

Elevated Factor VIII

Determination of elevated levels of factor VIII activity in plasma.

Background

Historically, factor VIII (FVIII) has always been associated with bleeding, since the well known pathological condition Haemophilia A is a consequence of very low plasma levels of FVIII. On the other hand, FVIII is also an acute phase reactant, and hence elevated levels are associated with conditions such as trauma, infection and exercise. Common to many other coagulation factors, FVIII also rises during pregnancy. FVIII is a key procoagulant factor and recent studies have shown the association between elevated levels of FVIII activity and an increased risk of venous and seemingly also arterial thrombosis (1-4). A concomitant increase was also noticed for von Willebrand factor (1-3). Importantly, FVIII activity shows a high correlation to FVIII antigen (FVIII:Ag), thereby attributing the increased activity to an increased FVIII synthesis (2, 5). So far the increased plasma FVIII:Ag has not been linked to any polymorphism of the FVIII gene promoter 5', but the search for a genetic contribution is still under investigation. FVIII activity in thrombotic patients is often above 1.5 IU/ml and might reach levels of 4-5 IU/ml (2), sometimes in connection with highly inflammatory conditions.

Therefore, a specific adaptation of Coamatic Factor VIII has been developed to allow accurate determination of elevated FVIII activity. The advantages in using a chromogenic method as compared to one-stage clotting methods are numerous. In particular the chromogenic method is not sensitive to preactivation of FVIII (6), thereby avoiding overestimation of FVIII activity.

Furthermore, due to its linear dose-response, it has a higher resolution at elevated levels and also a high precision. These features make Coamatic Factor VIII ideal as a tool for thrombophilia screening in addition to its established use for diagnosis of hemophilia and FVIII potency estimation of concentrates.

Reagents: Coamatic Factor VIII

Kit configuration

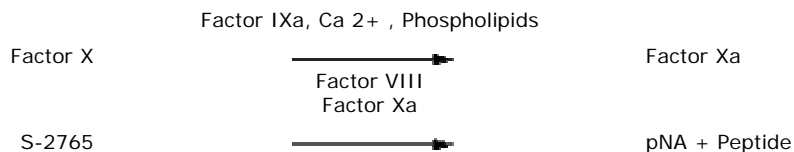
S-2765 + I-2581 1 vial

Factor reagent 2 vials

Buffer, stock solution 1 vial

Measurement principle

Factor VIII acts as an enzymatic cofactor of factor IXa during the activation of factor X to factor Xa in the presence of calcium ions and phospholipids. Factor Xa hydrolyses the chromogenic substrate S-2765 thus liberating the chromophore pNA. The colour is then read spectrophotometrically at 405 nm. The generated factor Xa and thus the intensity of colour is proportional to the factor VIII activity in the sample. Thrombin, that is contained in the factor reagent, brings about a rapid and complete activation of the FVIII present in the sample.



Specimen collection

Follow the instructions described in the Coamatic Factor VIII package insert.

Determination of elevated levels of Factor VIII activity

The applications of the Coamatic Factor VIII kit are currently referred to a low assay range and to a normal assay range. The upper measuring limit using the procedure for the normal assay range is 1.42 IU/ml for the microplate method and 1 IU/ml for the ACL method. The determination of FVIII activities higher than these limits, can be performed by pre-diluting the plasma samples 1:4 and assaying the diluted samples following the protocol described for the normal assay range but restricting this range to 0-1 IU/ml. The results should be multiplied by 4 to obtain the final value of FVIII activity.

- Pre-dilute the samples using the buffer contained in the Coamatic Factor VIII kit as follows:
1 vol plasma sample + 3 vol diluted buffer
- Dilute further as detailed in the package insert
- Follow the instructions contained in the Coamatic Factor VIII package insert (microplate procedure) or in the instrument application sheet (automated)

instruments)

Microplate method

Reagent preparation

Factor reagent: 3.0 ml of sterile water

Substrate: 6.0 ml of sterile water

Buffer: dilute 1:10 with sterile water

Standard curve

The standard curve 0-1 IU/ml is prepared by using a human normal plasma calibrated against an International Standard for plasma FVIII. In case the normal plasma does not contain exactly 1 IU/ml FVIII, the values of the standard must be recalculated accordingly.

