

Detection of A and B Subunits of Factor XIII and Detection of an Anti-Factor XIII by ELISA (Reference 2014.01.003)

Notice of Assessment

June 2014

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1 GENERAL INFORMATION

1.1 Requester: CHU Sainte-Justine

1.2 Application for Review Submitted to MSSS: July 15 and 16, 2013

1.3 Application Received by INESSS: March 1, 2014

1.4 Notice Issued: June 30, 2014

Note:

This notice is based on the scientific and commercial information submitted by the requester and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY, AND LICENCE(S)

2.1 Name of the Technology

Detection of A and B subunits of factor XIII and detection of non-neutralizing¹ antibodies directed against factor XIII ("routine" and "stat"²) using the ELISA method (enzyme-linked immunosorbent assay).

2.2 Brief Description of the Technology, and Clinical and Technical Specifications

Detection of A and B Subunits

The requester uses the TECHNOZYM® FXIII-A and FXIII-B SUB ELISA (Technoclone) kit. The ELISA assay kit determines the A (FXIII-A) and B (FXIII-B) subunits of factor XIII (FXIII) and uses monoclonal antibodies specific to FXIII-A and B. The kit includes:

- a solid support consisting of 12 x 8-well strips coated with streptavidin;
- a biotinylated mouse monoclonal capture antibody (anti-FXIII-A "A1" or anti-FXIII-B "B1");
- a monoclonal detection antibody conjugated to horseradish peroxidase (HRP) (anti-FXIII-A "A₂" or anti-FXIII-B "B₂");
- a concentrated buffer (phosphate-buffered saline or PBS, pH = 7.4) containing a detergent (0.01% merthiolate) for the washing phase and a sample dilution buffer (PBS, pH = 7.4) containing proteins (0.03% Proclin 300) for the stabilization phase.

A chromogenic substrate, TMB (tetramethylbenzidine), is added during the incubation period. The reaction is stopped with sulphuric acid 0.45 mol/L. The intensity of the colour is expressed as optical density at 450 nm [Technoclone, 2010a].

¹ A non-neutralizing antibody is an antibody that binds to the "factor" protein, thus leading to the creation of a complex that is eliminated by the reticuloendothelial system and responsible for a secondary deficiency of this factor [Biomnis, 2012].

² Stat: Test that can be carried out and its results communicated during a medical emergency. It is important to make the following distinctions when requesting tests and to clearly indicate the information on the requisitions: medical emergency ("Stat"): the test will be performed and its results provided immediately; "Stat" sampling: the sample must be taken immediately, but the results are not urgent.

Detection of Non-neutralizing Anti-FXIII Antibodies

The ELISA method is used to detect non-neutralizing antibodies directed against the FXIII A subunit in patients treated with FXIII-A concentrate. The requester uses an in-house protocol that comprises the following steps:

- dispensing of recombinant FXIII (TRETTEN^{MD}³) in a 96-well polystyrene microplate;
- detection of antibodies directed against FXIII-A bound with a peroxidase-labelled anti-human IgG detection antibody;
- addition of a chromogenic substrate composed of tetramethylbenzidine and hydrogen peroxide to obtain a colour reaction;
- measurement of colour intensity as optical density using a microplate reader at 492 nm (spectrophotometer).

To establish the positive range, six negative control plasmas are used.

2.3 Company or Developer

TECHNOZYM® FXIII-A and FXIII-B SUB ELISA commercial kits, from the Austrian company Technoclone, are used to detect the A and B subunits of FXIII.

Other ELISA kits can be used to measure FXIII-A (Zymutest Factor XIII-A: manufactured by HYPHEN BioMed [Aniara, 2009]).

2.4 Licence: Not applicable.

2.5 Patent, If Any: Not applicable.

2.6 Approval Status (Health Canada, FDA)

The commercial kits TECHNOZYM® FXIII-A SUB ELISA [Technoclone, 2010a] and TECHNOZYM® FXIII-B SUB ELISA [Technoclone, 2010b] are not approved by Health Canada. The product monographs indicate that they are "For Research Use Only."

2.7 Weighted Value

- Detection of A subunit of FXIII: 31.0 units
- Detection of B subunit of FXIII: 31.0 units
- Detection of an anti-FXIII: 33.29 units ("routine") and 389.96 units ("stat").

