Activated Partial Thromboplastin Time and Prothrombin Time Mixing Studies: Current State of the Art

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Abstract

Mixing studies have long been in the clinical laboratory armamentarium for investigating unexpected, prolonged activated partial thromboplastin time (aPTT) or prothrombin time (PT). The purpose of the mixing study is to identify whether the aPTT/PT prolongation is secondary to a factor deficiency versus an inhibitor, which would present as a "corrected" and "noncorrected" mixing study, respectively. The differentiation between a factor deficiency and inhibitor may likely further direct clinical decisions, including additional diagnostic testing or factor replacement therapy. While aPTT/PT mixing studies are simple tests to perform, there is a lack of standardization for both the testing protocol and the interpretation of what is considered to be a corrected or noncorrected mixing study result. This review will describe the common indications for the mixing test, preanalytic variables that may affect mixing study performance, and describe several methods for interpreting the results of aPTT and PT mixing tests.

Keywords
- activated partial thromboplastin time
- prothrombin time
- mixing study
- factor deficiency
- factor inhibitor

The activated partial thromboplastin time (aPTT) and prothrombin time (PT) are global hemostasis assays used in the evaluation of congenital or acquired bleeding disorders, for monitoring intravenous (e.g., heparin with the aPTT) or oral (e.g., warfarin with the PT) anticoagulant therapy, in the identification of a lupus anticoagulant (LA), and are often used as a preoperative screen to identify the potential for an increased bleeding risk.[1,2] There are several hemostasis factors, when deficient, as well as a variety of inhibitors, either endogenous or exogenous, that can lead to prolongation of the aPTT, PT, or both (see ►**Table 1**).[3] Plasma mixing tests are a useful and frequently performed investigative tool in the hemostasis laboratory to aid in the determination of the cause(s) of a prolonged clotting time and can help determine if the abnormal result reflects the presence of a factor deficiency or inhibitor.[4–6] This distinction is clinically important and may direct further evaluation and treatment. The mixing test is most commonly applied to the aPTT but can be applied to the PT, TT, as well as other clot-based assays such as factor activity assays.[6] The standard mixing test is performed by

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Table 1 Some known causes of prolonged aPTT

Factor deficiencies	Inhibitors
Inherited factor deficiencies	Immunoglobulin-type inhibitors
FVIII/FIX/FXI/FXII/PK/HMWK	Lupus anticoagulant
FII/FV/FX	FVIII inhibitor
Fibrinogen or dysfibrinogenemia	FIX or FXI inhibitor
von Willebrand disease when there is associated FVIII deficiency	FII or FV or FX inhibitor[53]
Acquired factor deficiency	Therapeutic agents
Liver dysfunction	Heparin, heparinoids—unfractionated heparin, low-molecular-weight heparins, danaparoid
Vitamin K deficiency	Thrombin inhibitors: hirudin derivatives, argatroban, dabigatran
Vitamin K antagonists	FXa inhibitors: fondaparinux, apixaban, rivaroxaban, edoxaban, betrixaban
Disseminated intravascular coagulation	Other
Amyloidosis with loss of FX[54]	Dengue fever
Nephrotic syndrome with loss of FXII	
Thrombolytic therapy	

Abbreviation: aPTT, activated partial thromboplastin time.
Notes: The degree to which the aPTT is prolonged depends on the degree of factor deficiency, or on the strength of the inhibitor, and the sensitivity of the aPTT reagent to the deficiency or inhibitor effect.

combining equal amounts of patient plasma (either with a prolonged clotting time or abnormal low factor activity result) with normal pooled plasma (NPP) and repeating the assay that yielded the abnormal result.[5] As a general rule, correction of a mixing test suggests in favor of a factor deficiency, while lack of correction or incomplete correction suggests the presence of an inhibitor. There are a variety of methods that are used to interpret the mixing test (see ►**Table 2**). Variations on the

