Welcome
Jim DeMase, Senior National Technical Sales Manager
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CAMLT/UC Davis Medical Center

Welcome
Your Presenter Today
Jim DeMase, Senior National Technical Sales Manager, Precision BioLogic

Account Manager:
• Western and Central USA
• Western CANADA

Hemostasis Is Unique-The Cascade, the Diseases and the Tests-All Mixed Up
Part 1- What is Hemostasis
Part 2- Intro to Hemophilia
Part 3- Mixing Studies
Objectives

1. Describe the stages of hemostasis as well as common bleeding and thrombotic disorders.
2. Explain the types of hemophilia, symptoms, diagnosis and common treatments.
3. Relate the methods and clinical application for a mixing study and the steps to perform one.

What is Hemostasis?

Pathology

Study of diseases
Especially the structural and functional changes (to the body) caused by the diseases

Many disciplines
Biochemistry
Blood Transfusion Services
Histology
Microbiology
Hematology
Hematology
Science of blood and its diseases
Sub-disciplines:
Immunology
Microscopy
Molecular Biology
Hemostasis (Coagulation)

Hemostasis
The process of stopping bleeding
Greek roots heme, blood + stasis, halt = halt of the blood

Blood
Red viscous liquid in arteries, veins and capillaries
Pumped by the heart
Irrigates every tissue
Transport of gases, nutritive materials and elements for immunity
Blood composition

Cellular
Red cells: hemoglobin
White cells: neutrophils, monocytes, lymphocytes
Platelets: small cells, essential role in prevention of blood loss

Liquid
Plasma: yellow liquid, composed mainly of lightly salted water containing nutritional materials, waste products and numerous different proteins

Proteins in the Plasma
Albumin
Globulins
Coagulation proteins
Procoagulant (e.g. FVII, FIX)
Anticoagulant (e.g. PC, PS, AT)
Fibrinolytic (e.g. Plasmin, PAI)
Hemostasis
A delicate balance
Complex process that stops bleeding at the site of an injury while maintaining normal blood flow elsewhere
When out of balance, hemorrhage or thrombosis can be life-threatening

**BLEEDING:** Anticoagulants

**CLOTTING:** Procoagulants

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**Hemostasis**
Two main phases
Primary hemostasis
Secondary hemostasis

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**Primary Hemostasis**
Vasoconstriction and platelet plug formation
Initial, rapid, short-lived response to vessel damage
Secondary Hemostasis
Activation of coagulation proteins to form a stable fibrin clot
Followed by fibrinolysis, gradual digestion & removal of clot as healing occurs

Coagulation Proteins
A co-dependant group of serine proteases (enzymes) known as "factors"
These factors work together in a pro-coagulant manner to form a clot which will stop bleeding
Factors are typically inactive in circulation until activated
Once activated they form a "cascade", activating each other until a clot is formed

Coagulation Proteins
Each factor belongs to a specific pathway
The Intrinsic and Extrinsic pathways are activated in different ways
Both pathways converge at a "Common" pathway where the coagulation process accelerates into fibrin clot formation
Coagulation Cascade

Inhibitors of Coagulation

Protein C and Protein S
Cofactors that work together to slow down the coagulation process
The activated form of Protein C inhibits Factor V and Factor VIII

Antithrombin
Powerful natural inhibitor that down regulates coagulation
Mostly inhibits thrombin (FIIa) and FXa, FIXa, FXIa and FXIIa are also inhibited to a certain degree
Fibrinolysis
Body’s way of keeping coagulation from becoming excessive and blocking blood vessels
Circulating Plasminogen is activated to become Plasmin, which breaks down the clot into small pieces (FSP, FDP). These pieces are then removed from the body by the liver.

Fibrinolysis

Bleeding Disorders
Hemorrhage
Severe bleeding requiring physical intervention
May be localized or generalized, acquired or congenital
Localized (from a single location) commonly indicates injury, infection, tumor or isolated blood vessel defect
Generalized (from multiple sites, spontaneous/recurring, or requiring medical attention) may indicate defect or disorder and warrant hemostasis laboratory testing
Bleeding Disorders

Congenital vs. Acquired

Congenital are:
- Diagnosed early in life
- Uncommon (<1 in 100 people)

Likely acquired if patient’s bleeding episodes:
- Began after childhood
- Are associated with disease or trauma
- Not present in relatives

Bleeding Disorders

Congenital

Most common congenital:
- von Willebrand disease (VWD)
- Hemophilia A & B (FVIII & FIX deficiencies)
- Platelet function disorders

Bleeding Disorders

Hemophilia A & B

Congenital single-factor deficiencies causing prolonged bleeding

Quite rare
- Affects approximately 1 in 10,000

Usually inherited
- About 30% of those with hemophilia have no family history

Two types
- Hemophilia A, FVIII deficiency
- Hemophilia B, FIX deficiency
Bleeding Disorders
Acquired disorders often associated with bleeding:
- Liver disease
- Vitamin K deficiency
- Renal failure
- Factor VIII inhibitor (auto-antibody against FVIII)

Bleeding may also occur as a result of anticoagulant therapy

Bleeding Disorders
Treatments
- Factor Concentrates
- Frozen Plasma
- Cryoprecipitate
- Red Blood Cell Transfusions
- Plasma Transfusions
- Platelet Concentrate

Bleeding Disorders
Factor Concentrates
- Various sources
  - Plasma-derived (human and porcine)
  - Recombinant

New generation of extended half-life products
Clotting Disorders

Thrombosis

Inappropriate formation of platelet or fibrin clots that obstruct blood vessels causing:

- ischemia (loss of blood supply)
- necrosis (tissue death)

Multifaceted disorder resulting from:

- abnormalities in blood flow
- abnormalities in coagulation system, platelet function, leukocyte activation molecules & blood vessel wall

Venous Thromboembolism (VTE)

Blood clots that form in veins:

- Deep Vein Thrombosis (DVT)
- Pulmonary Embolism (PE)

Occurs when DVT breaks off and flows into the lungs

Arterial Thrombosis

Blood clots that form in arteries:

- Stroke
- Myocardial Infarction

1 in 4 deaths worldwide is related to thrombosis
**Clotting Disorders**

**Thrombophilia**

Predisposition to thrombosis secondary to a congenital or acquired disorder

Theoretical causes:

- Physical, chemical or biological events such as chronic or acute inflammation
- Inappropriate & uncontrolled platelet activation
- Uncontrolled blood coagulation system activation
- Uncontrolled fibrinolysis suppression

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**Clotting Disorders**

**Risk Factors**

**Acquired/non-disease** e.g. age (<50 years); oral contraceptives; diet; femoral or tibial fracture; smoking

**Disease related** e.g. antiphospholipid syndrome; hepatic disease; chronic inflammation (diabetes, cancer, obesity, etc.)