³ This pharmaceutical was produced by **Novo Nordisk Canada Inc.**, and contains the medicinal ingredient catrideracog, a recombinant human coagulation factor composed of two FXIII A subunits (rFXIII-A₂) that is structurally identical to the human FXIII A subunit. Upon injection, rFXIII-A₂ dimers bind to endogenous circulating B subunits to form a stable rA₂B₂ heterotetramer. On July 19, 2012, Health Canada issued a Notice of Compliance to Novo Nordisk Canada Inc. for this product (Summary Basis of Decision (SBD) for TRETTEN^{MD}, available at: http://www.hc-sc.gc.ca/dhp-mps/prodpharma/sbd-smd/drug-med/sbd_smd_2012_tretten_152228-eng.php).

3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patient Group

Patients with congenital FXIII deficiency:

- In whom the subunit (FXIII-A or B) involved needs to be determined to classify the type of deficiency, in order to choose the appropriate replacement therapy (FXIII concentrate or recombinant FXIII);
- treated with FXIII concentrate with assessment of immune response (three times a year) by testing for and titrating non-neutralizing antibodies directed against FXIII. For cases presenting clinically with bleeding, an emergency test ("stat") is required.

3.2 Targeted Disease(s)

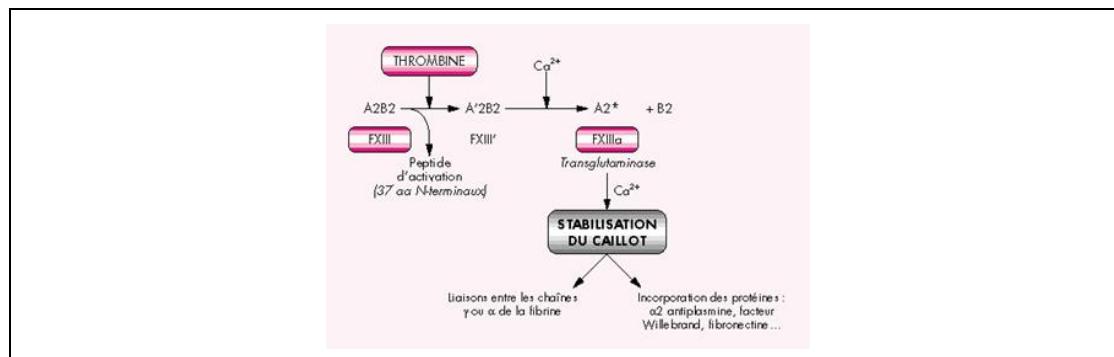
Congenital Factor XIII Deficiency

Congenital Factor XIII (FXIII⁴) deficiency is a rare coagulation disorder with an incidence of approximately 1 in 2 million cases. It is inherited in an autosomal recessive manner and has a high prevalence in regions where consanguineous marriages are common. Factor XIII is a protransglutaminase that plays a major role in the final stage of blood coagulation and an important role in wound healing and tissue repair. In plasma, circulating FXIII (pFXIII) has a tetrameric structure composed of A subunits (FXIII-A with a molecular weight of 83 kDa), produced by medullary cells, and B subunits (FXIII-B with a molecular weight of 80 kDa), synthesized by hepatocytes. These two subunits are held together by noncovalent bonds and, under normal conditions, circulate in plasma as complexes (FXIII-A₂B₂). Factor XIII is also present in cells (cFXIII) such as platelets, monocytes/macrophages, and bone marrow precursor cells. On average, 50% of FXIII-B is found in its free form, and helps stabilize FXIII-A by preventing it from being rapidly eliminated from circulation. Plasma thrombin, with its fibrin cofactors (fibrinogen) and Ca²⁺ ions, initiates the activation of FXIII during the final stage of the coagulation cascade. Thrombin cleaves the FXIII-A activation peptide and, in the presence of Ca²⁺, A and B subunits dissociate, and the FXIII-A₂ dimer transforms into an active transglutaminase, activated FXIII [FXIIIa] (Figure 1). The role of FXIIIa is to catalyze the formation of covalent bonds between α- and β-fibrin monomers to increase the strength of the fibrin clot and prevent it from being rapidly eliminated by plasmin [Kohler et al., 2001; Muszbek et al., 2011].