FVIII IU/ml	Predilution		Final Dilution	
	Plasma μ l	Buffer μ l	Dil. Plasma μ l	Buffer μ l
1.00	-	-	25	2000
0.70	100	100	25	1400
0.50	100	100	25	2000
0.25	50	150	25	2000
0.00	-	-	-	2000

Sample Dilution

1. Pre-dilute the sample by mixing 1 vol plasma with 3 vol of Coamatic Factor VIII Buffer
2. Dilute further as follows: 25 μ l Sample + 2000 μ l Buffer

Assay procedure

Diluted samples/controls/standards	50 μ l
Incubate at 37°C	3-4 min
Factor reagent (37°C)	50 μ l
Incubate at 37°C	2 min
Substrate	50 μ l
Incubate at 37°C	2 min
Acetic acid, 20%	50 μ l

Read the absorbance at 405 nm, using a reference wavelength of 490 nm.

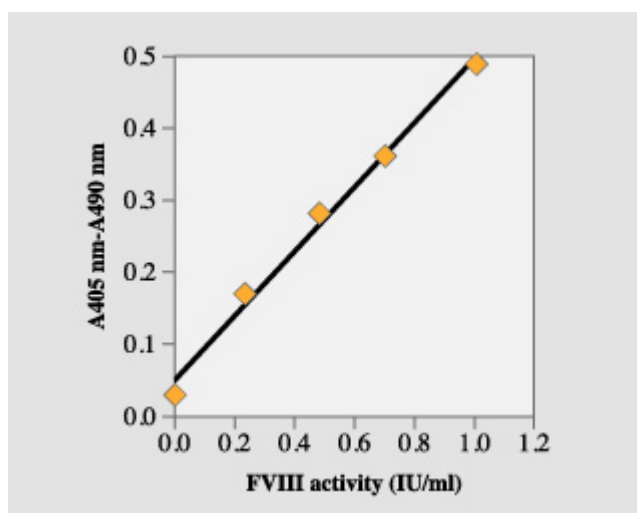


Fig. 1. Standard curve with the microplate method.

ACL method

This method is applicable to the ACL™200/300/3000/6000/7000.

Reagent preparation

Factor reagent: 3.0 ml of sterile water

Substrate: 5.25 ml of sterile water

Buffer: dilute 1:10 with sterile water

Standard curve

The standard curve is prepared by using a human normal plasma calibrated against an International Standard for plasma FVIII.

Dilute the standard as follows: 25 μ l plasma + 2000 μ l buffer

Sample Dilution

1. Pre-dilute the sample by mixing 1 vol plasma with 3 vol of Coamatic Factor VIII Buffer
2. Dilute further as follows: Sample 25 μ l Buffer 2000 μ l

Assay procedure

Select the test Plasminogen (channel).

Place diluted normal plasma in POOL position.

Place buffer working solution in DIL position.

Place factor reagent in position 2.

Place substrate in position 3.

Place sample cups with diluted plasmas.

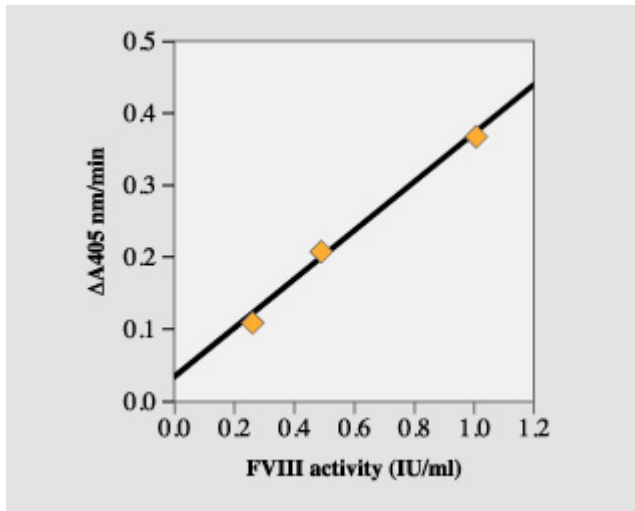


Fig. 2. Standard curve with the ACL method.

