Article published online: 2020-08-31



# Laboratory Assessment of Coagulation Factor XIII

Verena Schroeder[1]

[1] Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland

Hämostaseologie 2020;40:467–471.

Address for correspondence Verena Schroeder, PhD, Experimental Haemostasis Group, Department for BioMedical Research (DBMR), University of Bern, Murtenstrasse 40, 3008 Bern, Switzerland (e-mail: verena.schroeder@dbmr.unibe.ch).

**Abstract**

Laboratory diagnosis of congenital and acquired deficiencies of coagulation factor XIII (FXIII) can be challenging. Determination of FXIII function requires specific and sensitive assays which are not always available. This brief review article summarizes currently used FXIII assay methods, their principles and difficulties, and discusses the recommended diagnostic workup in case of a suspected FXIII deficiency. The article also briefly touches on experimental methods used in FXIII research.

**Keywords**

- coagulation factor XIII
- factor XIII assay
- FXIII deficiency

## Introduction

Coagulation factor XIII (FXIII) circulates in blood as a tetramer of two protransglutaminase A-subunits and two carrier B-subunits ($A_2B_2$) and acts at the end of the plasmatic coagulation cascade. Thrombin initiates FXIII activation by cleaving off the activation peptide which, in the presence of $Ca^{2+}$ and enhanced by fibrin, is followed by the dissociation of the tetramer and conformational changes to yield the active transglutaminase FXIIIa*. FXIIIa* biochemically cross-links fibrin molecules by introducing isopeptide bonds between glutamine and lysine side chains and also binds other plasma proteins, including antifibrinolytic proteins, to fibrin. This makes the fibrin clot more stable and resistant toward premature fibrinolysis. FXIII also facilitates incorporation of blood cells into the clot, which is necessary for clot retraction. Furthermore, FXIII has crucial functions in extracellular matrix stabilization, tissue remodeling and wound healing, and pregnancy.[1,2]

## Difficulties in FXIII Laboratory Assessment

Congenital and acquired FXIII deficiencies are rare but severe bleeding disorders with potentially life-threatening bleeding complications.[3] Therefore, a timely and accurate diagnosis is of utmost importance. However, there are several difficulties that make FXIII laboratory assessment challenging. Because FXIII acts at the end of the coagulation cascade when fibrin is already polymerizing, FXIII action is not picked up by routine global coagulation assays such as prothrombin time and activated partial thromboplastin time. Instead, specific FXIII assays have to be performed, but they are not available in every laboratory, or they are not routinely performed due to the small number of cases and hence the small number of analyses.

Among FXIII assays, clot solubility assays are still widely used as screening tests. In these assays, a plasma sample is clotted followed by observation of the dissolution of the clot, which is soluble in reagents such as urea or diluted monochloroacetic acid if not biochemically cross-linked by FXIII. These assays are cheap and do not require special reagents or infrastructure. However, the type of clotting and solubilizing agents, their concentrations, and assay procedures are not standardized and there are substantial differences in sensitivity between different protocols.[4] In addition, the result gives no quantitative information on FXIII activity or FXIII antigen levels. And importantly, very small residual FXIII amounts, e.g., from treatment with a FXIII-containing blood product within the last 4 to 6 weeks prior to blood sampling, are sufficient to give normal results (i.e., the clot does not dissolve). Therefore, clot solubility assays are associated with a high risk of missing a diagnosis of FXIII deficiency and are not recommended. Yet, it must be acknowledged that alternatives are often not available, in particular in countries with limited healthcare resources. When the limitations of the clot solubility assays are well known and taken into account and the patients are well characterized clinically, clot solubility assays may still have a place in FXIII diagnostics.[4,5]

Commercially available quantitative FXIII activity assays are also not without problems. The so-called photometric assays measure the amount of ammonia released during the transglutaminase reaction catalyzed by activated FXIIIa* in a subsequent indicator reaction of ammonia with NADH or NADPH catalyzed by glutamate dehydrogenase.[6] However,