**Congenital** e.g. protein C or S deficiency; hyperfibrinogenemia

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**Most Common Heritable Thrombophilias**

**General Population**

<table>
<thead>
<tr>
<th>Heritable Thrombophilia</th>
<th>Prevalence in general population</th>
<th>Incident VTE prevalence</th>
<th>Relative risk (95% CI)</th>
<th>Recurrent VTE prevalence</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden</td>
<td>7%-1%</td>
<td>12%-20%</td>
<td>4.1±0.9 (5.0)</td>
<td>40%-50%</td>
<td>1.31±0.32</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>60%-1%</td>
<td>5%-6%</td>
<td>16.9±5.4 (4.1)</td>
<td>15%-20%</td>
<td>1.60±0.26</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>60%-1%</td>
<td>1%-5%</td>
<td>25.4±8.1 (16.1-46.4)</td>
<td>5%-10%</td>
<td>2.3±0.7</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>60%-1%</td>
<td>3%-5%</td>
<td>11.3±2.2 (2.2)</td>
<td>5%-10%</td>
<td>2.3±0.7</td>
</tr>
<tr>
<td>Antithrombin deficiency</td>
<td>40%-60%</td>
<td>1%-3%</td>
<td>11.0±3.1 (1.5-23.0)</td>
<td>5%-4%</td>
<td>2.3±0.7</td>
</tr>
</tbody>
</table>

Clotting Disorders

Treatments

First antithrombotic: Heparin
Developed in 1916; FDA-cleared in 1936

Anticoagulants: suppress coagulation & reduce thrombin formation
Oral anticoagulants (e.g. warfarin, direct oral anticoagulants)

Antiplatelet drugs: suppress platelet formation
Oral antiplatelets (e.g. Aspirin)

Fibrinolytics: disperse/reduce existing clots

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# Examples

<table>
<thead>
<tr>
<th>Antithrombotic</th>
<th>Indication</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coumadin</td>
<td>Prevent post-VTE rethrombosis, ischemic stroke</td>
<td>Oral VK antagonist</td>
</tr>
<tr>
<td>UFH</td>
<td>Prevent post-VTE &amp; ACS rethrombosis; intraoperative anticoagulation</td>
<td>IV AT activation, anti-IIa &amp; -Xa</td>
</tr>
<tr>
<td>LMWH</td>
<td>Prevent thromboembolic post surgery, in medical conditions or ACS; DVT/PE</td>
<td>SC AT activation, anti-Xa</td>
</tr>
<tr>
<td>Rivaroxaban</td>
<td>Prevent stroke in NVAF; Treatment of DVT/PE; Reduce risk of DVT/PE; Recurrence; DVT/PE prophylaxis in hip or knee replacement surgery</td>
<td>Oral direct anti-Xa</td>
</tr>
<tr>
<td>Apixaban</td>
<td>Same as rivaroxaban</td>
<td>Oral direct anti-Xa</td>
</tr>
<tr>
<td>SAVAYSA (Edoxaban)</td>
<td>Prevent stroke in NVAF; Treatment of DVT/PE</td>
<td>Oral direct anti-Xa</td>
</tr>
<tr>
<td>Dabigatran</td>
<td>Prevent stroke in NVAF; Treatment of DVT/PE; Reduce risk of DVT/PE; Recurrence; DVT/PE prophylaxis in hip replacement surgery</td>
<td>Oral DTI</td>
</tr>
</tbody>
</table>

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The Other Anticoagulants

- Oral Anticoagulants
  - Warfarin
The New Anticoagulants

- Once a day with the evening meal
- No regular blood monitoring
- No known dietary restrictions

Watch Kevin’s Story

The New Anticoagulants

Eliquis®
(apixaban) tablets
5mg
2.5mg
The New Anticoagulants

Reversal Agents

UPDATE: On October 15, 2015, the FDA granted accelerated approval to Praxbind (andexanet alfa) for use in patients who are taking the anticoagulant Pradaxa (dabigatran) during emergency situations when there is a need to reverse Pradaxa’s blood thinning effects.

ONLY FDA-approved specific reversal agent for a NOAC

Portola Pharmaceuticals is in the process of developing Andexanet alfa, a reversal agent or antidote for all Factor Xa inhibitors, such as Eliquis, Savaysa, or Xarelto.
Laboratory Assessment of Hemostasis

Clinical assessment
Patient presentation and history should drive testing direction

Routine, screening tests
Generally used to rule out the presence of any abnormalities
Typically don’t identify the cause of abnormalities
Further investigation is often required
Also used to monitor anticoagulation therapy

Specialty coagulation tests
Specialized tests that identify the cause of abnormalities
Less frequently performed
Generally more complex than screening tests

Sample Collection
To assess coagulation in vitro, measure the time taken to form a clot
Blood is collected in a 3.2% sodium citrate tube to prevent clotting during transport to lab
Plasma separated from platelets by centrifugation

Potential sources of error in coagulation testing
- Sodium citrate collection tubes not used
- Incorrect plasma to citrate ratio (e.g. under filled tube or patient’s hematocrit >0.55 L/L)
- Heparin contamination of sample (incorrect order of collection or sample taken from central lines)
- Clotting in tube from traumatic venipuncture or inadequate mixing
- Hemodilution of sample

Routine Hemostasis Assays
Prothrombin Time (PT)
Assesses the extrinsic and common coagulation pathways

Activated Partial Thromboplastin Time (APTT)
Assesses the intrinsic and common coagulation pathways

Thrombin Time and Fibrinogen
Assess the function of fibrinogen and possible contamination by heparin

D-dimer
Elevated levels of D-dimers could be indicative of thrombosis
Normal D-dimer levels may rule out DVT and PE
Specialty Hemostasis Assays

Mixing Studies
Patient sample is mixed with Pooled Normal Plasma to assess the presence of an inhibitor or factor deficiency