In the normal population, the rate of FXIII activity varies considerably from 53.2% to 221.3% (mean ± standard deviation: 105% ± 28.56%) [Tahlan and Ahluwalia, 2014]. However, there is some uncertainty regarding the low values of plasma FXIII activity, which leads to a lack of correlation between the severity of hemorrhagic clinical manifestations and laboratory results [Muszbek et al., 2011]. In most cases, the rates of FXIII activity that define the severe clinical form range between < 1% [Jennings et al., 2003], < 3% [Karimi et al., 2009], and < 5% [Tahlan and Ahluwalia, 2014]. It is therefore necessary for the tests used to be sufficiently sensitive to detect a decrease in the levels of FXIII activity.

⁴ All abbreviations used to designate the different forms of factor XIII comply with the recommended terms and abbreviations for factor XIII from the Subcommittee of the Scientific and Standardization Committee [Muszbek et al., 2007].

Figure 1: Functions of factor XIII [Alhenc-Gelas, 2000]



Congenital FXIII deficiency is caused by a deficiency in FXIII-A resulting from a mutation in the *F13A1* gene located on chromosome 6, or by a deficiency in FXIII-B, resulting from a mutation in the *F13B* gene located on chromosome 1. Up until 2009, more than 70 mutations in the FXIII-A gene and four mutations in the FXIII-B gene had been described [Muszbek et al., 2011]. The homozygous and heterozygous forms represent the severe forms of the disease. Factor XIII-A deficiency may be quantitative (type I), resulting from decreased protein synthesis, or qualitative (type II), which occurs when the concentration of FXIII-A is normal or near normal but the protein is functionally defective (Figure 2). Factor XIII-B deficiency is relatively rare, and symptoms of hemorrhage are mild [Kohler et al., 2011; Muszbek et al., 2011].

Figure 2: Classification of FXIII deficiencies [Karimi et al., 2009]

Plasma Activity	Plasma Antigen		Platelet Antigen	
	A ₂ B ₂	A	B	A
FXIII-A deficiency				
Type I (quantitative)	↓↓↓	↓↓↓	↓↓↓	>30% ↓↓↓
Type II (qualitative)	↓↓↓	↓-n	↓-n	>30% ↓-n
FXIII-B deficiency	↓↓	↓↓↓	↓↓	↓↓↓ n

n, normal.

In 2011, the International Society on Thrombosis and Haemostasis (ISTH) recommended an algorithm to improve diagnostic efficiency and classification of FXIII deficiencies that included FXIII activity measurements. If FXIII activity is decreased, the subtype of FXIII deficiency is established by measuring FXIII-A2B2 antigen concentration. If the concentration is decreased, FXIII-A and FXIII-B subunit antigen concentration as well as FXIII activity and FXIII antigen in platelet lysate should also be measured. The presence of non-neutralizing antibodies against FXIII-A and FXIII-B may be responsible for the decrease in FXIII-A2B2 and should be determined [Kohler et al., 2011; Muszbek et al., 2011].

Most patients with congenital FXIII deficiency will, during their lifetime, experience hemorrhagic diathesis,⁵ which has variable clinical manifestations. The clinical signs are

⁵ Hemorrhagic syndrome caused by an abnormality of the vascular, platelet or plasma systems, which are essential for normal homeostasis.

unpredictable and may manifest as long periods of mild symptoms followed by severe hemorrhagic complications with a very high risk of early mortality. These may include umbilical cord bleeding a few days after birth (56%), intradermal (57%) or intramuscular (49%) bleeding, and intracranial hemorrhage (34%). Approximately 80% of deaths are attributable to intracranial hemorrhage. Factor XIII also plays a role in the maintenance of pregnancy, the formation of the cytotrophoblast, and the implantation of the placenta, which explains the risk of early miscarriage (> 80%) in women with severe FXIII deficiency [Fadoo et al., 2013].

Factor XIII-B deficiency may secondarily cause plasma FXIII-A deficiency as a result of the loss of FXIII-B's protective effect. Very few cases of severe congenital FXIII-B deficiency have been reported worldwide [Wada et al., 2013]. Clinically, FXIII-B deficiency is characterized by mild bleeding caused by the presence of small quantities of plasma FXIII-A (< 10%) and normal concentrations of intracellular FXIII-A (Table 1). For this reason, the diagnosis of congenital FXIII-B deficiency may easily be missed. However, when there are other concurrent hemostatic disorders, severe or deadly hemorrhagic episodes may be observed [Schroeder and Kohler, 2013].