Table 2 Some approaches to mixing test interpretation

Methods of mix test interpretation
1. Compare the 1:1 mix aPTT to the aPTT upper limit of the RI (URI)[5] – Correction is when the mix aPTT falls within the RI – Noncorrection is when the mix aPTT remains above the URI
2. Calculate the ratio of the 1:1 mix aPTT with the pooled normal aPTT – Correction is when the ratio is below or equal to a predetermined cut-off, e.g., 1.1 or 1.2 – Noncorrection is when the ratio is above a predetermined cut-off, e.g., 1.1 or 1.2
3. Compare the difference between the 1:1 mix aPTT and the pooled normal aPTT to a fixed level, such as 5 seconds – Correction is when a 1:1 mix aPTT falls within 5 seconds of the normal pool aPTT – Noncorrection is when a 1:1 mix aPTT is >5 seconds higher than normal pool aPTT
4. Calculate the Rosner index (ICA),[43] where: ICA $=$ [(1:1 mix aPTT – pooled normal aPTT) / patient aPTT] $\times$ 100 – Correction is when the ICA is below or equal to the predetermined cut-off, e.g., 12.0% – Noncorrection is when the ICA is above the predetermined cut-off, e.g., 12.0%
5. Calculate the percent correction,[42,45] where: %Correction $=$ [(patient aPTT – 1:1 mix aPTT) / (patient aPTT – pooled normal aPTT)] $\times$ 100 – Correction is when the calculated value is above or equal to a predetermined cut-off, e.g., 70% – Noncorrection is when the calculated value is below a predetermined cut-off, e.g., 70%
6. Compare the 1:1 mix aPTT to reference interval derived from aPTT mixing tests on normal donors (MTC).[38–40] The URI will be lower than that for undiluted plasma and thus more sensitive to inhibition – Correction is when the mix aPTT falls within the URI MTC – Noncorrection is when the mix aPTT remains above the URI MTC

Abbreviations: aPTT, activated partial thromboplastin time; ICA, index of circulating anticoagulant; MTC, mixing test-specific cut-off; URI, upper reference interval (value).
Notes: These vary in their complexity and in the amount of available information used in the calculation, such as the aPTT reference interval, the aPTT values of the patient's plasma, the pooled normal plasma, and the 1:1 mixture. Correction is normally expected when the test plasma has factor deficiency. Noncorrection is normally expected when the test plasma contains an inhibitor.

standard mixing tests, such as using four parts patient plasma to one part NPP or adding an incubation step, have value in certain situations. Furthermore, there are also several variables, both patient and laboratory related, that can influence the results and hence analysis of the mixing test results. Currently, standardization of mixing test performance and analysis of mixing test results is lacking. The need for standardization is evidenced through an international study of 990 responding laboratories which revealed large variations in mixing study methodologies with only 49% of participants accurately interpreting all mixing study scenarios correctly.[7] Inappropriate application, performance, or interpretation of mixing tests can lead to improper classification of the cause of the abnormal clotting study, with the potential for patient misdiagnosis and mismanagement.[8] This review will describe the common uses of the mixing test, describe the patient and laboratory variables that can influence mixing test results, and provide common methods for performing and interpreting aPTT and PT mixing tests.

Appropriate Use of the Mixing Test

A mixing test is often indicated in the investigation of an unexpectedly prolonged aPTT or PT, and is used in the investigation of a LA to meet recommended diagnostic criteria that an inhibitor is present.[1,2,9,10] In patients with hereditary or acquired factor deficiency, a mixing test is used to determine the presence or absence of a specific factor inhibitor.[5] A mixing test is not indicated when the baseline clotting time is within the normal reference interval (RI) and many laboratories do not perform mixing tests if the clotting time falls just outside this interval, although guidance as to how prolonged the clotting time should be before the mixing test is performed is lacking.

