

## APTT Mixing Study

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### Purpose

- This document describes the procedure for performing an activated partial thromboplastin time (APTT) mixing study to screen patient plasmas for inhibitors of clotting in the intrinsic and common pathways.
  - The rationale for performing a mixing study is to differentiate between a factor deficiency and an inhibitor.
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### Policy

In order to perform the APTT mixing study, the baseline APTT result should exceed the upper limit of the laboratory's defined reference range by 5 seconds or more.

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### Principle

- The APTT mixing study is performed to detect inhibitors of clotting in the intrinsic and common pathways, and to determine whether the prolongation of the APTT is due to deficiency in factor levels or due to a circulating inhibitor, often referred to as a circulating anticoagulant.
  - An APTT is performed on a 1:1 mixture of one part normal pooled plasma (NPP) and one part patient plasma. If the APTT does not correct to within the normal reference range on the immediate mix, the presence of an inhibitor is indicated.
  - If there is correction to within the normal range on the immediate mix, a second APTT must be performed on a timed incubation of the patient and NPP mixture. If the APTT remains corrected to within 3 seconds of the upper limit of the reference range following incubation, a factor deficiency is indicated. If the correction disappears following incubation, the presence of an inhibitor is indicated. This may occur because certain inhibitors, such as Factor VIII inhibitors and about 15% of lupus anticoagulant inhibitors are time-or temperature-dependent.
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### Scope

The intended users of this document include Clinical Laboratory Scientists (CLS) and Laboratory Technical Supervisors handling APTT mixing study samples, issues, or concerns.

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### Specimen sources

Plasma from citrated whole blood (blue top) drawn by venipuncture

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## APTT Mixing Study, Continued

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### Specimen collection and transport

Citrated whole blood (blue top) should be collected, handled, transported and processed in accordance with CLSI Document H21-A5 *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-5<sup>th</sup> Edition*.

- Centrifuge within one hour of collection.
  - Specimens maintained as plasma-based whole blood are stable up to 4 hours.
  - Spun citrated plasma is stable for four hours.
  - If testing cannot be performed within 4 hours of collection, prepare platelet-poor plasma by double centrifugation, then freeze.
  - Refrigeration and transportation of whole blood specimens on ice is not recommended because cold temperatures may lead to a gradual loss of von Willebrand Factor and factor VIII activity.
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### Preanalytical variables

- The plasma should be evaluated to exclude micro clots or fibrin strands by passing a small wooden stick through the sample or by gently inverting the sample tube for possible clotting. The presence of micro clots or fibrin threads could indicate a difficult venipuncture and pre-activation of some of the factors.
  - A high hematocrit or short draw can result in falsely prolonged APTT due to excess citrate anticoagulant.
  - Grossly hemolyzed specimens should be rejected, if possible. APTT values may increase or decrease because cell lysis products include tissue factors that may activate coagulation.
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### Technical Considerations

- NPP should be made from a pool of donors with normal factor levels, and must be fresh frozen and cell free.
  - It is recommended that commercial NPP be used in mixing studies.
  - Alternatively NPP can be prepared in-house from a minimum of 20 donors. Plasma should be stored frozen in 1 or 0.5 ml aliquots. At -70° C, stored NPP will be stable for 6 months. NOTE: Plasma aliquots can alternatively be maintained in a -20°C freezer for up to 6 weeks. If the -20°C freezer has automatic defrost cycles, aliquots must be placed inside a small Styrofoam container inside the freezer.
  - Regardless of source, laboratory must ensure that the APTT of the plasma pool used falls within the laboratory's normal reference ranges.
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## APTT Mixing Study, Continued

**Specialty Products Needed** (either one of these two commercial NPP could be used)

Description	Vendor	Product Number
Cryocheck Pooled Normal Plasma	Precision Biologic	CCN-10, available in 0.5 mL or 1 mL aliquots
Pooled Normal Plasma	George King Biomedical, Inc.	0010-1, available in 0.5 mL or 1.0 mL aliquots