**received**
April 15, 2020
**accepted after revision**
May 18, 2020

© 2020 Georg Thieme Verlag KG Stuttgart · New York

DOI https://doi.org/10.1055/a-1181-0327.
ISSN 0720-9355.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

other FXIII-independent chemical reactions in plasma may interfere with the indicator reaction, which can lead to an overestimation of the actual FXIII activity, in particular in the low-activity range (below 20–30% FXIII activity). It is therefore crucial to include a plasma blank, i.e., each plasma sample is measured twice, once with FXIII activation and once without FXIII activation or with addition of a FXIII inhibitor, and the difference between the two measurements is used to calculate the actual FXIII activity.[7–9]

## Quantitative FXIII Assays

Quantitative FXIII assays can be divided into antigen assays and functional (activity) assays, as summarized in ►Table 1.

Antigen assays use specific polyclonal or monoclonal antibodies against the FXIII $A_2B_2$ tetramer or the individual A- and B-subunits. The most common assay format is the enzyme-linked immunosorbent assay (ELISA). Several ELISA kits are commercially available, and their specificity and sensitivity depend largely on the antibodies used. A special format is the latex agglutination immunoassay that allows automation (HemosIL Factor XIII Antigen, Instrumentation Laboratory, Bedford,Massachusetts, United States). The results from antigen assays are expressed usually in percentage relative to a normal plasma or standard material such as the International Standard for factor XIII plasma.[10] Antigen assays allow the quantification of the actual amount of FXIII protein present in the sample irrespective of its activity. Usually, antigen and activity are strongly correlated so that it may be enough to determine either antigen or activity levels. However, in cases of congenital FXIII type II deficiency, i.e., normal protein levels but reduced function,[11] and acquired FXIII deficiency due to inhibiting antibodies,[12,13] it is essential to measure both FXIII antigen and activity. A significantly reduced FXIII activity at normal FXIII antigen levels is also found in patients with afibrinogenemia,[14] again arguing for the determination of both FXIII activity and antigen.

Functional assays are based either on the detection of ammonia released during the transglutaminase reaction, the detection of (small) substrate molecules and peptides that become cross-linked to protein substrates (also termed amine incorporation assays), or the isopeptidase activity of transglutaminases. Among the functional tests, the photometric ammonia release assay,Berichrom Factor XIII assay (Siemens Healthineers, Erlangen, Germany), is automatable and it is widely used, but as discussed above, the protocol should be adjusted to include a plasma blank. In contrast, the Technochrom FXIII assay (Technoclone, Vienna, Austria) does contain a blank reagent and is therefore also reliable in the low-activity range. An incorporation assay that detects incorporation of a FXIII-specific glutamine peptide substrate, one of the so-called Hitomi peptides, into an amine substrate[15] is commercially available (Covalab, Villeurbanne, France). An in-house incorporation assay that detects incorporation of biotin-pentylamine into fibrinogen is very sensitive also in the low-activity range. In addition, it picks up the common FXIII Val34Leu polymorphism which accelerates the FXIII activation rate.[16] However, when FXIII is fully activated, the specific activity of the FXIII Val34 and

**Table 1** Quantitative FXIII assays

| Assay type | Principle | Examples of commercial assays | Assay is automatable |
|---|---|---|---|
| FXIII antigen assays | Sandwich-type ELISAs detecting $FXIIIA_2B_2$, FXIII-A, or FXIII-B depending on antibody specificity | Zymutest Factor XIII-A (Hyphen BioMed, France) | |
| | Latex agglutination immunoassay for FXIII-A antigen | HemosIL Factor XIII Antigen (Instrumentation Laboratory, Bedford,Massachusetts, United States) | Yes |
| Functional FXIII assays (activity assays) | Ammonia released during the transglutaminase reaction reacts with NADH or NADPH which is recorded photometrically | Berichrom Factor XIII (Siemens Healthineers, Germany) | Yes |
| | | Biophen Factor XIII (Hyphen BioMed, France) | Yes |
| | | Technochrom FXIII (Technoclone, Austria) | Yes |
| | Incorporation of small molecules (e.g., biotin-labeled pentylamine) or peptides into protein substrates (e.g., fibrinogen), detection of the incorporated substrate with a labeled marker (e.g., streptavidin) or antibody | Specific Plasmatic Transglutaminase Factor XIII Colorimetric Microassay Kit (Covalab, France) | |
| | Fluorogenic assay based on isopeptidase activity: transglutaminases can also cleave isopeptide bonds. This cleavage releases a quencher molecule, leading to an increase in fluorescence from a fluorophore attached to a substrate | Isopeptidase-Fluorogenic Factor XIII Assay (Zedira, Germany) | Yes |