Before a mixing test is performed, patient history including medications should be obtained. Prior to performance of a mixing test, it should always be determined that the sample was properly collected in accordance with appropriate guidelines.[11–13]

Patient History

A detailed patient history, including medications, should be obtained when possible. It should be ascertained if the patient has a known history of a factor deficiency or an inhibitor. Investigating the patient's current list of medications is of critical importance, as certain agents are known to cause prolongation of various clotting times. Most anticoagulant agents, when administered in therapeutic doses, result in prolongation of the aPTT, PT, TT, or a combination of these three assays (see ►**Table 1**). Several of these agents, including heparin, heparinoids, direct thrombin inhibitors (DTIs), and direct activated factor X (Xa) inhibitors, function as inhibitors resulting in failure to correct the mixing test.[14,15] Mixing tests therefore offer no clinically useful information when patients are on inhibitor-based anticoagulant agents, other than to perhaps confirm potential presence of such agents. If the presence of these inhibitor-acting anticoagulants is not known or suspected and a mixing test performed, significant misinterpretation of mixing study results may occur. DTIs, for example, can perfectly mimic a factor VIII–specific inhibitor in the hemostasis laboratory.[16] Specifically, DTIs may interfere with the FVIII assay, falsely indicating a reduced level, and both FVIII inhibitor and DTI may result in prolongation of a mixing test when incubated.

Some lipoglycopeptide antibiotics active against methicillin-resistant *Staphylococcus aureus* (MRSA) such as daptomycin, telavancin, or other lipoglycopeptides may cause prolongation of the aPTT and/or PT and concentration-dependent lack of correction upon mixing with NPP.[17–19] In addition, certain PEGylated drugs may cause prolongation of the aPTT depending on the type of PEG and the aPTT reagent used.[20,21] C-reactive protein, a protein produced in the liver and elevated in inflammatory conditions, has also been reported to factitiously prolong the aPTT in certain reagents.[22]

Patient Sample

Mixing studies should be performed on the same sample as was reported to exhibit PT and/or aPTT prolongation. The patient sample and NPP should be collected into the same concentration of sodium citrate, either 3.2 or 3.8%, with 3.2% the preferred concentration.[6,12] Serum and heparin- or EDTA-anticoagulated plasma samples are not acceptable sample types for clot-based testing because they result in factitious prolongation of the aPTT and/or PT.[11] Heparin and EDTA plasmas moreover act as inhibitors in the mixing test, while a mixing test using serum leads to correction that is often shorter than the RI due to activation of factors in the clotting process. It is important to note that similar to DTIs, EDTA samples (from an otherwise normal individual) can mimic the presence of a factor VIII–specific inhibitor.[16]

Samples should be collected from a peripheral vein rather than an intravenous line. If samples must be obtained from an intravenous line, local institutional processes should be followed for collecting venous blood from a line to assure adequate intravenous line clearance. If mixing tests will be performed on a newly collected plasma sample (i.e., the original sample was consumed or has exceeded stability), persistence of the abnormality in unmixed plasma should be confirmed before proceeding with the mixing test. Samples for mixing tests should be collected prior to initiating therapy for bleeding or thrombotic disorders as such therapy may alter the patient's clotting studies and thus mask the underlying causes for the prolonged baseline aPTT/PT.

Mixing Study Components

aPTT and PT Reagents

There are a variety of aPTT and PT reagents available commercially and these reagents vary in their sensitivity profiles to each factor deficiency as well as responsiveness to LA and anticoagulant agents.[23] The sensitivity profile reflects the level of factor deficiency or concentration of anticoagulant (e.g., LA or anticoagulant drug) present that is needed to cause prolongation of the aPTT and/or PT.[24–26] According to

the Clinical and Laboratory Standards Institute (CLSI) guideline, aPTT and PT reagents should be manufactured such that an isolated deficiency of factor VII, VIII, IX, or XI in the range of 30 to 35% results in PT or aPTT prolongation, respectively.[6] However, the realities, especially of aPTT reagents that have been commercially available for decades, is that they are often more sensitive than desired or recommended.[24–28] If a reagent has inadequate sensitivity such that prolongation occurs only when an isolated factor falls below 35 to 40%, a normal clotting time could miss a mild factor deficiency. If a reagent is too sensitive (or the normal range established incorrectly), the clotting time will be prolonged when an isolated factor level is 50% or greater such that unnecessary evaluations of prolonged clotting times may be performed. The normal RI for the PT and aPTT should be established following appropriate guidance documents, such as from the CLSI, and must be verified with each change in reagent lot.[29] Appropriate determination of the normal RI is critical to determine when a mixing study may be indicated and for the interpretation of mixing test results.