Treatment of Congenital FXIII Deficiency

When a diagnosis of severe congenital FXIII deficiency is confirmed, prophylactic replacement therapy (10 U/kg to 20 U/kg of FXIII) every 4 to 6 weeks is strongly recommended, particularly when FXIII concentrations are below 1 U/dL. Plasma-derived FXIII concentrates and fresh frozen plasma are very effective treatment options, which, however, carry a risk of infection and allergic reaction, as is the case with all plasma-derived products. In recent years, the development of highly purified, plasma-derived recombinant FXIII (rFXIII) has represented a major improvement in the treatment of congenital FXIII deficiency [Tahlan and Ahluwalia, 2014; Fadoo et al., 2013; Inbal et al., 2012; Lovejoy et al., 2006]. The effectiveness of a new rFXIII (Tretten^{MD})⁶, produced in *Saccharomyces cerevisiae* (yeast), has been reported in relation to congenital FXIII-A deficiency [Inbal et al., 2012]. However, unlike plasma-derived FXIII concentrates, FXIII does not contain FXIII-B and is not indicated for FXIII-B deficiencies [Biswas et al., 2014].

Non-neutralizing Anti-factor XIII Antibodies

Unlike neutralizing antibodies,⁷ non-neutralizing antibodies form immune complexes, thus decreasing levels of FXIII subunits, which are rapidly eliminated by the reticuloendothelial system. Older publications refer only to the development of neutralizing anti-FXIII-A antibodies, which may be due to the difficulty in detecting non-neutralizing antibodies [Tahlan and Ahluwalia, 2014; Muszbek et al., 2011]. The latter are transient, low-titre, and occur in very few patients [Schroeder et Kohler, 2013].

⁶ Catridercog, the active ingredient of Tretten^{MD}, is a recombinant human coagulation factor XIII-A₂ (rFXIII-A₂). It is a homodimer composed of two FXIII A-subunits. It is structurally identical to human FXIII A-subunit (A₂). Upon injection, the rFXIII A2 dimers bind to endogenous circulating B-subunits that circulate in excess to form a stable rA2B2 heterotetramer (Notice of Compliance issued by Health Canada on July 19, 2012 for Tretten^{MD}). Available at: http://www.hc-sc.gc.ca/dhp-mps/prodpharma/sbd-smd/drug-med/sbd_smd_2012_tretten_152228-eng.php.

⁷ Antibodies directed against the active site of the hemostasis factor and capable of neutralizing the same factor of a control plasma in vitro [Biomnis, 2012]. These antibodies can inhibit the activation of FXIII, FXIIIa activity, or the binding of FXIII to fibrinogen [Muszbek et al., 2011].

3.3 Number of Patients Targeted

Congenital FXIII deficiency showing a deficit of subunit A or B: the requester estimates the provincial volume to be approximately 20 to 30 patients per year over the next three years.

Based on the information provided by the requester, the detection of non-neutralizing anti-FXIII antibodies will be limited by the small number of patients with severe FXIII deficiency in Quebec (currently: 13 patients). The requester anticipates approximately 40 tests per year over the next 3 years.

According to the Canadian Hemophilia Registry,⁸ FXIII deficiency was diagnosed in 2013 in 54 Canadians, 31 of whom had severe forms of the disease.

3.4 Medical Specialties and Other Professions Involved

Hematology, hemostasis, obstetrics, gynecology and surgery.

3.5 Testing Procedure

Blood samples are obtained in a sampling centre using venipuncture.

4 TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology: Single tests.

4.2 Brief Description of the Current Technological Context

Factor FXIII has traditionally been measured using a qualitative method, a test based on the solubility of a fibrin clot in a 5 M urea or 1% monochloroacetic acid solution (clot solubility test). In the case of FXIII deficiency, the clot dissolves rapidly, within a few minutes to 1 hour. In the absence of FXIII deficiency, the clot remains insoluble. However, this method detects only very severe FXIII deficiency and is difficult to standardize. Its sensitivity depends on the fibrinogen level, the clotting reagent (thrombin and/or Ca^{2+}), and the solubilizing agent and its concentration. The detection limit varies between 0.5% and 5% of FXIII activity. The large number of cases that remain undiagnosed or are diagnosed late is partly attributable to the use of this test, which is no longer recommended [Kohler et al., 2011]. However, it may be the only test available within the context of a limited health care budget [Schroeder and Kohler, 2013].

Quantitative methods for the measurement of FXIII activity are based on two assays:

- 1) the measurement of ammonia released during the transglutaminase reaction;
- 2) the measurement of labelled amine incorporated into a protein substrate. In both types of assay, pFXIII is activated by thrombin and Ca^{2+} .