**Equipment**

- Diagnostica Stago Coagulation Analyzer
- Pipettes

**Materials and supplies**

- Pipette tips
- Micro vials
- Micro vial adapters

**Safety**

*Refer to the safety manual for general safety requirements.*

**Quality Control**

- Refer to Stago Quality Control and Start-up Procedures for specific guidelines for performing quality control for APTT assay.
- The APTT assay should be performed and documented on the NPP used in mixing study at the beginning of each mixing study run.
- The APTT result on the NPP used in mixing study should fall within the established normal reference range of the laboratory.
- If performing the incubation study, patient plasma and NPP should also be incubated separately for one hour at 37°C without mixing, and then mixed together for the APTT to be performed. This will serve as a control for the timed incubation, which may affect the stability of factors V and VIII.

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## APTT Mixing Study, Continued

**Procedure:** Follow the steps below to perform the APTT mixing study.

### 1:1 Immediate Mixing Study

Step	Action								
1	Perform APTT on patient plasma alone. Record the result on the APTT mixing study worksheet. <table border="1" style="margin-left: 20px;"> <thead> <tr> <th style="text-align: center;">If...</th> <th style="text-align: center;">Then...</th> </tr> </thead> <tbody> <tr> <td>a. patient <b>baseline APTT</b> is normal (within reference range)</td> <td>a mixing study is not indicated and should not be performed. Choose <b>APTT1</b> as canned comment for the result.</td> </tr> <tr> <td>b. patient <b>baseline APTT</b> is minimally prolonged (&lt;5 seconds from the upper limit of reference range)</td> <td>a mixing study is not performed. Choose <b>APTT2</b> as canned comment for the result.</td> </tr> <tr> <td>c. patient <b>baseline APTT</b> is prolonged (≥5 seconds from the upper limit of reference range)</td> <td>proceed to perform a 1:1 immediate mixing study.</td> </tr> </tbody> </table>	If...	Then...	a. patient <b>baseline APTT</b> is normal (within reference range)	a mixing study is not indicated and should not be performed. Choose <b>APTT1</b> as canned comment for the result.	b. patient <b>baseline APTT</b> is minimally prolonged (<5 seconds from the upper limit of reference range)	a mixing study is not performed. Choose <b>APTT2</b> as canned comment for the result.	c. patient <b>baseline APTT</b> is prolonged (≥5 seconds from the upper limit of reference range)	proceed to perform a 1:1 immediate mixing study.
If...	Then...								
a. patient <b>baseline APTT</b> is normal (within reference range)	a mixing study is not indicated and should not be performed. Choose <b>APTT1</b> as canned comment for the result.								
b. patient <b>baseline APTT</b> is minimally prolonged (<5 seconds from the upper limit of reference range)	a mixing study is not performed. Choose <b>APTT2</b> as canned comment for the result.								
c. patient <b>baseline APTT</b> is prolonged (≥5 seconds from the upper limit of reference range)	proceed to perform a 1:1 immediate mixing study.								
2	Gently mix 200 uL of patient plasma and 200 uL of NPP together in a single plastic tube or instrument micro vial.								
3	Immediately after preparation, perform APTT on mixture. <table border="1" style="margin-left: 20px;"> <thead> <tr> <th style="text-align: center;">If after immediate mixing study...</th> <th style="text-align: center;">Then...</th> </tr> </thead> <tbody> <tr> <td>a. the <b>immediate mix APTT</b> corrects to within normal reference range for APTT</td> <td>an additional mixing study with incubation at 37°C should be performed (see Step 4)</td> </tr> <tr> <td>b. the <b>immediate mix APTT</b> does not correct to within the normal reference range for APTT</td> <td>incubation study is not applicable. Results are suggestive of an inhibitor. Choose <b>APTT5</b> as canned comment for the result.</td> </tr> </tbody> </table>	If after immediate mixing study...	Then...	a. the <b>immediate mix APTT</b> corrects to within normal reference range for APTT	an additional mixing study with incubation at 37°C should be performed (see Step 4)	b. the <b>immediate mix APTT</b> does not correct to within the normal reference range for APTT	incubation study is not applicable. Results are suggestive of an inhibitor. Choose <b>APTT5</b> as canned comment for the result.		
If after immediate mixing study...	Then...								
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## APTT Mixing Study, Continued