Normal Pooled Plasma

NPPs can be sourced commercially as frozen or lyophilized material or can be prepared locally from a pool of normal plasma donors. There are limited data to suggest that a frozen format has advantages to lyophilized NPP due to alterations related to the process of lyophilization. Some NPPs may be buffered while others are not. There are no adequate published data to suggest an advantage to buffered or unbuffered NPPs. The average factor activity of a NPP varies based on the number of donors in the pool and their respective factor activity levels. In the ideal circumstance, NPP factor levels are present at greater than 80% activity.[5] Larger numbers of donors used to make the NPP will lead to a greater likelihood that more than 80% activity will be achieved. Commercial sources of NPP should ideally provide information on the factor activity levels within the product insert. If the NPP is to be employed for LA detection, the NPP must be prepared to be sufficiently platelet-poor ($<10 \times 10^9$/L) to reduce occurrences of false negatives arising from preanalytic binding of LAs to platelet phospholipid.[13] If NPPs are prepared locally, a minimum of 15 to 20 ostensibly healthy adult donors who are devoid of anticoagulant treatment for at least 1 week, no strenuous exercise within the past 4 hours, and not on oral contraceptives or hormone replacement therapy, is commonly recommended.[30] Adult donors should be carefully screened and used in the pool only if they have a normal aPTT and PT. A very elevated factor(s) in an NPP such as FVIII and fibrinogen could mask a weak inhibitor and/or mild factor deficiency.

Factor Deficiency(ies)

Factor deficiency, whether hereditary or acquired (by non-inhibitory mechanisms), generally results in correction of the mixing test, although this depends on the number and severity of the deficient factors, factor levels present in the NPP, the aPTT and/or PT reagent sensitivity, and whether the normal RI was appropriately determined.[4,5,29] This is because the added NPP provides the deficient factor typically resulting in a normal clotting time. A mixing test performed on plasmas with multiple factor deficiencies has the chance for false noncorrection, as incomplete (or sometimes referred to as partial) correction of PT mixing tests may be seen in vitamin K deficiency or vitamin K antagonists (e.g., warfarin), which is likely due to multiple low factor levels and the presence of PIVKA proteins.[5]

Factor Specific and Nonspecific Inhibitors

Inhibitors, whether specific or nonspecific, tend to lead to lack or incomplete correction in a mixing test. This is because the inhibitor can inhibit the NPP as well as the patient plasma. Inhibitors can be exogenous or endogenous in origin. Endogenous inhibitors are typically immunoglobulins or immunoglobulin-binding proteins that act as either a nonspecific or specific factor inhibitor, such as LA, or a factor VIII–specific inhibitor, respectively.[16] Factor-specific inhibitors furthermore can be neutralizing or nonneutralizing (clearing). Neutralizing inhibitors cause loss of the target factor activity in the patient's plasma and can inhibit the same target factor in the NPP, so that upon mixing there is failure to achieve complete correct. This behavior is seen with most factor-specific inhibitors (e.g., factors XII, XI, X, IX, some VIII, VII, V, and some II).[31] Neutralizing autoantibodies that occur in patients with acquired factor VIII deficiency, however, may demonstrate correction in the immediate aPTT mixing test, especially when residual functional FVIII levels are apparent in the patient's plasma. Residual factor activity is seen when the autoantibodies possess second-order kinetics and are therefore unable to neutralize the FVIII activity completely.[32] Furthermore, FVIII-specific inhibitors are often time and temperature dependent, such that their neutralizing activity may require incubation at 37 °C for a period of time to be evident.