The advantage of the first assay is that it is quick and easy to automate; disadvantages include low sensitivity and a quantitation limit between 3% and 5%. The second assay is very sensitive, but it is more time-consuming and difficult to standardize [Kohler et al., 2011].

⁸ Canadian Hemophilia Registry. Rare Coagulation Disorders. 2013. Available at: <http://fhs.mcmaster.ca/chr/pdf/13/CHRRareCoag130517.pdf>.

4.3 Brief Description of the Advantages Cited for the New Technology

The ELISA assay can be used to directly detect FXIII-A or B. This method is very sensitive and reliable for low values of FXIII, but it is more time-consuming and cannot be automated [Schroeder et al., 2007]. According to a survey on the performance of laboratory tests conducted from 2010 to 2011 in laboratories that participated in the External Quality Control of Diagnostic Assays and Tests (ECAT) program, only 20% of laboratories use the ELISA method to quantify FXIII-A and B antigens [Hsu et al., 2014]. It is often difficult to integrate this method into a coagulation laboratory's workflow, either because the technique is manual, or because it involves batch processing; that is, the samples must be collected—sometimes for several days—before the test is carried out, and only whole strips (usually eight tests) can be used for one assay [Kappel and Ehm, 2010]. These methods may also be difficult to integrate into a perioperative environment, where results must be obtained quickly. Generally, in-house immunological tests are used to measure FXIII subunits, FXIII-A or the FXIII-A₂B₂ complex [Raut et al., 2007].

5 EVIDENCE

5.1 Clinical Relevance

5.1.1 Other Tests Replaced: The tests will not replace an existing test.

5.1.2 Diagnostic or Prognostic Value

The detection of FXIII-A and B allows FXIII deficiency to be classified and helps guide the choice of replacement therapy. The ELISA method is recommended for the diagnosis and classification of FXIII, as it allows FXIII-A and B to be directly measured, using specific antibodies, and allows therapeutic monitoring of FXIII deficiency [Fadoo et al., 2013; Kessel et al., 2013; Kohler et al., 2011; Muszbek et al., 2011; Schroeder et al., 2007].

The development of non-neutralizing antibodies is rarely observed, and the clinical effect of these antibodies is unknown. Some authors consider the ELISA method, or the method referred to as "dot blot," to be necessary for the detection of these antibodies [Kessel et al., 2013; Kohler et al., 2011; Muszbek et al., 2011].

5.1.3 Therapeutic Value

The therapeutic value of the new tests concerns the choice of replacement therapy based on test results and on the FXIII subunit responsible for the deficiency. In fact, the effectiveness of treatment depends on whether non-neutralizing anti-FXIII antibodies are present.

5.2 Clinical Validity

No studies on the clinical validity of the detection of FXIII A and B subunits or non-neutralizing anti-FXIII antibodies were identified. It should be noted that congenital FXIII deficiency is an extremely rare disease. According to the Canadian Hemophilia Registry,⁹ the deficiency was diagnosed in 2013 in 54 Canadians, 31 of whom had severe forms of the disease. According to the Factor XIII Registry Database,¹⁰ approximately 300 cases have been reported worldwide.

⁹ Canadian Hemophilia Registry. Rare Coagulation Disorders. 2013. Available at: <http://fhs.mcmaster.ca/chr/pdf/13/CHRRareCoag130517.pdf>.

¹⁰ Factor XIII Registry Database. Introduction to FXIII Deficiency [website]. Available at: [http://www.f13-database.de/\(i4njjn5451kccdtjngc31n45\)/content.aspx?menu=1,39](http://www.f13-database.de/(i4njjn5451kccdtjngc31n45)/content.aspx?menu=1,39).

5.3 Analytical (or Technical) Validity

COMPONENT	PRESENCE	ABSENCE	NOT APPLICABLE
Repeatability	X		
Reproducibility	X		
Analytical sensitivity			
Analytical specificity	X		
Matrix effect			
Concordance	X		
Correlation between test and comparator			
Other, depending on type of test			

Very few studies have published results on the analytical validity of the ELISA method used to detect A or B subunits of FXIII. No published studies on the detection of non-neutralizing anti-FXIII antibodies were identified.