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FXIII, factor XIII.
Notes: The list of commercial assays contains typical examples but makes no claim to be complete. Not all of the commercially available assays are registered for diagnostic purpose, some are for research purpose only.



This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

Leu34 variants does not differ. More recently, a fluorogenic assay has become commercially available that allows sensitive quantification of FXIII activity also in the low-activity range[17] (fluorescent factor XIII assay, Zedira, Darmstadt, Germany), but it has not yet been used widely in clinical conditions. The assay is based on the isopeptidase activity of transglutaminases. It has also been used to monitor FXIIIa generation over time upon triggering the coagulation cascade.[18] The isopeptidase assay has recently been implemented and validated for use on an automated platform.[19]

## Recommended Diagnostic Workup

The Factor XIII and Fibrinogen SSC Subcommittee of the International Society on Thrombosis and Haemostasis has published recommendations on the diagnosis and classification of FXIII deficiencies.[20] As summarized in ►Fig. 1, when FXIII deficiency is suspected, a quantitative functional assay should be performed as first-line screening assay. Antigen assays are recommended to establish the type of deficiency. Since most cases among congenital FXIII deficiencies reported so far have been congenital FXIII-A deficiencies, an ELISA to measure the FXIII-A subunit is usually sufficient. Identification of the underlying genetic mutation in congenital FXIII deficiency has usually no influence on patient treatment, so molecular genetic analysis is mostly of interest to research to learn more about FXIII structure and function. Acquired FXIII deficiency can be caused by consumption during heavy blood loss, prolonged bleeding, thrombosis, or acute or chronic inflammation.[3] In recent years, a large number of autoimmune FXIII deficiencies have been reported.[13,21] In suspected autoimmune acquired FXIII