**Procedure:** Follow the steps below to perform the APTT incubation mixing study

**Mixing Study  
 with Incubation**

Step	Action						
4	<p><b>Test incubation:</b></p> <ul style="list-style-type: none"> <li>Incubate a 1:1 mixture (e.g. 300µL + 300µL) of patient test plasma and NPP in a single plastic tube for 1 hour at 37 °C. Perform this incubation at the same time as the control incubation step (see Step 5 below).</li> <li>After the 1 hour incubation, run the <b>incubated test APTT</b> on the incubated mixed sample of patient plasma and NPP.</li> </ul>						
5	<p><b>Control incubation:</b></p> <ul style="list-style-type: none"> <li>Incubate 300 µL of patient plasma alone and 300 µL NPP alone in separate plastic tubes for 1 hour at 37 °C. Perform this incubation step at the same time as the patient test incubation step (see Step 4 above).</li> <li>After the 1 hour incubation, gently mix the patient plasma and the NPP from their separate tubes into a single plastic tube or instrument microvial. Load plastic tube or micro vial with the mixture onto instrument and run the <b>incubated control APTT</b>.</li> </ul>						
6	<ul style="list-style-type: none"> <li>For the run to be valid, the <b>incubated control APTT</b> will not increase by more than 3 seconds from the <b>immediate mix APTT</b>.</li> <li>If this is the case and the run is valid, use the following to interpret the patient test result.</li> <li>If this is not the case, see Step 7.</li> </ul> <table border="1" style="margin-left: 20px;"> <thead> <tr> <th style="width: 50%;">If after incubation ...</th> <th>Then...</th> </tr> </thead> <tbody> <tr> <td>a. the <b>incubated test APTT</b> remains corrected to within 3 seconds of the upper limit of the reference range (see <b>Interpretation / Results / Alert Values</b> block below)</td> <td>Mixing studies are suggestive of factor deficiency. Choose <b>APTT3</b> as canned comment for the result.</td> </tr> <tr> <td>b. the <b>incubated test APTT</b> does not remain corrected to within 3 seconds of the upper limit of the reference range (see <b>Interpretation / Results / Alert Values</b> block below)</td> <td>Mixing studies indicate a time- or temperature-dependent factor inhibitor such as factor VIII inhibitor or some lupus anticoagulants. Choose <b>APTT4</b> as canned comment for the result.</td> </tr> </tbody> </table>	If after incubation ...	Then...	a. the <b>incubated test APTT</b> remains corrected to within 3 seconds of the upper limit of the reference range (see <b>Interpretation / Results / Alert Values</b> block below)	Mixing studies are suggestive of factor deficiency. Choose <b>APTT3</b> as canned comment for the result.	b. the <b>incubated test APTT</b> does not remain corrected to within 3 seconds of the upper limit of the reference range (see <b>Interpretation / Results / Alert Values</b> block below)	Mixing studies indicate a time- or temperature-dependent factor inhibitor such as factor VIII inhibitor or some lupus anticoagulants. Choose <b>APTT4</b> as canned comment for the result.
If after incubation ...	Then...						
a. the <b>incubated test APTT</b> remains corrected to within 3 seconds of the upper limit of the reference range (see <b>Interpretation / Results / Alert Values</b> block below)	Mixing studies are suggestive of factor deficiency. Choose <b>APTT3</b> as canned comment for the result.						
b. the <b>incubated test APTT</b> does not remain corrected to within 3 seconds of the upper limit of the reference range (see <b>Interpretation / Results / Alert Values</b> block below)	Mixing studies indicate a time- or temperature-dependent factor inhibitor such as factor VIII inhibitor or some lupus anticoagulants. Choose <b>APTT4</b> as canned comment for the result.						