Precision

A Subunit of FXIII

The monograph for the TECHNOZYM® ELISA kit reports an inter-assay variability of 3.7% to 4.2% (2 plasma samples and 4 assays) for concentrations of $7.45 \pm 0.28 \mu\text{g/mL}$ to $1.16 \pm 0.06 \mu\text{g/mL}$, respectively, for the detection of the A subunit. Intra-assay variability is estimated at 3.1% to 5.3% (2 plasma samples and 5 assays) for concentrations of $8.28 \pm 0.26 \mu\text{g/mL}$ to $1.14 \pm 0.06 \mu\text{g/mL}$, respectively [Technoclone, 2010a].

A study published as an abstract used the same kits and also reported a high degree of precision based on tests conducted on two control plasma concentrations: inter-assay coefficient of variation (CV) of 6.4% and intra-assay CV of 4.6% [Vetr et al., 2010].

The ELISA method, developed by Katona et al. [2001], has a high degree of precision for three categories of plasma (20 measurements each) and an intra-assay variability of 5.9% (low-concentration control plasma provided by the company Dade Behring), 5.98% (plasma from a patient with FXIII deficiency), and 3.11% (normal plasma provided by Dade Behring). The daily accuracy for 16 measurements was 7.15% and 9.98% for normal and low-concentration plasmas (provided by Dade Behring).

B Subunit of FXIII

The monograph of the TECHNOZYM® ELISA kit reports an inter-assay variability of 1.1% to 7.3% (2 plasma samples and 3 assays) for concentrations of $14.04 \pm 0.15 \mu\text{g/mL}$ to $2.01 \pm 0.15 \mu\text{g/mL}$, respectively, for the detection of the B subunit. The intra-assay CV ranges from 2.2% to 3.4% (2 plasma samples and 5 assays) for concentrations of $13.27 \pm 0.29 \mu\text{g/mL}$ to $1.93 \pm 0.07 \mu\text{g/mL}$, respectively [Technoclone, 2010b].

Vetr et al. [2010] used these same kits and reported an inter-assay CV of 6.4% and intra-assay CV of 4% for two control plasma concentrations.

Repeatability

Murdock et al. [1992] developed an ELISA procedure to detect FXIII-A and B subunits. Standards were established using a pool of 20 normal plasma samples with A and B subunit concentrations of 100 U/dL (100%). The variations observed in the measurements of A and B subunits were expressed as mean plasma concentrations along with a standard curve in duplicate. However, the authors of this study did not clearly indicate whether the results that were provided represent a mean of the values obtained or the observed variation.

Intra-assay variability (normal and abnormal plasma, measured 10 times):

- A subunit of FXIII:
 - Normal plasma (mean): $110.7 \text{ U/dL} \pm 3.3$
 - Abnormal plasma (mean): $50.6 \text{ U/dL} \pm 3.4$
- B subunit of FXIII:
 - Normal plasma (mean): $109.9 \text{ U/dL} \pm 1.3$
 - Abnormal plasma (mean): $34.3 \text{ U/dL} \pm 1.5$

Inter-assay variability (normal plasma measured 4 times in 10 separate tests):

- A subunit of FXIII:
 - Normal plasma (mean): $104.1 \text{ U/dL} \pm 3.9$
- B subunit of FXIII:
 - Normal plasma (mean): $104.8 \text{ U/dL} \pm 2.9$

Reproducibility

Katona et al. [2000] reported that the intralot reproducibility of the ELISA method, for 20 diluted plasma samples with normal (mean: $19.4 \pm 0.38 \text{ mg/L}$) and low (mean: $1.94 \pm 0.064 \text{ mg/L}$) FXIII concentrations, is good: the resulting coefficients of variation are 2.0% and 3.3%, respectively. The inter-daily variability for 10 plasma samples with normal concentrations of FXIII (mean of $19.4 \pm 1.06 \text{ mg/L}$) was 5.5% and 8.7% for 9 plasma samples with low concentrations (mean: $2.38 \pm 0.21 \text{ mg/L}$).

Linearity

The results of the two Technozym FXIII ELISA assays used to measure FXIII-A and B are linear over a wide range of concentrations.

Monograph of the TECHNOZYM®ELISA Technoclone [2010] kit:

- A subunit: 1 $\mu\text{g/mL}$ to 20 $\mu\text{g/mL}$ (9.5% to 195%) for a plasma dilution of 1:1,000.
- B subunit: 2 $\mu\text{g/mL}$ to 40 $\mu\text{g/mL}$ for a plasma dilution of 1:1,000.

