criticized this position. The reviewer strongly conveyed the view that we were not adhering to the CLSI⁵ and CAP⁶ guidance, which ultimately mandates this default position for laboratories seeking CAP accreditation, no doubt including all US accredited laboratory sites. As a laboratory accredited to Australian standards, we instead need to adhere to guidelines from other organizations, namely, the National Pathology Accreditation Advisory Council and the National Association of Testing Authorities, and according to the International Organization for Standardization standard 15189,¹³ none of which mandate assessment of parallelism in patient samples.

So, in 2022, should all laboratories still perform 2 to 3 dilution points for *all* patient factor assays to assess for parallelism? What are the benefits and limitations of such an approach? Some arguments for and against are summarized in Table 1. Within our laboratory, and our broader associated laboratory network, we have invested strongly in routine coagulation test autovalidation processes that in part comprise information gathering up front in relation to anticoagulation status.¹⁴ When routine coagulation tests (ie, PT, aPTT) are electronically ordered, clinicians are required to select, from a drop-down menu, whether a patient is on anticoagulant therapy; notably, electronic orders account for >95% of such orders in our network. Thus, we already know (assuming appropriate clinician compliance) whether a patient is on an anticoagulant and can address subsequent test results accordingly. The autoverification process works to autoverify tests in an equivalent way to what an experienced technician would do in the same situation – that is, normal coagulation test results or results from anticoagulated patients within “expected therapy limits” (Figure 1). Abnormal routine coagulation test results from patients not indicated to be on anticoagulation therapy are instead automatically followed up with additional tests (mixing studies, fibrinogen and/or thrombin times), and these therefore create a composite test panel that often provides sufficient information about likely causes of prolongation, or else will provide guidance to the requesting clinicians around further recommended testing.¹⁴⁻¹⁶ Thus, performing multiple factor dilutions to assess for anticoagulant related nonparallelism has limited value in our network, since most patients on anticoagulant therapy have already been identified, and, indeed, such known presence of anticoagulation may explain any abnormal factor test results.

Another argument against universal assessment of parallelism by means of multiple dilutions for *all* patient samples is that the vast majority of factor assays performed by laboratories will yield normal values; identification of nonparallelism in samples yielding normal values will not be clinically useful in most cases, and instead may lead to adverse events (eg, cause anxiety in requesting clinicians with limited knowledge of hemostasis who are told that their patient

TABLE 1 Arguments for and against assessment of parallelism using multiple patient dilutions for all patients in factor assays

Arguments for	Arguments against
<ul style="list-style-type: none"> Permits assessment of parallelism, which may be lost in the presence of “inhibitors” and that may lead to false low factor levels. “Nonparallelism” may identify LA, certain factor inhibitors, anticoagulants, as the source of abnormal routine coagulation tests (ie, PT, aPTT). Evaluation of parallelism may clarify the situation of patients with concomitant defects (eg, inhibitor or interference + factor deficit, or alternatively inhibitor or interference + specific inhibitor). 	<ul style="list-style-type: none"> Most factor assays performed by laboratories will yield normal values, and identification of nonparallelism associated with normal factor levels may lead to adverse consequences. Thus, further unnecessary testing will be performed to identify the source of the apparent inhibitor. This is costly and a potential waste of laboratory resources, including technician time and test reagent. In the case of clinicians with limited knowledge of hemostasis, this may also lead to anxiety and cancellations in planned surgery, while everyone awaits clarification of the inhibitor by further testing. In 2022, there are better, more direct ways to assess for the presence of inhibitors (ie, preanalytical clinical information requested for routine coagulation orders, specific anticoagulant assays performed on the basis of routine coagulation test results, specific factor/inhibitor assays performed when low factor levels are detected, specific LA assays if required) than indirectly through parallelism checks. Thus, parallelism checks can be more selectively applied. Use of LA-insensitive aPTT reagents, in line with LA guidelines, will avoid generation of abnormal aPTT due to LA that may then require factor testing.

Abbreviations: aPTT, activated partial thromboplastin time; LA, lupus anticoagulant; PT, prothrombin time.