Although the mixing test is widely used, performance and interpretation are not entirely sufficient to rule out the presence of a specific factor inhibitor. Many studies have evidenced the heterogeneity of methodologies applied to mixing tests that provide a high variation of results among laboratories leading to both false-negative and -positive results.[7,8,16]

Not all inhibitors lead to noncorrection in the mixing test. Specifically, nonneutralizing antibodies bind to the factor and are removed as an antibody–antigen complex by the reticuloendothelial system.[33] For this reason, their behavior in the laboratory is more like that of a factor deficiency. A nonneutralizing (sometimes also referred to as a clearing antibody) antibody can bind factor in the NPP, but this complex cannot be cleared in vitro leading to correction in a mixing test (unless there is a concurrent inhibitor, such as LA causing noncorrection). Correction of a mixing test, therefore, does not consistently rule out the presence of an inhibitor. Antibodies to prothrombin that occur in some patients with LA are often nonneutralizing.[34] To determine the presence of nonneutralizing antiprothrombin antibodies, an immunoassay to detect immunoglobulin G (IgG) antiprothrombin antibodies should

be performed. Not all antibodies identified in the IgG–anti-prothrombin antibody assay, however, result in prothrombin clearance in vivo and, in fact, only a small percentage is functional clearing antibodies. Factor II activity levels should be determined and low factor II activity confirmed. Other methods to detect and measure nonneutralizing antibodies include counterimmunoelectrophoresis assays or the performance of an *in vivo* pharmacokinetic study to calculate the circulating half-life of the affected factor.

Exogenous inhibitors include some anticoagulant drugs and certain antibiotics effective against MRSA.[14,15,17–19] The anticoagulant agents that have in vivo inhibitor effects often have a corresponding in vitro effect resulting in prolonged clotting times and include heparins, hirudin-based anticoagulants, parenteral DTIs, and direct oral anticoagulants.[14,15] These typically yield noncorrection in mixing tests, but there should be no need for a mixing test if the history of drug administration is known. A caveat here is when the degree of aPTT prolongation exceeds that expected from the effect of the drug alone on the aPTT, and further investigation is necessary. It is theoretically possible to neutralize heparins and direct oral anticoagulants from plasma samples prior to performing mixing studies, but these in vitro neutralizing techniques have not been fully vetted for use in aPTT/PT mixing studies.[35,36]

How to Perform the Mixing Test

The mixing test should be performed with the same assay and reagent used to detect the prolonged aPTT and/or PT. The patient mix should ideally be accompanied by two controls, one consisting of factor-deficient plasma and the other inhibitor plasma. Controls are performed with each assay or within an 8-hour time period. The mixing test is most commonly performed using a 1:1 ratio, meaning one-part patient plasma combined with one-part NPP.[4,5] Mixing studies performed with a 4:1 ratio of patient to pooled normal plasma has been recommended in the evaluation of potential weak inhibitors because a weak inhibitor may be masked by the volume of normal plasma present in a 1:1 mix.[37] A caveat is that the addition of only 20% pooled normal plasma may not provide sufficient factor to correct a more marked factor deficiency. Most mixing tests are performed as an immediate mix but in certain circumstances, such as a suspected factor VIII inhibitor, the addition of an incubation step is necessary.

Immediate 1:1 Mixing Test

The immediate 1:1 mixing test is performed by combining equal volumes of patient and NPP. The aPTT and PT are performed in the mix immediately. Certain correction methods may require the NPP to be tested concurrently.

Incubated Mixing Test

The incubated mixing test can be performed based on the aPTT or PT, although it is most commonly applied to the aPTT only. Most mixing tests are performed as an immediate mix, as this will detect most factor deficiencies and inhibitors. Some inhibitors and importantly FVIII-specific inhibitors are time and temperature dependent.[16] This means that the inhibitory effect may not be seen in the immediate mix and requires time at 37 °C to see its effect. Incubated mixing studies should be considered in patients with or suspected of having hereditary or acquired factor VIII deficiency. Acquired factor VIII inhibitors should be considered in patients with an elevated aPTT who present with current bleeding but no prior history of bleeding.

Incubation of the mix is performed in a 37 °C water bath. Sample tubes must be capped during incubation to prevent evaporation. A control should be performed to account for the possible loss of factor over time at 37 °C and change in sample pH over time, although there are insufficient published studies to provide detail as to how this should be accomplished and whether running such a control influences result interpretation.[5,6,32]

A control mixture may also be considered for incubated testing. For control mixture, the patient test plasma and NPP are incubated separately, and then at the conclusion of the incubation period, the two samples are mixed together and retested for aPTT/PT as appropriate. The result of this control mixture should be compared with the result of incubated mixture to assess prolongation secondary to factor's lability associated with the incubation step.