Vetr et al. [2010]:

- A subunit: 1 $\mu\text{g/mL}$ to 20 $\mu\text{g/mL}$ (9.5 % to 195% of the normal concentration) $R^2 = 0.998$;
- B subunit: 2 $\mu\text{g/mL}$ to 40 $\mu\text{g/mL}$, $R^2 = 0.997$.

Detection Limit

A Subunit of FXIII

The lower limit of detection is 0.35 $\mu\text{g/mL}$ for a plasma dilution of 1:1,000. This corresponds to 3.3% of the mean normal concentration of FXIII-A plasma. The sensitivity may be increased

to 0.033% with a plasma dilution of 1:10 for samples with extremely low concentrations [Technoclone, 2010a].

The lower limit of detection is 0.35 µg/mL, regardless of the plasma dilution. It is 0.4 µg/mL to 40 µg/mL for a recommended 1,000-fold dilution of plasma and as low as 4 ng/mL for a 10-fold dilution of plasma, which corresponds to a detection limit of FXIII-A concentration of 0.04% in healthy subjects [Katona et al., 2001].

B Subunit of FXIII

The lower limit of detection is 0.9 µg/mL for a plasma dilution of 1:1000. The sensitivity can be increased to 0.009 µg/mL with a plasma dilution of 1:10 for samples with extremely low concentrations [Technoclone, 2010b].

Analytical Specificity

Katona et al. [2000] tested the analytical specificity of the ELISA method by: 1) studying the cross-reactivity with free FXIII subunits; 2) assessing the effect of the presence of free FXIII subunits; and 3) assessing the effect of the bond between FXIII and fibrinogen. No reaction with cellular FXIII-A₂ or free FXIII-B was observed, even when the subunits of FXIII were present in molar concentrations four times higher than the linearity limit of the FXIIIA₂B₂ tetramer, which is 40 µg/L. Adding the free FXIII-A and B subunits to the FXIII-A₂B₂ tetramer, even at a molar concentration of 80 µg/L, had no effect on the concentration of plasma FXIII measured (results not reported).

To test whether the bond between fibrinogen and plasma-derived FXIII affects the availability of the respective epitopes that react with the anti-FXIII-A and B antibodies used, the test was carried out using purified plasma-derived FXIII in the presence or absence of fibrinogen. The presence of 2 g/L of purified fibrinogen had no effect on the absorbance measured at various concentrations of FXIII (from 1 mg/L to 20 mg/L).

In another series of experiments, concentrations of FXIII were determined using plasma from a patient with severe FXIII deficiency (0.061 mg/L) that contained a normal quantity of fibrinogen to which was added purified FXIII (1.5 mg/L and 15.0 mg/L) in two separate samples. Since plasma-derived FXIII, due to its low dissociation constant (K~10 to 8 M), is fully associated with fibrinogen, it was important to exclude fibrinogen's potential effect on the assay. The resulting concentrations of FXIII (1.48 mg/L and 15.0 mg/L, respectively) indicate an added recovery of FXIII of 94.7% (1.42 mg/L) and 100% (15.0 mg/L). Moreover, neither fibrinogen nor other plasma components interfere with the FXIII bound to the monoclonal antibodies used in this method. The authors conclude that even traces of FXIII (0.061 mg/L or 61 µL or 0.29%) in plasma could be measured reliably.

The manufacturer of the TECHNOZYM®ELISA (Technoclone) kit reports that the results of FXIII-A or FXIII-B are not affected by the plasma concentrations of fibrinogen or by the presence of any of the subunits, depending on the type of assay.

Yorifuji et al. [1988] tested the ELISA method for FXIII-A and B and FXIIIA₂B₂ complex by using polyclonal anti-A IgG antibodies (a capture antibody and a detection antibody) to measure FXIII-A₂ and a polyclonal anti-B (IgG) antibody to measure total B protein (free and bound to the complex). No cross-reactivity occurred with the antibodies: anti-A antibody did not react with FXIII-B, and anti-B antibody did not react with FXIII-A. High concentrations of fibrinogen (400-

fold dilution of plasma) interfere with the association between FXIII-A and its antibody, but have no effect on the association between FXIII-B and its antibody. Therefore, the plasma samples must be diluted (concentration of fibrinogen of approximately 75 µg/mL to 100 µg/mL).