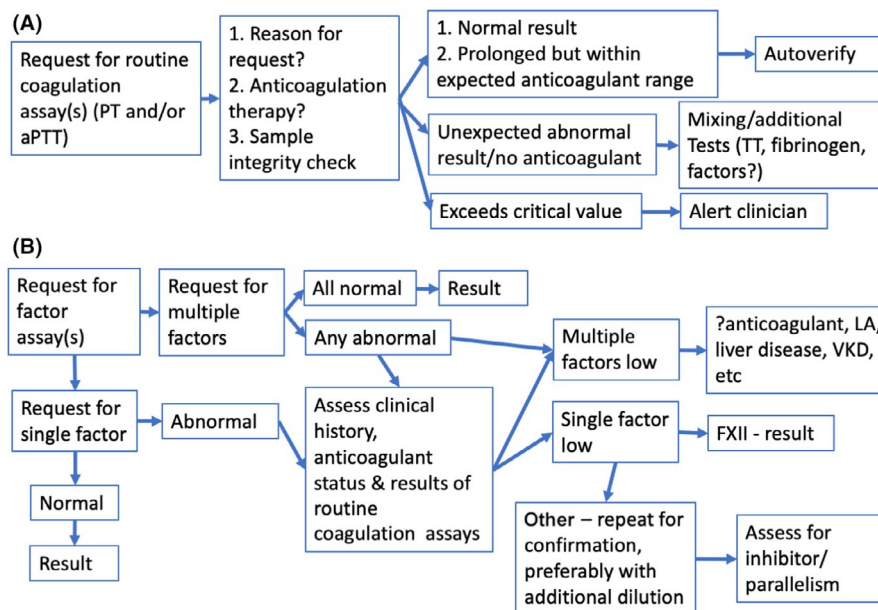


FIGURE 1 One potential algorithm to the investigation of an abnormal coagulation screening assay (A) or to follow-up of factor assay test results (B). This algorithm essentially reflects our current approach, as also based on an expert autoverification process.^{14,15} For part B, we assert that assessment of parallelism should progress only for investigation of abnormal factor test results, and only in cases where anticoagulant status is identified as “none.” As an alternative to parallelism assessment, laboratories could instead perform specific investigations for factor inhibitors, LA, or presence of anticoagulant, as in part differentially informed by findings of part A. Use of an LA-insensitive APTT reagent, in line with LA guideline recommendations,¹⁸ will further reduce the need for factor assay assessments of LA-driven aPTT prolongations. This algorithm may provide a starting point for future discussions regarding parallelism assessments, and hopefully moving us towards more selective use of such assessments. aPTT, activated partial thromboplastin time; FXII, factor XII; LA, lupus anticoagulant; PT, prothrombin time; TT, thrombin time; VKD, vitamin K deficiency

has an inhibitor; this may also lead to delay or cancellation of planned surgeries and/or lead to further otherwise unnecessary testing to identify the source of the inhibitor, which is often an undisclosed anticoagulant or an LA or a factor XII deficiency in an asymptomatic

patient). This situation also reflects a costly waste of laboratory resources including technician time and test reagents.¹⁶ For example, a recent 10-year data capture audit of our practice identified performance of 7561 factor VIII assays, of which 83.3% were normal.

What, if any, value is there in ultimately finding an undisclosed anticoagulant or an LA or indeed a FXII deficiency in an asymptomatic patient in the case of normal factor results? Thus, we would assert that rather than having parallelism as the default position for all patient samples, a case can be made to target such assessments only to abnormal factor results (Figure 1), thereby reducing the burden of multiple factor dilutions from 100% of patients (7561 factor VIII assays in our example) to 16.7% (ie, only abnormal factor assay results). Thus, we propose that only abnormal factor levels should be further followed up in some way, be it by repeat or extended testing or by assessment of LA or performance of specific anticoagulant assays (eg, when multiple factor levels are low and the results of other tests are suggestive of same), or assessment of specific inhibitor assays (ie, when this is indicated by laboratory test results) (Figure 1). Automated analyzers are very accurate; normal factor levels, even if obtained using a single patient dilution, are not likely to be falsely normal. Barring preanalytical issues,¹⁷ even abnormal factor levels are unlikely to be falsely abnormal, but of course these should be further checked and followed up.¹⁴

Another way to avoid the possibility of abnormal aPTT test results by LA is to use an LA-insensitive aPTT reagent as the general screening reagent, in line with current guidelines.¹⁸ Thus, follow-up of abnormal aPTT assays (and need to assess factor assays) due to LA can be avoided. We can highlight two additional potentially useful references.^{19,20} Lawrie et al¹⁹ challenged another CLSI guideline²¹ providing advice on determining factor sensitivity. They reported that aPTT factor sensitivity performed in accordance with this guidance can yield inconsistent and misleading results. They performed factor assays using samples tested at multiple dilutions, thereby facilitating an assessment of linearity and parallelism of dose response and thus detection of “possible inhibitory activity or falsely high results caused by sample activation.” They cited the 2013 guidance document¹² in support of this approach, but again we will highlight another circular argument, given that this document cites the CLSI guidance⁵ in support. Riley et al²⁰ described an automated autoverification process incorporating the use of multiple factor dilutions for factor assays. They cite both the CLSI⁵ and CAP⁶ documents in support of multiple dilutions, again creating more circular arguments that continue to promote use of multiple factor dilutions for all factor assays, but without actual evidence that this practice actually benefits the majority of patients.

We would certainly like to hear of the experience of others in this space, even if views are in opposition to ours. Perhaps this is a topic that can be taken up by an interested ISTH Scientific Standardization Committee. Additional studies could be initiated to appropriately assess the value (or not) of multiple dilutions for all or for select patients. Let the debate begin!

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The authors have no competing interests.

AUTHOR CONTRIBUTIONS

EJF wrote the original draft of this article. All authors contributed content, helped revise the manuscript, and approved its submission. The views expressed herein are those of the authors and are not necessarily those of NSW Health Pathology.

DATA AVAILABILITY STATEMENT

Please contact the lead author.

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