Measuring range

With pre-dilution of the sample the measuring range is 1 – 4 IU/ml with both the microplate and the ACL method.

Results

The evaluation of Coamatic Factor VIII with samples from thrombotic patients has been performed both with the microplate and the ACL applications. The standard curves are shown in figures 1 and 2 respectively. The upper limit of the standard curve is 1 IU/ml in both methods resulting in an upper measurement limit of 4 IU/ml, with plasma samples diluted 1:4. The precision of the method has been evaluated by using plasma samples diluted according to the protocol described above.

FVIII IU/ml	Within series		Between series		
	CV%	n	CV%	n	N
1.0	3.0	35	6.0	5	7
4.0	3.0	35	6.0	5	7

The FVIII activity of 130 patient samples has been determined with Coamatic Factor VIII on ACL, by pre-diluting or not the plasma samples. The samples have been obtained from patients about three months after the thrombotic episode. The following results were obtained from linear regression analysis (figure 3):

Slope = 1.52

Intercept = -0.57

R = 0.96

Range (x) = 0.45 – 3.28 IU/ml FVIII

Range (y) = 0.33 – 4.50 IU/ml FVIII

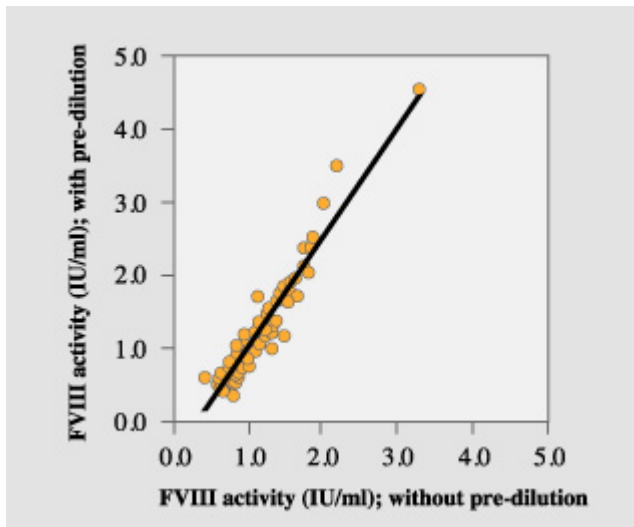


Fig. 3. Comparison of FVIII activities obtained with and without sample pre-dilution using Coamatic Factor VIII.

For FVIII activities higher than 1 IU/ml, the samples can be under-estimated if the pre-dilution is not performed.

Coamatic Factor VIII has been compared with a one-stage clotting method on the ACL analyser. For the Coamatic Factor VIII assay, the samples were pre-diluted 1:4 as recommended in the protocol described above. For the clotting method the plasma samples were pre-diluted 1:4 (with 0.05 mol/l imidazol, 0.1 mol/l NaCl, pH 7.3; buffer recommended by the clotting reagent manufacturer) followed by the prescribed sample dilution 1:5. 71 plasma samples from thrombotic patients were analysed. The results are shown in figure 4.