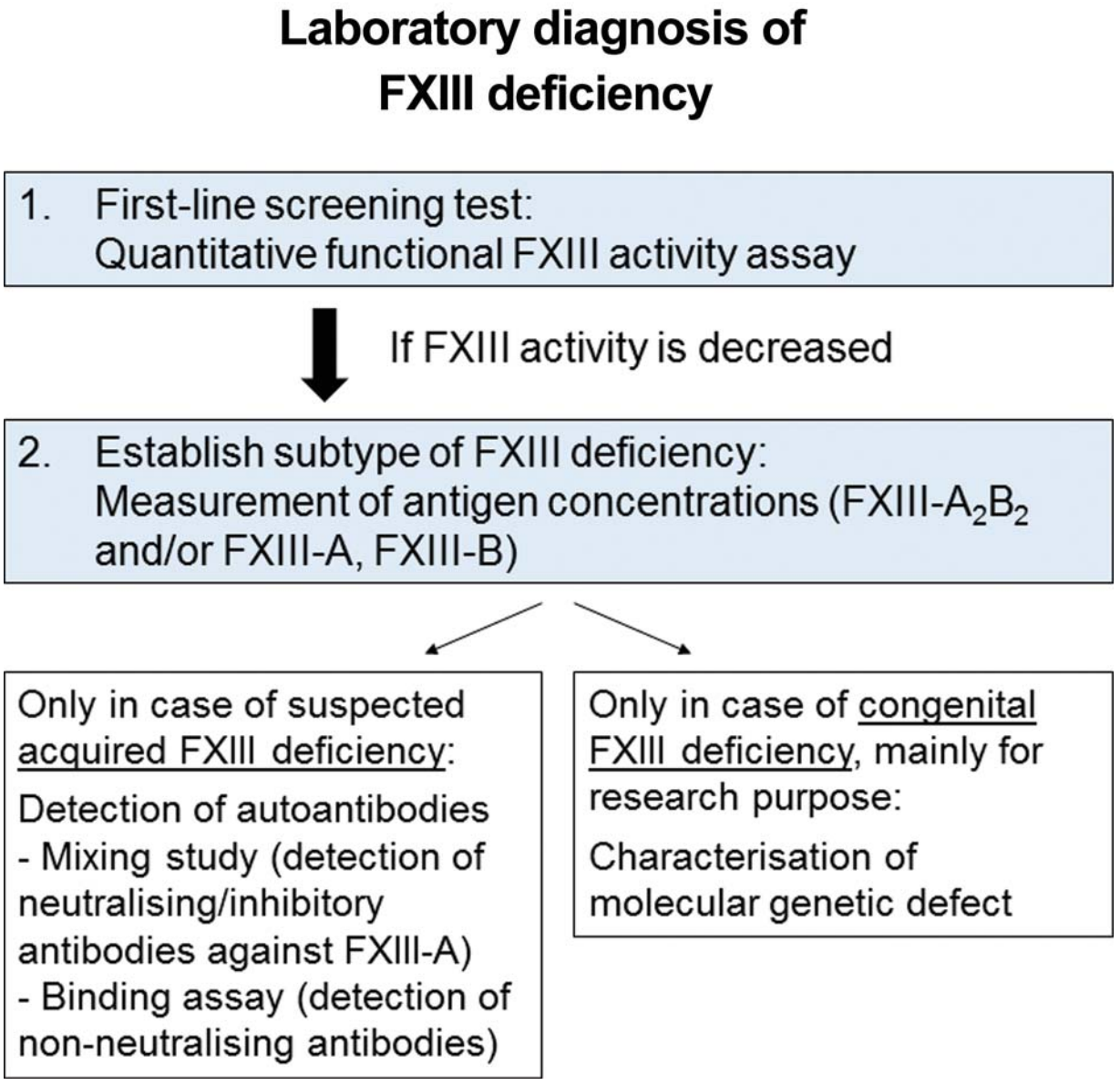


**Fig. 1** Recommended diagnostic workup for suspected FXIII deficiency, based on the recommendations on the diagnosis and classification of FXIII deficiencies published by the Factor XIII and Fibrinogen SSC Subcommittee of the ISTH.[20] ISTH, International Society on Thrombosis and Haemostasis.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

deficiency due to autoantibodies, further assays can be performed to characterize the type of autoantibody.[12,22]

The recommended diagnostic workup[20] is followed ideally to thoroughly characterize FXIII deficiency in addition to the clinical symptoms and family history of a patient. However, as mentioned above, the specific assays and resources are not always available. In regions where consanguineous marriages are common, leading to a high frequency of a few genetic mutations, genotyping for these mutations may represent a valuable yet inexpensive tool in the diagnosis of congenital FXIII deficiency.[23]

## Further (Experimental) Assay Methods

In FXIII research, a variety of methods have been applied to test FXIII function in vitro and in vivo. These methods, which can only be briefly summarized in the following paragraph, range from detection of FXIII activation to visualization of FXIII action in vivo.

When FXIII is activated by thrombin, the FXIII activation peptide (AP-FXIII) is cleaved off from the N-terminus of the FXIII-A subunit. Our group developed an ELISA method to quantify AP-FXIII in plasma and monitor FXIII activation.[24] However, many questions such as the in vivo half-life of AP-FXIII in plasma and its clinical value are still open and under further investigation.