Methods to Determine Correction

Several different methods of evaluating mixing test results have been proposed for determining correction versus noncorrection (see ►**Table 2**). The methods vary in complexity depending on how much test information (such as clotting time of the NPP and the patient's baseline clotting time) is incorporated into the calculations. There is no consensus as to which method is the best. Furthermore, no single method can perfectly discriminate factor deficiency from inhibitor with 100% sensitivity and specificity.[5] Some level of overlap in mixing test results from samples with factor deficiency versus inhibitors is inevitable, whichever method of setting a cut-off is used as was demonstrated in a direct comparison of four different methods.[8,38]

Mixing Test Result Falls within the Normal Reference Interval

One common method of result interpretation is simply to determine if the clotting time of the 1:1 mix falls within the PT or aPTT normal RI for unmixed plasma, the correction to normal suggesting a factor deficiency. This method is easy to implement, although it is not always successful in distinguishing a deficiency from inhibitor. Other subtraction methods to determine correction of the mixing test include comparing the ratio of the 1:1 mix result to the clotting time of the NPP, or subtracting the clotting time of the NPP from the 1:1 mix clotting time.

Moore et al and others have proposed that a specific 1:1 mix RI (one-part normal donor plasma and one-part NPP) be

established to improve efficacy in the detection of weaker inhibitors, particularly LA.[39,40] The mixing study RI is often narrower than the aPTT normal RI, because the NPP partially compensates for clotting times of normal donors at RI extremes. A potential complication when using a mixing test–specific cut-off (MTC) is that a severe factor deficiency may correct to below the upper limit of the RI and not fall within the MTC.[40] Thus, interpretation in light of clinical presentation is crucial. The MTC can be determined in the same fashion as any locally generated RI except that each normal donor plasma is first diluted 1:1 in the same NPP. The MTC must be verified with each new lot of reagent. Use of the same NPP that will be employed in diagnostic testing for mixing with normal donor plasmas is a critical step in the generation of the MTC RI.

On rare occasions, the results of the immediate mixing study are prolonged beyond the baseline aPTT. This is referred to as "LA cofactor effect" and generally occurs in the presence of an LA, but it is not pathognomonic for an LA and can occur in the presence of a factor VIII–specific inhibitor or some anticoagulant drugs. The LA cofactor effect is due to antibody excess and reflects a prozone effect, also known as hook effect.[41] The LA cofactor effect is more likely to occur with a low phospholipid content LA-responsive reagent.

Index of Circulating Anticoagulant and Percent Correction Formula

Some interpretive methods apply equations that evaluate various ratios. Calculating the Rosner index, also known as the index of circulating anticoagulant (ICA), or the percent correction formula, uses the clotting times of the NPP, patient plasma, and the 1:1 mix clotting time[42–45] (see ►**Table 2**). Some calculations can be performed by the coagulation analyzer or middleware, or on occasion the laboratory information system. While like any method, these formulas are not 100% successful in distinguishing factor deficiency from inhibitor.

Variables in the Performance and Interpretation of the Mixing Test

Coagulation testing, including the mixing test, is subject to preanalytic, analytic, and postanalytic variables. Pre-analytic variables of hemostasis testing are well described in other publications and only those specific or pertinent to the mixing test are described here.[46–50] Acknowledging and minimizing these variables will improve the reliability of the mixing test result. These variables are summarized in ►**Table 3**.