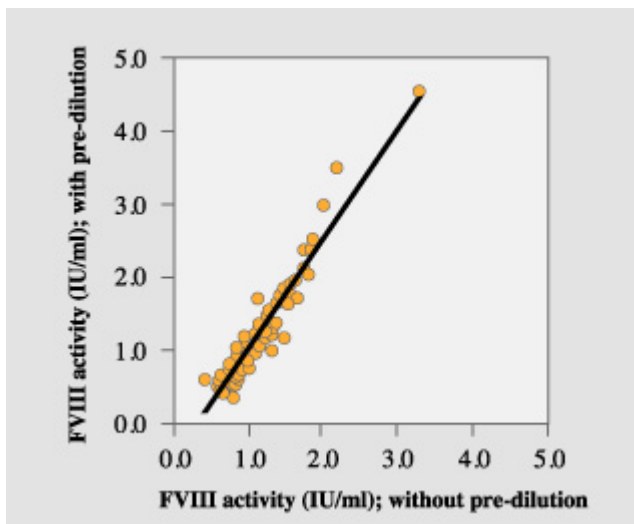


Fig. 4. Comparison of FVIII activities obtained by a clotting assay and Coamatic Factor VIII.

The following results were obtained from linear regression analysis:

Slope = 1.28

Intercept = -0.43

R = 0.92

Range (x) = 0.50 – 2.32 IU/ml FVIII

Range (y) = 0.18 – 2.51 IU/ml FVIII

Conclusions

The results described here represent a preliminary evaluation of Coamatic Factor VIII applied for the screening of samples from thrombotic patients. From the population of samples tested, about 25% had a FVIII activity higher than 1.4 IU/ml, thus confirming earlier published data 1,2 . These results have been obtained by a simple modification of the existing applications and protocols, consisting in the pre-dilution 1:4 of the plasma samples.

Coamatic Factor VIII is a kit suitable for use on a number of automated instruments as well as on microplates. The data presented here show its applicability on the ACL instrument for determination of elevated FVIII activity. In case the pre-dilution is done manually, the current application notes for automated instruments can then be adhered to, with the only exception of restricting the assay range to 0-1 IU/ml. Indeed, some instruments offer the possibility of also performing the predilution step.

Bibliography

1. Koster T, Blann AD, Briët E, Vanderbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. *Lancet* 345; 152-155 (1995).
2. O'Donnell J, Tuddenham EGD, Manning R, Kembell-Cook G, Johnson D, Laffan D. High prevalence of elevated factor VIII levels in patients referred for thrombophilia screening: role of increased synthesis and relationship to the acute phase reaction. *Thromb Haemost* 77; 825-828 (1997).
3. Gorog DA, Rkhit R, Parums D, Laffan M, Davies GJ. Raised factor VIII is associated with coronary thrombotic events. *Heart* 80; 415-417 (1998).
4. Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WRS, Haines AP, Stirling Y, Imeson JD, Thomsom SG. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart study. *Lancet* ii: 533-537 (1986).
5. Mansvelt EPG, Laffan M, McVey JH, Tuddenham EGD. Analysis of the F8 gene in individuals with high plasma factor VIII:C levels and associated venous thrombosis. *Thromb Haemost* 80; 561-565 (1998).
6. Rosén S, Andersson M, Blombäck U, Hägglund U, Larrieu MJ, Wolf M, Boyer C, Rothschild C, Nilsson IM, Sjörin E, Vinazzer H. Clinical application of a chromogenic substrate method for determination of factor VIII activity.

Additional publications

- Kraaijenhaagen RA et al. High plasma concentration of factor VIII:C is a major risk factor for venous thromboembolism. *Thromb Haemost* 83, 5-9 (2000).
- O'Donnell J et al. Elevation of FVIII:C in venous thromboembolism is persistent and independent of the acute phase response. *Thromb Haemost* 83, 10-13 (2000).
- Schambeck CM et al. Venous thromboembolism and associated high plasma factor VIII levels : Linked to Cytomegalovirus infection? *Thromb Haemost* 83, 510-511 (2000).
- Rosendaal FR. High levels of factor VIII and venous thrombosis. *Thromb Haemost* 83, 1-2 (2000).