Katona et al. [2001] used two monoclonal antibodies, one biotinylated, the other conjugated with horseradish peroxidase, that were directed against different epitopes on FXIII-A (3B2H12; anti-FXIII-A-1 and 3A6H7; anti-FXIII-A-2). The authors wanted to show that identical results were obtained with FXIII-A bound to the FXIIIA₂B₂ complex and unbound FXIII-A. The absorbance obtained from combining the saturating concentrations of the two antibodies showed an additive effect, with an additive index ELISA of 82%. The Deming regression method was used to compare the results of FXIII-A with those of the FXIIIA₂B₂ complex and then with those of FXIII activity. The first comparison, which included the plasma of 35 healthy subjects and 179 patients, showed a strong correlation ($r = 0.965$), with a mean concentration of FXIII-A of 52%, close to that of the FXIIIA₂B₂ complex (51%). The second comparison (FXIII-A with FXIII activity), carried out using the plasma of 166 patients, showed a correlation of $r = 0.870$. Factor XIII-A was not affected by the addition of FXIII-B. The formation of an A₂B₂ tetrameric complex therefore does not affect the availability of anti-FXIII-A-1 and anti-FXIII-A-2 antibodies for the respective epitopes, and the bond between FXIII and fibrinogen does not interfere with the assay. The authors therefore concluded that the ELISA method shows good reproducibility and high sensitivity [Katona et al., 2001].

Stability of FXIII-A

Once the plasma samples are stored, FXIII is protected against damage caused by freezing and thawing and retains its antigenicity and activity for at least 6 months at -20°C and -70°C (98.5% and 106%, respectively) [Kárpáti et al., 2010]. Factor XIII-A recovery after the plasma samples were stored for 6 months at -20°C was 99.2% [Katona et al., 2001].

Correlation

The correlation between the measurements of FXIII (mg/L) concentrations obtained with ELISA and the measurements of FXIII (%) activity obtained with UV spectrophotometry in 60 healthy subjects: $r = 0.935$ [Katona et al., 2000].

5.4 Recommendations from Other Organizations

In the United Kingdom, the British Committee for Standards in Haematology recommends the use of immunological methods (immunoturbidimetric tests or ELISA) for the detection of FXIII-A and B, the diagnostic confirmation, and the classification of FXIII deficiency, while specifying that this step is deemed important particularly if a treatment with recombinant FXIII-A protein is anticipated. The Committee indicates that the clot solubility test for FXIII deficiency shows low sensitivity and may not be reliable [Mackie et al., 2012]. These practice guidelines do not address therapeutic follow-up or the issue of non-neutralizing antibodies.

6 ANTICIPATED OUTCOMES OF INTRODUCING THE TEST

- 6.1 Impact on Material and Human Resources:** Not assessed.
- 6.2 Economic Consequences of Introducing Test Into Quebec's Health Care and Social Services System**
 - Not assessed.
- 6.3 Main Organizational, Ethical, and Other (Social, Legal, Political) Issues**
 - Not assessed.

7 IN BRIEF

7.1 Clinical Relevance

The results of the new test are used for the classification of congenital FXIII deficiency and the prompt implementation of an available replacement therapy for FXIII-A, for the most common and severe clinical form of FXIII-A deficiency.

The development of non-neutralizing antibodies in patients treated with recombinant FXIII is rare; consequently, the clinical effect of the presence of this type of antibody is unknown.

7.2 Clinical Validity

No studies on the clinical validity of these tests have been identified.

7.3 Analytical Validity

The analyzed data from the monographs of the kits used by the requester and several validation studies indicate good analytical performance.

7.4 Recommendations from Other Organizations

Proven clinical utility of the ELISA method for the detection of A and B subunits of FXIII with a recommendation from the British Committee for Standards in Haematology in the United Kingdom. No recommendations for non-neutralizing antibodies were identified.

8 INESSS NOTICE IN BRIEF

Detection of A and B Subunits of Factor XIII and Detection of a Non-neutralizing Anti-factor XIII byELISA

Status of the Diagnostic Technology:

- Established
- Innovative
- Experimental (for research purposes only)
- Replacement for technology: _____ which becomes obsolete

INESSS Recommendation:

- Inclusion of test in the Index conditional upon the implementation of external quality control measures for the detection of anti-factor XIII if the volume is insufficient
- Do not include test in the Index
- Reassess test

Additional Recommendation:

- Draw connection with listing of drugs, if companion test
- Produce an optimal use manual
- Identify indicators, when monitoring is required

Notes

- External quality control measures are essential considering the low volume expected.
- The test kit is not licensed in Canada.
- The requester's laboratory has the expertise required to perform the test in Quebec.

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