Factor Assays
Tests that detect the presence of a specific factor deficiency

Thrombophilia Tests
Assays that identify the underlying cause for thrombosis

Abnormalities of natural anticoagulant proteins
  • Protein C, Protein S, Antithrombin

Antiphospholipid Syndrome (APS) assays
  • Lupus Anticoagulants (LA), Antiphospholipid Antibodies

Genetic Abnormalities
  • e.g. Activated protein C resistance (APCR) caused by FV Leiden

Bleeding Disorders

Screening Tests

Prothrombin Time (PT)
Prolonged clotting time may be indicative of a factor deficiency in the Extrinsic and common pathways

Activated Partial Thromboplastin time (APTT)
Prolonged clotting time may be indicative of a factor deficiency in the Intrinsic and common pathways

Thrombin Time / Fibrinogen
Assesses potential for fibrinogen abnormalities

Bleeding Disorders

Confirmatory Tests

Mixing Studies
When either or both the PT/PTT screening assays exceed upper limit of lab's defined reference range
Detect factor deficiencies, LAs and specific inhibitors

Factor Assays
Detect and measure coagulation factor deficiencies

Bethesda Titers
Detect and measure coagulation factor inhibitors
Clotting Disorders

Antiphospholipid Syndrome (APS) Assays

Lupus Anticoagulant

ISTH subcommittee recommends two different tests that represent different assay principles:

1. Diluted Russell Viper Venom Time (dRVVT) widely used in clinical labs & believed to be specific for detecting LA in patients at high risk of thrombosis
2. aPTT test with silica as an activator and low PL content because of sensitivity to LA

Antiphospholipid Antibodies

ISTH recommends Anti-Beta2Glycoprotein 1 and Anticardiolipin antibody tests

- Moderate to high titres (IgG and IgM)

Clotting Disorders

Natural Inhibitor Assays

Protein C, Protein S, Antithrombin

Activity assays measure function
Antigen assays measure quantity
Defects can be either functional or quantitative (or both)

Clotting Disorders

Assays for Other Genetic Abnormalities

Activated Protein C Resistance / FV Leiden

Clot-based screening assays can offer very good sensitivity / specificity
Genetic assays for FV Leiden mutation may reveal specific defect

Prothrombin Gene Mutation

No screening test exists
Genetic assays required

Summary
Hemostasis can be compared to a balance
Careful equilibrium between coagulation and fibrinolysis
maintains blood fluidity
An imbalance can result in either a bleeding or clotting disorder
An accurate diagnosis is important in order to decide on the
correct course of treatment
Screening and specialty tests are available to help clinicians
make a diagnosis
Choosing the correct assays can have a meaningful impact on
patient care

Resources
Publications
Bloody Easy: Coagulation Simplified, 2013, ORBCoN
Journal of Thrombosis and Haemostasis, Wiley
Quick Guide to Hemostasis, 2015, AACC Press
Rodak’s Hematology: Clinical Principles and Applications, 2016,
Elsevier
Websites
hematology.org
worldthrombosisday.org
managedcarehemo.com
fristmaphactor.com

Introduction to Hemophilia
What is Hemophilia?
X-linked congenital bleeding disorder
Those with hemophilia bleed for longer than normal

Quite rare
• Frequency: approx. 1 in 10,000 births
• Estimated 400,000 worldwide

Usually inherited
• About 30% of those with hemophilia have no family history (acquired hemophilia)

Two types
• Hemophilia A, FVIII deficiency
• Hemophilia B, FIX deficiency

When Dinosaurs Still Walked
65 million years ago
The mutation giving rise to hemophilia occurs in at least three orders of placental mammals that existed at the end of the Cretaceous period

2nd Century AD
Rabbi Judah the Patriarch rules 3rd son exempt from circumcision if his two elder brothers died of bleeding after circumcision

Family Ties
1791
Obituary of Isaac Zoll, aged 19, the sixth brother to bleed to death following minor injuries; half-siblings born to a different mother unaffected
1820
Nasse’s law: German physician C.F. Nasse defines the inheritance pattern
1828
Term Haemophilia (love of blood) is first used
The Royal Disease

Queen Victoria: suspected spermatogenesis mutation in her father
Prince Leopold: died of cerebral hemorrhage at 31
Beatrice, carrier: 2 of 3 sons had hemophilia; 1 daughter (carrier) who married Alfonso XIII of Spain – 2 of their sons had hemophilia

Alice, carrier: 1 of her sons had hemophilia & two of her daughters had sons with hemophilia. One of these was Tsarevitch Alexei Nikolaevich

Royal Disease

Tsarevitch Alexei Nikolaevich

Alexei was born on August 12, 1904. He was the youngest child and only son of Emperor Nicholas II and Empress Alexandra Feodorovna. Alexei was the heir to the throne of the Russian Empire. He was born with Hemophilia B (which could be traced back to his maternal great grandmother Queen Victoria). Alexei was killed with his parents and sisters during the Russian Civil War on July 17, 1918.

Famous Faces

Ryan Wayne White
How is Hemophilia Inherited?
X-linked inheritance; males predominantly affected

- Father has hemophilia; Mother not a carrier
  - Sons will not have hemophilia; all daughters will be carriers
  - Father does not have hemophilia; Mother is a carrier
  - Sons will have hemophilia; 50% chance sons will have hemophilia; 50% chance daughters will be carriers

Severity of Hemophilia
Classified on plasma levels of FVIII or FIX activity

<table>
<thead>
<tr>
<th>Level</th>
<th>% of normal factor activity</th>
<th>Occurrences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td>50 – 150%</td>
<td></td>
</tr>
<tr>
<td>Mild hemophilia</td>
<td>&gt; 5 – &lt; 40%</td>
<td>• Might bleed for long time after surgery/injury</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Do not bleed without reason</td>
</tr>
<tr>
<td>Moderate hemophilia</td>
<td>1 – 5%</td>
<td>• Might bleed about 1x/month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Rarely bleed for no obvious reason</td>
</tr>
<tr>
<td>Severe hemophilia</td>
<td>&lt; 1%</td>
<td>• Might bleed 1 or 2x/month</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Might bleed for no obvious reason</td>
</tr>
</tbody>
</table>