An international study of 990 responding laboratories revealed that only 56% of responding laboratories included an incubated mix in those instances where immediate correction of the 1:1 mix occurred, hence leading to the possibility of missing the presence of a time- and temperature-dependent antibody.[7] Rather than progressing to incubated mixing studies, some laboratories may choose to perform factor activity testing. This study, however, did not take the patient's clinical presentation into account and some laboratories may perform an incubated mix only

Table 3 Summary of variables affecting the performance and interpretation of mixing tests

Preanalytic	
Blood collection tube	Use only trisodium citrate[11,12]
Transport	Transport at room temperature and test within 4 hours of collection[11,12,48]
Centrifugation	Removal of platelets to a count of $<10 \times 10^9$/L to prevent neutralization of lupus anticoagulants especially after freeze-thawing[9,25,48]
Presence of anticoagulant drugs	Samples should ideally be free of anticoagulant drugs including heparin and DOACs
Normal pooled plasma	Has factor levels in the region of 100% of normal and is free of inhibitors[5]
Analytic	
aPTT and PT reagents	Each has its own factor deficiency and inhibitor sensitivity profile that will impact results. Responsiveness may vary with lot number change[23–28]
Analyzer type	Changes in clot detection algorithms or methodology need to be validated before use
Normal pooled plasma	Changes in lot number may vary clotting times and hence interpretation, so must be verified before use. Plasma must be used within its established stability window
Timing of immediate and incubated mixing studies	Delays in performing aPTT after mixing test and NPP may reduce the potential difference in aPTTs between immediate and incubated mixing test in the presence of some FVIII inhibitors[5]
Postanalytic	
Calculations and interpretation	Correct calculations must be performed and interpreted according to established cut-off levels[5]
Reporting	Proper selection of interpretive comments for reporting based on calculated parameters

Abbreviations: aPTT, activated partial thromboplastin time; DOACs, direct oral anticoagulants; NPP, normal pooled plasma; PT, prothrombin time.

Table 4 Summary of correct interpretation percentages of mixing tests based on a study using patient samples containing either lupus anticoagulants or factor inhibitors ($n = 26$), or factor deficiency ($n = 62$)[a]

Cut-off method	Cut-off	Inhibitors: number with correct interpretation at designated cut-off	Factor deficiencies: number with correct interpretation at designated cut-off
Difference between 1:1 mix CT and NPP CT	4 s	26/26 (100%)	55/62 (89%)
	8 s	21/26 (81%)	62/62 (100%)
Percent correction	70%	19/26 (73%)	62/62 (100%)
Rosner index	11%	20/26 (77%)	62/62 (100%)

Abbreviations: CT, clotting time; NPP, normal pooled plasma.

[a]Data are derived from this study using immediate mixes and are reported in Kitchen et al.[12] For method 1, the effect of varying the cut-off on the percent of correct interpretations is also shown. For example, at <4 s all lupus anticoagulant samples showed noncorrection, i.e., showed a 1:1 mix value of more than 4 s above the NPP CT, but some of the factor-deficient samples appear to show noncorrection.[31]

in those with a bleeding history. Different models to determine correction can lead to differing classification depending on the method used and the underlying abnormality (see ►**Table 4**). A 2022 international study of external quality assessment programs revealed that up to 50% of participants did not identify the presence of a factor VIII–specific inhibitor using the aPTT mixing study, demonstrating that a screening APTT mix is not sufficient to rule out factor VIII–specific inhibitors.[51] If factor VIII activity levels are low, laboratories may choose to progress to factor inhibitor evaluation. Analytic variability may occur related to the establishment of the cut-offs used to determine correction. Cut-offs that are not correctly established may result in incorrect classification of both factor deficiencies and inhibitors.

Correct interpretation of mixing tests is paramount. Written interpretation should be provided and must be performed by an individual trained and with the understanding of the procedure. Misinterpretation of mixing test results has been reported in a significant percentage of laboratories participating in a large international study as well as external proficiency programs leading to the potential for misdiagnosis and treatment.[52]

Conclusion

Mixing studies for evaluating unexpected, prolonged aPTT and PT have been available for many decades as an aid to practitioners to determine whether the abnormality is secondary to factor deficiency or the presence of an inhibitor. The laboratory should be cognizant of their reagent sensitivity to factor levels as well as patient and test analytic variables that may influence the mixing study result. Due to the lack of standardization for performing mixing studies, as well as limited studies to determine the optimal method for determining mixing study correction or noncorrection, mixing studies are not 100% reliable. Until a more standardized approach and interpretation of mixing studies is achieved, clinical decisions for further testing should not be guided by mixing study results in isolation.

Conflict of Interest
None declared.

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