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## APTT Mixing Study, Continued

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### Mixing Study with Incubation, continued

Step	Action
7	<ul style="list-style-type: none"><li>• If the <b>incubated control APTT</b> increases by more than 3 seconds from the <b>immediate mix APTT</b>, check the temperature of the heating element to see that it is at 37 °C.</li><li>• Repeat the mixing study with incubation (Steps 4-6) at the correct temperature.</li><li>• If the <b>incubated control APTT</b> remains increased by more than 3 seconds from the <b>immediate mix APTT</b> on the repeat study, stop the study and consult a supervisor.</li><li>• The sample may need to be referred to the Regional Reference Laboratories if additional testing is still required.</li></ul>

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## APTT Mixing Study, Continued

**Interpretation /  
 Results / Alert  
 Values**

Use the following guidelines for interpretation of mixing study results (the results for the incubation study assume that the run was valid, such that the **incubated control APTT** does not differ from the **immediate mix APTT** by more than 3 seconds):

<b>Immediate Mix Result</b>	<b>Incubation Study Result</b>	<b>Interpretation</b>
Complete correction: the <b>immediate mix APTT</b> corrects to within APTT reference range	Correction: the <b>incubated test APTT</b> corrects to within 3 seconds of the upper limit of the normal APTT reference range	Results of these studies are suggestive of factor deficiency. ( <b>APTT3</b> )
Complete correction: the <b>immediate mix APTT</b> corrects to within APTT reference range	No correction: the <b>incubated test APTT</b> does not correct to within 3 seconds of the upper limit of the normal APTT reference range	Results of these studies indicate a time-or-temperature-dependent factor inhibitor such as factor VIII inhibitor or some lupus anticoagulants. ( <b>APTT4</b> )
Partial or no correction: the <b>immediate mix APTT</b> does not correct to within APTT reference range	Not applicable	Results are suggestive of an inhibitor. The presence of anticoagulant inhibitor drugs such as heparin or direct thrombin inhibitors cannot be excluded. ( <b>APTT5</b> )

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## APTT Mixing Study, Continued

### Results

- Result APTT 1:1 Mixing Study in LMS by accessing RES,SPE.
- Report clotting time in whole seconds.
- Enter appropriate **Comment Result Value** in result field to attach interpretation text to mixing study results.

**RESULT EXAMPLE**

Result name	Result Value			
PATIENT APTT	68	seconds	←	Patient baseline APTT result
APTT 1:1 MIX (IMMEDIATE)	31	seconds	←	APTT Immediate 1:1 Mix
APTT 1:1 MIX (1HR)	54	seconds	←	APTT 1:1 Mix-1Hr. Incubation
APTT 1:1 MIX CNTL (1HR)	32	seconds	←	APTT1:1 Mix-1Hr. Incubation Control
COMMENT	APTT4	Result Value	←	Select correct Message (Result Value) to attach interpretation text

### Limitations

While this procedure can broadly identify whether a factor deficiency or an inhibitor may be present in the patient sample, it does not identify any one specific factor deficiency or inhibitor by name. Identification of specific factor deficiencies or inhibitors may be performed at the Regional Reference Laboratory if clinically necessary.

### Non-Controlled Documents

The following non-controlled document supports this policy.

Clinical and Laboratory Standards Institute (CLSI). *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition*. CLSI document H21-A5 (ISBN 1-56238-657-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2008.

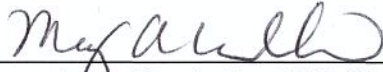


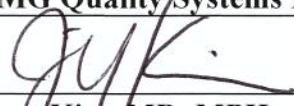
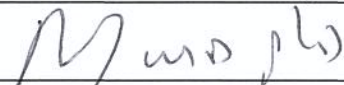
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## APTT Mixing Study, Continued

Reviewed and approved by:

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