Symptoms
Bleeds can occur inside or outside the body; they may begin immediately, after a delay of several hours or spontaneously
- Large/unexplained bruises
- Bleeding into muscles and joints causing swelling, pain and stiffness
- Spontaneous internal bleeding for no obvious reason
- Prolonged bleeding after injury, dental work or surgery
Hemophilia in Women
It's not just a male disease

• Regardless of gender, anyone with < 40% of the normal clotting factor has hemophilia
• Some carriers have symptoms even though their clotting factor levels are above 40%
• A woman with levels of 40-60% who experiences abnormal bleeding is called a symptomatic carrier
• In addition to the usual symptoms, symptomatic carriers and women with hemophilia might experience:
  • Heavy or prolonged menstrual bleeding
  • Postpartum bleeding
  • Other gynecological problems

World Hemophilia Day 2017
April 17 is World Hemophilia Day – a day to raise awareness about hemophilia and other inherited bleeding disorders
This year’s focus is on the women and girls who live with a bleeding disorder or have someone in their lives who does

Hemophilia in Developing Countries
Lack of access to care and treatment is an urgent and important public health challenge due to the cost of products
Globally, 75% of people with bleeding disorders receive inadequate treatment or no treatment at all
Diagnosis is also a challenge
Organizations such as WFH are working to close the gap between the
• number of people born with hemophilia and those who reach adulthood
• estimated and actual number of people diagnosed
• amount of treatment product needed and what is available
Complications

Physical complications
Frequent bleeds may result in debilitating and progressive musculoskeletal lesions and deformations
Neurological deficiencies after intracranial hemorrhage
Infection (drastically reduced since the introduction of sterilized and recombinant factor concentrates)

Psychological and economic complications
Stress, low self-esteem, depression
Limited productivity, time away from work/school
Development of inhibitors

Diagnosis

Prenatal diagnosis can be done at 9-11 weeks by chorionic villus sampling (CVS) or by fetal blood sampling at 18 weeks or more
Newborns to a mother with family history of hemophilia are tested at birth
Severe hemophilia is usually diagnosed before in first year
Mild hemophilia may not be suspected until triggering event in late childhood or later

Diagnosis

Accurate measure of activity is necessary to:
Make a diagnosis
Classify the severity
Monitor therapy

Clinical assessment: detailed bleeding and family history

Routine, screening tests
APTT usually prolonged
PT/INR is normal
TT is normal

Specialty coagulation tests
Mixing studies
Factor assays
Break Time!

Mixing Studies
A first-line investigation
Uses normal pooled plasma mixed with patient plasma to either correct a factor deficiency or be influenced by an inhibitor in that patient plasma when using PT and/or APTT test system. Once differentiation is made, the lab can use algorithms leading to identification of deficient factor or type of inhibitor present.

Factor Assays
Diagnose or monitor treatment
Hemophilia A & B are commonly diagnosed through the use of a modified APTT assay. When a patient sample is mixed with FVIII/FIX deficient plasma, the degree of correction of the APTT is proportional to the level of FVIII/FIX in the patient plasma.
Factor Assays
One-stage clot assay
Based on APPT
Used by majority of clinical labs for all factor activity assays
Many instrument and reagent combinations available

Two-stage clot assay
Rarely performed: complex, cannot be automated, no kit available

Chromogenic assay
Based on two-stage clot assay
Limited availability in clinical labs, considered expensive, often
performed as batched analysis
FVIII: multiple FDA-cleared kits; offered by few labs
FIX: no FDA-cleared kit, offered by few to no labs

Carrier Detection
Hemophilia A
Approximately 90% are detected by measuring the ratio of factor
activity to VWF:Ag value (VIII:VWF)
Effective because VWF production unaffected by FVIII deficiency
Normalizes for variables that affect FVIII activity such as estrogen
levels, stress and exercise
• Establish reference interval using plasma from 30 normal
female donors
• If ratio of patient is below lower limit of interval, she's likely a
carrier
Genetic testing may be necessary to confirm
Carrier Detection

Hemophilia B

Determination of carrier status less successful in hemophilia B
Large number of FIX mutations
Lack of linked molecule such as VWF that can be used as normalization index
DNA analysis may be used when hemophilia B has been diagnosed & its mutation identified in a relative

Carrier Detection

Hemophilia C

Factor XI Deficiency

Common among people of Jewish decent
Prevalence estimated at up to 3% of Ashkenazi Jews
Autosomal recessive
Effects both males and females
Bleeding severity is not influenced by the level of factor XI

Management

Prevent bleeding
Avoid trauma such as:
- IM injections
- Arterial punctures
- Contact sports
Avoid antiplatelet agents and regular NSAIDs (e.g. aspirin)
Avoid herbal medicines suspected to cause bleeding (e.g. ginkgo biloba)
Replace missing factor prior to surgery and dental work
Patients, especially those with severe hemophilia, require regular prophylactic factor replacement therapy on a regular basis
Coordinate patient care with Hemophilia Treatment Center (HTC)
Management
When to Treat?
If serious bleeding or trauma is suspected, treat first
Bleeding into a joint/muscle
Injury to neck, mouth, tongue, face or eye
Severe head blows and unusual headaches
Heavy/persistent bleeding
Severe pain or swelling
Open wounds requiring stitches
Rest, compression, elevation for affected muscles/joints
Follow therapy recommendations; consult Hematology or HTC for advice
Quick treatment helps:
• Reduce pain and recovery time
• Prevent damage to joints, muscles and organs
• Minimize the amount of blood product required to stop the bleeding

Case Study
8-month old male
Uncircumcised
Learning to crawl
Mother noticed swollen knee, which seemed painful and was hot to the touch
Visit with doctor revealed:
Bruising on legs and arms
No definitive family history of bleeding disorders

8-month old male
Screening test results

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>12 sec</td>
<td>10 - 12 sec</td>
</tr>
<tr>
<td>PTT (APTT)</td>
<td>&gt;120 sec</td>
<td>25 - 35 sec</td>
</tr>
<tr>
<td>PLT count</td>
<td>200,000/µL</td>
<td>120 - 440,000/µL</td>
</tr>
</tbody>
</table>

What is the next step?
8-month old male
Factor assay results

<table>
<thead>
<tr>
<th>Factor Assay</th>
<th>Patient</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII</td>
<td>&lt; 1%</td>
<td>50-150%</td>
</tr>
<tr>
<td>FIX</td>
<td>80%</td>
<td>50-150%</td>
</tr>
<tr>
<td>FXI</td>
<td>95%</td>
<td>50-150%</td>
</tr>
<tr>
<td>FXII</td>
<td>93%</td>
<td>50-150%</td>
</tr>
</tbody>
</table>

