Chromogenic Factor VIII, Activity

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Chromogenic factor VIII activity is used for diagnosis of mild hemophilia A (in conjunction with one-stage clot-based factor VIII activity) and measurement of factor VIII activity in the presence of interfering drugs or lupus anticoagulants that result in underestimation by clot-based methods. It is also used for measuring factor VIII activity in patients treated with certain modified extended half-life factor VIII replacement products and emicizumab.

Featured ARUP Testing

Chromogenic Factor VIII, Activity 3002343

Method: Chromogenic Assay

- Measures hydrolysis of a p-nitroanilide (pNA) substrate
  - The rate of release of pNA is proportional to the factor VIII activity in the sample
- Can quantitate factor activity as low as 1% of normal

Disease Overview

Incidence

Hemophilia A in 1/4,000-5,000 live male births worldwide; rare in females[1]

Inheritance

X-linked recessive, factor VIII deficiency can also be acquired due to autoantibodies[1]

Visit the Hemophilia - Factor VIII or IX Deficiency Consult topic for additional information about factor VIII deficiency

Diagnostic Issues

Hemophilia A may be classified as mild, moderate or severe, based on factor activity.

Disease Classification	Expected Factor Activity
Mild	6-35%
Moderate	1-5%
Severe	<1%

Mild hemophilia A may require both one-stage clot-based factor VIII activity and chromogenic factor VIII activity for diagnosis, due to differences in how the underlying mutations affect factor VIII activity in the tests.[2]

Monitoring Issues

Modified extended half-life factor VIII replacement products may lead to underestimation of factor VIII activity in clot-based factor VIII assays using certain aPTT reagents.[3,4,5]

Extended Half-Life FVIII Replacement Product	Manufacturer	Modification Type	Effect on FVIII Activity
Adynovate	Shire/Baxalta, Baxter	PEGylated (random) rFVIII	Acceptable recovery with chromogenic or one-stage FVIII activity for all aPTT reagents tested to date Relatively higher recovery with aPTT reagents with ellagic acid activators (eg, Actin FS, Actin FSL) that is not clinically significant

Extended Half-Life FVIII Replacement Product	Manufacturer	Modification Type	Effect on FVIII Activity
Afstyla	CSL Behring	Single chain, B-domain truncated rFVIII	Underestimated with all one-stage FVIII activity assays; package insert recommends use of correction factor which is supported by a multicenter field study[5] Acceptable recovery with chromogenic FVIII activity assays
Eloctate	Biogen Idec	Fc fusion, B-domain deleted rFVIII	Acceptable recovery with chromogenic or one-stage FVIII activity for all aPTT reagents tested to date
Esperoct	Novo Nordisk	B-domain truncated, glycoPEGylated rFVIII	Understimated with STA-PTT A, SynthAFax, APTT Sp (chromogenic factor VIII activity recommended instead of one-stage assay using these aPTT reagents) Acceptable recovery with one stage FVIII activity for all other aPTT reagents tested to date
Jivi	Bayer Healthcare	PEGylated (site directed) B-domain deleted rFVIII	Underestimated with aPTT reagents with silica activators (eg. STA-PTT A, APTT Sp) Few other aPTT reagents studied to date, recovery appears acceptable with aPTT reagents with ellagic acid activators

Factor VIII activity cannot be accurately measured using a one-stage clot-based factor VIII activity assay in the presence of emicizumab.[6]

- Emicizumab is a bispecific antibody that bridges factor IX and factor X to produce activated factor X (FXa). Emicizumab effectively replaces the function of factor VIII in secondary hemostasis
- Emicizumab will substitute for factor VIII function in one stage clot-based factor VIII activity assays and will result in overestimation of factor VIII activity (either native factor VIII or factor VIII concentrate administered in an acute care setting)
  - Interference may last for up to to 6 months following end of therapy
- In patients receiving emicizumab, patient factor VIII activity (endogenous or factor VIII concentrate) can be accurately measured using chromogenic factor VIII activity with bovine reagents
  - Emicizumab can bind to factor IX and X in chromogenic assays using human factor-derived reagents and will still overestimate factor VIII activity
  - The chromogenic factor VIII activity assay at ARUP Laboratories uses bovine reagents and is not affected by emicizumab
- Measuring factor VIII inhibitors in patients receiving emicizumab requires use of a Bethesda assay based on a chromogenic factor VIII assay (bovine reagents)

Test Interpretation

Results

- Age-specific reference intervals are provided for each result on the patient chart
- Decreased factor VIII activity is expected in patients with hemophilia A (see table above for disease classification) and is associated with increased risk of bleeding

Limitations

- Decreased chromogenic factor VIII activity results may also be caused by:
  - von Willebrand disease
  - Specimen collection and storage issues:
    - Uncontrolled freeze-thaw cycles
    - Prolonged ambient storage
    - Activated or clotted specimens
  - Anticoagulant medications (assay interference)
    - Heparin (>2 U/mL)
    - Direct thrombin inhibitors
    - Direct Xa inhibitors
- Factor VIII activity may be elevated above usual baseline (normal or high result could mask underlying deficiency) in patients with acute phase responses
- Normal factor VIII activity does not exclude female hemophilia carrier status

References

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Related Information

Hemophilia - Factor VIII or IX Deficiency

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