Fibrin cross-linking by activated FXIII can be detected by gel electrophoresis (SDS-PAGE). Upon activation of fibrinogen and FXIII by thrombin, the fibrinogen γ-chains and α-chains disappear, forming γ–γ dimers and high-molecular-weight α- and γ-multimers.[25] Absence of γ-chain dimerization was shown in patients with FXIII deficiency.[12]

A global coagulation test that is sensitive to some extent to FXIII function is thrombelastography or its newer variation rotational thromboelastometry (ROTEM). Both kinetic parameters of clot formation and parameters describing the viscoelastic properties of plasma or whole blood clots are affected by FXIII.[26,27]

Visualization of FXIII action in vivo has been described in a mouse model. Using intra-vital microscopy, the influence of FXIII action on in vivo thrombosis, thrombus size, and fibrin cross-linking following $FeCl_3$-induced vessel injury was demonstrated.[28] Our group has recently reported an endothelialized, whole blood microvascular flow model[29] to study effects of complement components on clot formation.[30] In this model, we can also observe FXIII action during clot formation in realtime by applying specific fluorescent-labeled FXIII substrate peptides or an antibody against fibrin crosslinks (DD-XLink-mab, Zedira) (unpublished results).

## Conclusion

In conclusion, assessment of FXIII function and diagnosis of FXIII deficiencies remain challenging. Understanding FXIII function and knowledge of the available laboratory assays with their pitfalls and limitations are crucial for the correct diagnosis and management of patients with congenital or acquired FXIII deficiencies.

Conflict of Interest
The authors declare that they have no conflict of interest.

## References

1 Muszbek L, Bereczky Z, Bagoly Z, Komáromi I, Katona É. Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. Physiol Rev 2011;91(03):931–972
2 Schroeder V, Kohler HP. Factor XIII: structure and function. SeminThrombHemost 2016;42(04):422–428
3 Schroeder V, Kohler HP. Factor XIII deficiency: an update. SeminThrombHemost 2013;39(06):632–641
4 Jennings I, Kitchen S, Woods TAL, Preston FE; UK NEQAS. Problems relating to the laboratory diagnosis of factor XIII deficiency: a UK NEQAS study. J ThrombHaemost 2003;1(12): 2603–2608
5 Jennings I, Kitchen S, Menegatti M, et al. Detection of factor XIII deficiency: data from multicentre exercises amongst UK NEQAS and PRO-RBDD project laboratories. Int J Lab Hematol 2017;39 (04):350–358
6 Muszbek L, Polgár J, Fésüs L. Kinetic determination of blood coagulation factor XIII in plasma. Clin Chem 1985;31(01):35–40
7 Kárpáti L, Penke B, Katona E, Balogh I, Vámosi G, Muszbek L. A modified, optimized kinetic photometric assay for the determination of blood coagulation factor XIII activity in plasma. Clin Chem 2000;46(12):1946–1955
8 Ajzner E, Muszbek L. Kinetic spectrophotometric factor XIII activity assays: the subtraction of plasma blank is not omissible [corrected]. J ThrombHaemost 2004;2:2075–2077
9 Cini M, Legnani C, Frascaro M, et al. Measurement of factor XIII (FXIII) activity by an automatic ammonia release assay using iodoacetamide blank-procedure: no more overestimation in the low activity range and better detection of severe FXIII deficiencies. Clin Chem Lab Med 2016;54(05):805–809
10 Raut S, Merton RE, Rigsby P, et al; ISTH/SSC Factor XIII Subcommittee and the Factor XIII Standardization Working Party. A collaborative study to establish the 1st International Standard for factor XIII plasma. J ThrombHaemost 2007;5(09): 1923–1929
11 Li B, Billur R, Maurer MC, et al. Proline 36 of the factor XIII activation peptide plays a crucial role in substrate recognition and zymogen activation. ThrombHaemost 2018;118(12):2037–2045
12 Muszbek L, Katona É. Diagnosis and management of congenital and acquired FXIII deficiencies. SeminThrombHemost 2016;42 (04):429–439
13 Ichinose A; Japanese Collaborative Research Group on AH13. Autoimmune acquired factor XIII deficiency due to anti-factor XIII/13 antibodies: a summary of 93 patients. Blood Rev 2017;31 (01):37–45
14 Bridey F, Négrier C, Duval C, Ariëns R, de Moerloose P, Casini A. Impaired factor XIII activation in patients with congenital afibrinogenemia. Haematologica 2019;104(03):e111–e113
15 Hitomi K, Kitamura M, Alea MP, Ceylan I, Thomas V, El Alaoui S. A specific colorimetric assay for measuring transglutaminase 1 and factor XIII activities. Anal Biochem 2009;394(02):281–283
16 Kohler HP, Ariëns RA, Whitaker P, Grant PJ. A common coding polymorphism in the FXIII A-subunit gene (FXIIIVal34Leu) affects cross-linking activity. ThrombHaemost 1998;80(04):704
17 Oertel K, Hunfeld A, Specker E, et al. A highly sensitive fluorometric assay for determination of human coagulation factor XIII in plasma. Anal Biochem 2007;367(02):152–158
18 Dodt J, Volkers P, Seitz R. Factor XIIIa generation assay: a tool for studying factor XIII function in plasma. Anal Biochem 2013;439 (02):145–151
19 Leitner M, Büchold C, Pasternack R, Binder NB. Feasibility of an automated coagulation factor XIIIa test using its isopeptidase activity. Anal Biochem 2020;600:113757