Factor assays show severe FVIII deficiency; referred to Hemophilia Treatment Center for treatment

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**Treatment Options**

**Clotting Factor Replacement Therapy (prophylaxis)**

<table>
<thead>
<tr>
<th>Factor Replacement Therapy</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII</td>
<td>Replacement therapy given in the form of directlyr subcutaneous bleeding.</td>
</tr>
<tr>
<td>Factor IX</td>
<td>Replacement therapy given to prevent bleeding.</td>
</tr>
<tr>
<td>Factor VIII prophylaxis</td>
<td>Regular continuous replacement therapy started in the absence of documented joint disease, determined by history, examination and/or imaging studies, and defined as the period of time between scheduled prophylactic doses.</td>
</tr>
<tr>
<td>Factor IX prophylaxis</td>
<td>Regular continuous replacement therapy started after two or more joint bleeds following the onset of joint disease documented by physical examination and/or imaging studies.</td>
</tr>
<tr>
<td>Factor VIII episodic prophylaxis</td>
<td>Replacement therapy given to prevent bleeding for periods not exceeding 5 weeks in any year.</td>
</tr>
<tr>
<td>Factor IX episodic prophylaxis</td>
<td>Replacement therapy given to prevent bleeding for periods not exceeding 5 weeks in any year.</td>
</tr>
</tbody>
</table>

**Factor replacement therapy**

Calculation based on baseline level, desired level for clinical bleeding situation and rise in factor expected with replacement

**Factor VIII replacement**: each IU/kg results in 2% rise in factor activity; half-life of 8-12 hours

**Factor IX replacement**: each IU/kg results in 0.5-1% rise in factor activity; half-life of 18-24 hours

<table>
<thead>
<tr>
<th>Situation</th>
<th>Desired Factor Level (IU/mL)</th>
<th>Dose of Recombinant FVIII (IU/kg)</th>
<th>Dose of Recombinant FIX (IU/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor bleed</td>
<td>0.25-0.35</td>
<td>15-20</td>
<td>25-40</td>
</tr>
<tr>
<td>Severe bleed/minor surgery</td>
<td>0.8-1.0</td>
<td>40-50</td>
<td>80-120</td>
</tr>
</tbody>
</table>

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Blonder A: Coagulation Simplified, 2013; ORBCoN, 42.
Treatment Products
Factor Concentrates
Plasma-derived
Widely used in the late 1960s & 1970s
Quality of life improved: home therapy, life-expectancy increased
By the early 1980s, however, epidemic of blood-borne viruses (HBV, HCV, HIV) transmitted by these concentrates
• By 1984, 63% of US hemophilia patients had HIV

Efforts by patient advocacy groups & CDC resulted in donor screening and new manufacturing processes such as dry heat to kill viruses in plasma
CDC surveillance 1998-2002 reports no transmission
Safer treatments were sought
• Cloning of FIX gene in 1982 and FVIII gene in 1984 paved the way for recombinant products

Treatment Products
Recombinant Factor Concentrates
Manufactured using genetically engineered cells that carry a human factor gene
During 1990s, licensed rFVIII and rFIX products became available
Treatment Products
Recombinant Factor Concentrates con’t

2nd Generation
No added human/animal proteins in final product

3rd Generation
No human and animal proteins in growth medium or final product

4th Generation
Next step — extended half-life

FVIII New Generation & Longer Lasting Products

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novo Nordisk</td>
<td>Novoeight®</td>
<td>B-domain truncated recombinant FVIII</td>
</tr>
<tr>
<td>Pfizer</td>
<td>REFACTION®</td>
<td>B-domain deleted recombinant FVIII</td>
</tr>
<tr>
<td>Octapharma</td>
<td>NovoEight®</td>
<td>Human B-domain deleted recombinant FVIII (HEK 293 cells)</td>
</tr>
<tr>
<td>Shire (Baxalta/Baxter)</td>
<td>ADYNOVE®</td>
<td>PE/Glylated Advate – recombinant FVIII</td>
</tr>
<tr>
<td>Novo Nordisk</td>
<td>N8-GP</td>
<td>GlycoPEGylated Truncating Affa</td>
</tr>
<tr>
<td>Bayer</td>
<td>KG-N</td>
<td>PE/Glylated – domain deleted recombinant FVIII</td>
</tr>
<tr>
<td>CSL Behring</td>
<td>rFVIII-SingleChain CSL627</td>
<td>rFVIII-SingleChain (covalently bonded)</td>
</tr>
<tr>
<td>Biogen</td>
<td>ELOCTATE®</td>
<td>Recombinant FVIII FC fusion protein</td>
</tr>
</tbody>
</table>

FIX New Generation & Longer Lasting Products

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shire (Baxalta)</td>
<td>RIXUBIS®</td>
<td>Recombinant FIX with reduced FIXa content</td>
</tr>
<tr>
<td>Pfizer</td>
<td>BeneFIX®</td>
<td>Recombinant FIX (CHO)</td>
</tr>
<tr>
<td>Aptevo BioTherapeutics</td>
<td>IXINITY®</td>
<td>Recombinant FIX with post translational modifications produced in genetically modified CHO cells</td>
</tr>
<tr>
<td>Novo Nordisk</td>
<td>N9-GP</td>
<td>GlycoPEGylated rFIX</td>
</tr>
<tr>
<td>Biogen</td>
<td>ALPROLIX®</td>
<td>Recombinant FIX FC fusion protein</td>
</tr>
<tr>
<td>CSL Behring</td>
<td>IDELVION®</td>
<td>Recombinant FIX Albumin fusion protein</td>
</tr>
</tbody>
</table>
Alternative Treatment Products

Plasma

Cryoprecipitate
- derived from blood & contains moderately high concentration of FVIII
- effective for joint & muscle bleeds
- chance of viral contamination; challenging to store & administer
- can be made at local blood collection facilities

Fresh Frozen Plasma
- red cells removed, leaving blood proteins including FVIII and FIX
- less effective than cryoprecipitate for treating hemophilia A as FVIII is less concentrated
- large volumes of plasma must be transfused; can lead to circulatory overload
- still only product available in some countries

Treatment Products

Treatment For Hemophilia C

No purified concentrate
Must use Fresh Frozen Plasma (FFP)
Factor Inhibitors
Treatment-related complication
Antibodies directed against administered factor concentrates
IgG antibodies that neutralize clotting factors
Render replacement therapy ineffective
More frequently encountered in patients with severe hemophilia
Cumulative incidence:
- Hemophilia A patients
  - Severe: 20 – 30%
  - Moderate/mild: 5 – 10%
- Hemophilia B patients
  - < 5%

Detecting Factor Inhibitors
Replacement therapy patients should be screened for inhibitor development
Confirmation of the presence of an inhibitor and quantification of the titer is performed in the laboratory, preferably using the Nijmegen-modified Bethesda assay


Treating Factor Inhibitors
Greatest problem in the management of hemophilia today
Treatments include:
High-Dose Clotting Factor Concentrates
Bypassing Agents (e.g. NovoSeven®)
Immune Tolerance Induction (ITI) Therapy
Rituxan® (rituximab)

How does immune tolerance induction work?
With immune tolerance induction (ITI) therapy, factor concentrate is given regularly over a period of time until the body is trained to recognize the treatment product without reacting to it. When immune tolerance induction is successful, the inhibitors disappear and the patient’s response to factor concentrates returns to normal. The majority of people who undergo ITI therapy will see an improvement within 12 months, but more difficult cases can take two years or longer.

What factors influence the outcome of immune tolerance induction therapy?
It is still unclear why ITI works better in some people than in others. Factors that have been associated with successful ITI therapy include:
Beginning ITI in people whose inhibitor levels are below 10 BU/mL and ideally below 5 BU/mL.
Beginning ITI in people whose inhibitor levels have never gone higher than 200 BU/mL and have ideally stayed below 50 BU/mL.
Beginning ITI within five years of a person being diagnosed with the inhibitor.

Rituximab in the treatment of acquired factor VIII inhibitors
Blood 2002 100:3426-3428; doi: https://doi.org/10.1182/blood-2002-03-0765

Abstract
Autoantibodies against factor VIII (FVIII) are rare but can cause life-threatening bleeding requiring costly factor replacement and prolonged immunosuppression. We report 4 consecutively treated patients whose acquired FVIII inhibitors responded rapidly to immunosuppressive regimens that included rituximab, a monoclonal antibody against CD20+ B cells.
Summary
Hemophilia is a rare, X-linked congenital bleeding disorder
A - FVIII deficiency
B - FIX deficiency
Bleeds can occur inside or outside the body; may begin immediately, after a delay of several hours or spontaneously
Accurate measure of factor activity is necessary to:
Make a diagnosis
Classify the severity
Monitor therapy
Factor replacement therapy is the preferred treatment
Inhibitors are now the greatest problem in the management of hemophilia

Resources
Publications
Bloody Easy: Coagulation Simplified, 2013, ORBCoN
Journal of Thrombosis and Haemostasis, Wiley
Quick Guide to Hemostasis, 2015, AACC Press
Rodak’s Hematology: Clinical Principles and Applications, 2016, Elsevier
Websites
hematology.org
managedcarehemo.com
fritsmafactor.com
wfh.org
bloodcmecenter.org

Mixing Studies
Mixing Studies
A first-line investigation

Purpose: differentiate a factor deficiency from an inhibitor

Unexpectedly prolonged PT and/or APTT

Exclude pre-analytical variables, e.g.:
- Under-filled or over-filled tube
- Heparin contamination

Perform PT and/or APTT mixing test

CORRECTION:
Perform factor assays if required

NON-CORRECTION:
Perform LA screen or specific coag factor inhibitor assays

Use additional information, e.g.:
- Clinical history
- Anticoagulation therapy?
- APTT with alternate reagent

Case Study #1
32-YO female pre-op screen

Six weeks post-partum

Easy bruising, frequent nosebleeds, menorrhagia

<table>
<thead>
<tr>
<th>Assay</th>
<th>Patient</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>11.8 g/dL</td>
<td>12 - 15 g/dL</td>
</tr>
<tr>
<td>PT</td>
<td>12.4 s</td>
<td>9.8 - 12.6 s</td>
</tr>
<tr>
<td>PTT (APTT)</td>
<td>42.5 s</td>
<td>25 - 35 s</td>
</tr>
<tr>
<td>PLT count</td>
<td>310,000/µL</td>
<td>250 - 450,000/µL</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>320 mg/dL</td>
<td>220 - 498 mg/dL</td>
</tr>
</tbody>
</table>

Isolated, prolonged PTT response? 1:1 PTT mix

Case Study #1
Rule out Heparin, DOAC

R/O unfractionated heparin (UFH) and direct oral anticoagulant (DOAC)
Outpatient: consider DOAC
Inpatient: unrecorded UFH flush of vascular catheter
If DOAC, discontinue testing, cancel order
If UFH, treat w/ Hepsocb (polybrene) or Hepzyme and proceed
If no UFH, perform 1:1 PTT mix to differentiate factor deficiency from factor-specific inhibitor or “non-specific inhibitor” lupus anticoagulant (LA)

PTT Mixing Study
Cheap and Basic
Start testing within 2 h to avoid specimen degradation
- Factors V (FV) and VIII (FVIII) are labile
- Platelet factors (mostly FV) released
- Ensure patient plasma is platelet-poor, < 10,000/uL
Mix plasma 1:1 with pooled normal plasma (NP) and perform immediate PTT on mixture
PTT of 1:1 mix “corrects” to ≤10% above NP PTT
- Factor deficiency
No correction: 1:1 mix is >10% above NP PTT
- Non-specific inhibitor, usually LA
- Specific inhibitor (anti-FVIII), usually requires 37°C incubation

PTT Mixing Study

<table>
<thead>
<tr>
<th>Assay</th>
<th>Patient</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>34 s</td>
<td>&lt;21 s</td>
</tr>
</tbody>
</table>

Example Only — Laboratories should establish local value
PTT Mixing Study
Using 10% Rule