This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

20 Kohler HP, Ichinose A, Seitz R, Ariëns RAS, Muszbek L; Factor XIII And Fibrinogen SSC Subcommittee Of The ISTH. Diagnosis and classification of factor XIII deficiencies. J ThrombHaemost 2011;9 (07):1404–1406
21 Muszbek L, Pénzes K, Katona É. Auto- and alloantibodies against factor XIII: laboratory diagnosis and clinical consequences. J ThrombHaemost 2018;16(05):822–832
22 Ichinose A, Kohler HP, Philippou H; Factor XIII and Fibrinogen SSC Subcommittee of the ISTH. Recommendation for ISTH/SSC Criterion 2015 for autoimmune acquired factor XIII/13 deficiency. ThrombHaemost 2016;116(04):772–774
23 Dorgalaleh A, Farshi Y, Alizadeh SH, et al. Challenges in implementation of ISTH diagnostic algorithm for diagnosis and classification of factor XIII deficiency in Iran. J ThrombHaemost 2015; 13(09):1735–1736
24 Ortner E, Schroeder V, Walser R, Zerbe O, Kohler HP. Sensitive and selective detection of free FXIII activation peptide: a potential marker of acute thrombotic events. Blood 2010;115(24):5089–5096
25 Allan P, Uitte de Willige S, Abou-Saleh RH, Connell SD, Ariëns RAS. Evidence that fibrinogen γ′ directly interferes with protofibril growth: implications for fibrin structure and clot stiffness. J ThrombHaemost 2012;10(06):1072–1080
26 Schroeder V, Kohler HP. Thrombelastographic studies on factor XIII. ThrombHaemost 2010;104(06):1277–1279
27 Martinuzzo M, Barrera L, Altuna D, et al. Effects of factor XIII deficiency on thromboelastography. Thromboelastography with calcium and streptokinase addition is more sensitive than solubility tests. Mediterr J Hematol Infect Dis 2016;8(01): e2016037
28 Duval C, Ali M, Chaudhry WW, Ridger VC, Ariëns RA, Philippou H. Factor XIII A-subunit V34L variant affects thrombus cross-linking in a murine model of thrombosis. ArteriosclerThrombVasc Biol 2016;36(02):308–316
29 Tsai M, Kita A, Leach J, et al. In vitro modeling of the microvascular occlusion and thrombosis that occur in hematologic diseases using microfluidic technology. J Clin Invest 2012;122(01): 408–418
30 Jenny L, Dobó J, Gál P, Pál G, Lam WA, Schroeder V. MASP-1 of the complement system enhances clot formation in a microvascular whole blood flow model. PLoS One 2018;13(01):e0191292

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.