EXAMPLE ONLY — Laboratories should establish local value

Laboratories should establish local value

PTT ≤ 33 s: Correction
> 33 s: No correction

100 uL
1:1 mix
100 uL
PTT
rgt
163 uL
CaCl₂
1:1 mix
+ PTT rgt
+ CaCl₂

1:1 PTT Mix with Incubation
PTT of immediate mix ≤ 10% above NP
• Correction: factor deficiency? But first...
  • Incubate 1:1 mix 1–2 h and repeat
Correction after incubated mix = factor deficiency
No correction: PTT remains > 10% above NP
Specific inhibitor such as anti-FVIII
• IgG4: temp dependent, may require incubation
• However, some FVIII neutralization within 10 min
• May detect in immediate mix

1:1 PTT Mix with Incubation
Only when unincubated mix corrects
Must also incubate normal control plasma
Compare mix PTT to incubated normal control PTT
May also detect temp-dependent LA
• ~15% of LAs are temp-dependent
**37°C Incubated 1:1 PTT Mix**

Unincubated PTT 42.5 s

Incubated PTT 35 s

Incubated PTT of Mix ≤38.5 s: Correction
> 38.5 s: No correction

**Mixing Study Result**
32-YO female, 6 weeks post-partum

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result (s)</th>
<th>Normal Range</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTT control 1:1 mix</td>
<td>32.1 s</td>
<td>Control 30 s</td>
<td>Commercial platelet-free control plasma (NP)</td>
</tr>
<tr>
<td>PTT control 1:1 mix</td>
<td>37.3 s</td>
<td>Control 35 s</td>
<td>Incubate both 1:1 mix and NP</td>
</tr>
</tbody>
</table>

Conclusion: immediate and incubated mix PTTs correct, suspect factor deficiency, arrange for factor assays and von Willebrand disease workup

**Factor Assay Results**
32-YO female, 6 weeks post-partum

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result (%)</th>
<th>RI</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII (Fibrinogen)</td>
<td>39%</td>
<td>50 - 150%</td>
<td>VWD?</td>
</tr>
<tr>
<td>FIX</td>
<td>92%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FXI</td>
<td>131%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FXII</td>
<td>113%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Molecular Weight Kininogen (HMWK)</td>
<td>ND</td>
<td>65 - 135%</td>
<td>XII, HMWK &amp; PK deficiency not associated with bleeding</td>
</tr>
<tr>
<td>Prekallikrein (PK)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**PT and PTT Test Results in Inherited Coagulopathies**

<table>
<thead>
<tr>
<th>PT</th>
<th>PTT</th>
<th>Single Factor Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long</td>
<td>Normal</td>
<td>VII</td>
</tr>
<tr>
<td>Long</td>
<td>Long</td>
<td>X, V, II and fibrinogen(^1)</td>
</tr>
<tr>
<td>Normal</td>
<td>Long</td>
<td>VIII, IX, X(^2)</td>
</tr>
</tbody>
</table>

\(^1\)PT & PTT prolonged when fibrinogen is <100 mg/dL, perform fibrinogen assay

\(^2\)Contact factor deficiencies XII (1–3% prevalence), prekallikrein (PK, Fletcher), or high molecular weight kininogen (HMWK, Fitzgerald) also prolong PTT results, but no bleeding

---

**PTT Mix**

**Why Does This Work?**

- **Hypothetical 20% F VIII level prolongs PTT**
  - PTT reagents calibrated to prolong at 30 - 40% FVIII, IX, XI
  - Add NP with established 100% factor level
  - 1:1 mix, average of 100% and 20% = 60%

- **Hypothetical anti-FVIII or lupus anticoagulant**
  - With typical avidity, retains ability to prolong the mix
Case Study #2
52-YO female pre-op screen
Athletic
Total hip replacement

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>14.1 g/dL</td>
<td>12 - 15 g/dL</td>
</tr>
<tr>
<td>PT</td>
<td>11.2 s</td>
<td>9.8 - 12.6 s</td>
</tr>
<tr>
<td>PTT (APTT)</td>
<td>58 s</td>
<td>25 - 35 s</td>
</tr>
<tr>
<td>PLT count</td>
<td>170,000/µL</td>
<td>150 - 400,000/µL</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>410 mg/dL</td>
<td>220 - 498 mg/dL</td>
</tr>
</tbody>
</table>

Patient reports no bleeding or bruising, no thrombosis

Isolated Prolonged PTT
Possible reasons
Could be nothing: 5% of normals exceed limit
Preanalytical variable: green or lavender top tube, hemolysis, lipemia, clotted specimen
Outpatient: DOAC
Inpatient: unreported UFH
Congenital single factor deficiency: VIII, IX, or XI, hemophilia A, B, or C with bleeding, VWD
Congenital FXII, PK or HMWK without bleeding
Acquired FVIII inhibitor (acquired hemophilia) with severe bleeding
Lupus anticoagulant (LA)
Prolonged PT

Possible reasons

- Congenital deficiencies of II, V, VII, or X
- PT and PTT long: II, V, X
- PT only: VII, skip mixing and go to factor assay

Prevalence: 500,000 - 1:2,000,000

Liver disease: PT prolongs before PTT due to des-carboxy II, VII, and X, reduced factor V

Vit K deficiency: des-carboxy II, VII, and X (also IX for PTT)

Anti-Xa direct oral anticoagulants

- Rivaroxaban, apixaban, edoxaban

PTT Mixing Study Result

52-YO female

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>17 s</td>
<td>NR: &lt; 21 s, rules out DOAC</td>
</tr>
<tr>
<td>PTT</td>
<td>58 s</td>
<td>NR: 25 - 35 s</td>
</tr>
<tr>
<td>PTT NP</td>
<td>28 s</td>
<td>Correction if &lt; 30.8 s (10%)</td>
</tr>
<tr>
<td>1:1 mix</td>
<td>35 s</td>
<td>25% over NP = no correction</td>
</tr>
</tbody>
</table>

What is the next step?

Acute Care Mixing Study Algorithm

Isolated prolonged PTT

- TT long
  - Heparinase or polybrene
  - if DOAC, stop here

- TT normal
  - PTT normal
  - APTT normal
  - FVIII inhibitor
  - If DOAC, stop here
  - Factor assay
  - NA profile
  - LA profile
  - No correction

- Factor assay
  - FVIII inhibitor
  - NA profile
  - LA profile
  - No correction
Mixing Study Considerations
Preanalytical variables
- Anti-Xa rivaroxaban, apixaban, edoxaban prolong PT, PTT
- Dabigatran and UFH prolong PTT
- Clotted, hemolyzed, lipemic specimen
- Under filled tube, wrong anticoagulant
- Must be platelet-poor, <10,000/uL patient and NP
- Heparinase or polybrene neutralize only ≤ 1 unit/mL UFH
- Anti-FVIIIs may generate immediate neutralization
- 15% of LAs require incubation
- Weak LAs may be missed in 1:1 mix: ask for consult
- Select a more LA-sensitive PTT reagent or request 4:1 mix

Normal Plasma Source
Make home brew: ~20 normal plasmas, male ≅ female
- Ensure plasma is platelet-poor; < 10,000/uL, PTT ≅ NR
- Ensure ~100% of all factors, elevated FVIII causes false negatives
- Screen for LA, specific factor inhibitors, HBV, HCV, HIV
- Aliquot and freeze
- Time consuming & difficult to find normal donors
- Purchase commercial plasma
- GMP meets all criteria
- Frozen meets all criteria
- Lyophilized acceptable when validated; processed with stabilizers

Case Study #3
59-YO male pre-op screen
- Former hockey player
- Total knee replacement
Case Study #3
59-YO male pre-op screen

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>14.8 g/dL</td>
<td>12 - 15 g/dL</td>
</tr>
<tr>
<td>PT</td>
<td>11.2 s</td>
<td>9.8 - 12.6 s</td>
</tr>
<tr>
<td>PTT (APTT)</td>
<td>38 s</td>
<td>25 - 35 s</td>
</tr>
<tr>
<td>PLT count</td>
<td>310,000/µL</td>
<td>150 - 400,000/µL</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>390 mg/dL</td>
<td>220 - 498 mg/dL</td>
</tr>
</tbody>
</table>

Patient reports no bleeding or bruising, no thrombosis.

When to Perform Mixing Study
Any PTT > NR upper limit
Any PTT > NR upper limit + 5 seconds
Any PTT > NR upper limit with consult
Is patient bleeding or clotting?
Possible “weak” LA: use 4:1 mix
Lupus sensitive PTT reagent
Factor sensitive PTT reagent


PTT Mixing Study Result
59-YO male

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>17 s</td>
<td>NR: &lt; 21 s, rules out dabigatran</td>
</tr>
<tr>
<td>PTT</td>
<td>38 s</td>
<td>NR: 25 - 35 s</td>
</tr>
<tr>
<td>PTT NP</td>
<td>31 s</td>
<td>Correction if &lt; 34.1 s (10%)</td>
</tr>
<tr>
<td>1:1 mix</td>
<td>35 s</td>
<td>Correction? No Correction?</td>
</tr>
</tbody>
</table>

What is the next step?
Clinical Consult
59-YO male
Consult: if he is well, go no further
Thrombotic events: perform mix using 4:1 patient to normal plasma
Or choose PTT reagent that is LA-sensitive
If anatomic bleeding symptoms, test FVIII, FIX, FXI
Vitamin K deficiency
Renal insufficiency
Liver disease
Malignancy
VWD

Case Study #4
2-YO male hemophilic

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>11.8 g/dL</td>
<td>9.6 - 15.6 g/dL</td>
</tr>
<tr>
<td>PT</td>
<td>11.2 s</td>
<td>9.8 - 12.6 s</td>
</tr>
<tr>
<td>PTT (APTT)</td>
<td>65 s</td>
<td>25 - 35 s</td>
</tr>
<tr>
<td>PLT count</td>
<td>310,000/µL</td>
<td>150 - 400,000/µL</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>390 mg/dL</td>
<td>220 - 498 mg/dL</td>
</tr>
</tbody>
</table>

Inflamed, swollen knee and ankle
Mixing Study Result
2-YO male hemophilic

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>Normal Range</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTT</td>
<td>65 s</td>
<td>25 - 35 s</td>
<td>Confirms previous PTT</td>
</tr>
<tr>
<td>PTT/control 1:1 mix</td>
<td>33.5 s</td>
<td>Control 30 s</td>
<td></td>
</tr>
<tr>
<td>immediate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTT/control 1:1 mix</td>
<td>47.9 s</td>
<td>Control 35 s</td>
<td>Control is incubated</td>
</tr>
<tr>
<td>1 h at 37°C</td>
<td></td>
<td></td>
<td>alone and with mix</td>
</tr>
</tbody>
</table>

Conclusion: Anti-FVIII inhibitor

Factor VIII Assay
Dilute plasma 1:10, add factor VIII-depleted reagent plasma 1:1
Add PTT reagent, incubate 3 minutes
Add CaCl₂, record interval to clot formation
Compare result in seconds to dilution curve

![Factor VIII Activity Reference Curve]

FVIII Assay Dilutions
Parallelism indicates no inhibitor

<table>
<thead>
<tr>
<th>Plasma Dilution</th>
<th>Seconds</th>
<th>Raw FVIII Activity</th>
<th>Computed FVIII Activity (x dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10 &quot;undiluted&quot;</td>
<td>90 s</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>1:20</td>
<td>104 s</td>
<td>10%</td>
<td>20% (parallel)*</td>
</tr>
<tr>
<td>1:40</td>
<td>107 s</td>
<td>5%</td>
<td>20% (parallel)</td>
</tr>
<tr>
<td>1:80</td>
<td>130 s</td>
<td>2.5%</td>
<td>20% (parallel)</td>
</tr>
</tbody>
</table>

* <10% difference from undiluted indicates parallelism, no inhibitor
**Summary**

Mixing studies are a first-line investigation into the cause of an abnormal screening test (PT or APTT).

They can be done locally to differentiate a factor deficiency from an inhibitor and guide further investigation.

Patient plasma is mixed with normal plasma and screening test repeated.

If results correct, suggests factor deficiency and specific factor assays can be performed.

If results don’t correct, suggests an inhibitor or other interference and applicable assays can be performed.

---

**Reasons to Perform Mixing Studies Locally**

Unexpected isolated prolonged PTT or PT requires immediate action.

Delay results in specimen deterioration.

Results may immediately direct therapy or medical intervention.

If necessary, forward results to reference lab to direct follow-up.
Resources
Special thank you to George Fritsma who originally authored this presentation for the Precision BioLogic webinar entitled Improving Acute Care with Coagulation Mixing Studies
www.fritsmafactor.com

Thank you
Jim DeMase
jdemase@precisionbiologic.com