Coagulation Factor VIII Deficient Plasma

FACTOR VIII DEFICIENT

Revision bar indicates update to previous version.

CE 0197

Intended Use

FACTOR VIII DEFICIENT is an in vitro diagnostic reagent for use in assays for the quantitative, WHO-standardized determination of coagulation factor VIII (FVIII) activity as aid to diagnosis and monitoring of congenital or acquired FVIII deficiencies in patients with bleeding disorders or at risk for FVIII deficiency in human sodium citrated plasma by means of automated, semi-automated and/or manual coagulometric methods. In addition, the coagulometric FVIII assay can be used for monitoring FVIII substitution therapy.

Summary and Explanation

Hemophilia A is a congenital, X-linked bleeding disorder caused by coagulation factor VIII (FVIII) deficiency. Acquired hemophilia A FVIII can develop due to presence of FVIII specific inhibitors. In the circulation FVIII is bound to its carrier protein von Willebrand factor (vWF). Both, FVIII and vWF are acute phase proteins, which increase in response to inflammation and stress. High FVIII levels constitute a prevalent, dose-dependent risk factor for venous thromboembolism[1–3].

FVIII is involved in the intrinsic coagulation pathway; hence a prolongation of activated partial thromboplastin time (APTT) is seen in case of a FVIII deficiency. The APTT-based one-stage clotting assay using **FACTOR VIII DEFICIENT** is applied for determination of FVIII activity[4,5].

The determination of FVIII in plasma is indicated in the following cases:

- clarifying the cause of a prolonged APTT,
- diagnosing congenital or acquired factor deficiency states,
- monitoring FVIII substitution therapy in hemophilia A
- distinguishing between dysproteinemias and protein synthesis disorders (in conjunction with immunochemical methods).

Principles of the Procedure

A plasma deficient in any of the factors comprising the intrinsic pathway will result in a prolonged partial thromboplastin time (APTT). Coagulation factor deficient plasma can be used to confirm a factor deficiency, in general, and to identify and quantify coagulation factor deficiency in patient plasma. A mixture of the respective factor deficient plasma and the patient plasma is tested in the APTT assay, and the result is interpreted using a reference curve obtained with dilutions of **STANDARD PLASMA** or a normal plasma pool mixed with the deficient plasma. A patient plasma deficient in a specific factor will not be able to compensate for the absence of the factor in the corresponding coagulation factor deficient plasma and therefore result in a prolonged APTT.

Reagents

Note: **FACTOR VIII DEFICIENT** can be used manually or on automated coagulation analyzers. Sysmex provides Reference Guides (Application Sheets) for several coagulation analyzers. The Reference Guides (Application Sheets) contain analyzer/assay specific handling and performance information which may differ from that provided in these Instructions for Use. In this case, the information contained in the Reference Guides (Application Sheets) supersedes the information in

these Instructions for Use. Please also consult the instruction manual of the instrument manufacturer!

Reagent	Description	Storage	Stability
Coagulation Factor VIII Deficient Plasma FACTOR VIII DEFICIENT	Lyophilized reagent containing: • human plasma[a], FVIII activity ≤1 % • Stabilizer: • D-Mannitol (reconstituted: 20 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: reconstituted, 8 hours[b]; –20 °C: reconstituted, 4 weeks[b]

[a] from pooled plasma collected from selected healthy blood donor
[b] closed original vial

FACTOR VIII DEFICIENT is manufactured by immunoadsorption from normal plasma and is free from the antigen of the respective factor. Fibrinogen is present in a quantity greater than 1.5 g/L, and the remaining coagulation factors are present in an activity greater than 50 % of Norm.

Von Willebrand factor is present in an activity greater than 50 % of Norm.

FACTOR VIII DEFICIENT can be frozen and thawed once after reconstitution without a loss of coagulation activity. The plasma must be well sealed and frozen as quickly as possible. Thawing should be accomplished at 37 °C within 10 minutes. Thawed plasma should be used within 2 hours when held at 15 to 25 °C.

On-board stability
Information regarding on-board stability is specified in the Reference Guides (Application Sheets) for the different coagulation analyzers.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State through your local distribution representative in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available upon request.

Caution! Potential Biological Risk
Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests that are CE marked or FDA approved for this purpose. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Caution
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Summary of Safety and Performance (SSP) is available in the European database on medical devices (see Eudamed public website: https://ec.europa.eu/tools/eudamed). In case Eudamed is not available, SSP can be delivered by the manufacturer on request.

Preparing Reagents

FACTOR VIII DEFICIENT:

1. Reconstitute 1 mL by adding distilled or deionized water.
2. Allow to stand at 15 minutes for at least 15 to 25 °C.
3. Shake carefully to dissolve (without foam formation).
4. Before use, gently mix once more.

APTT reagent:

- Use according to the respective Instructions for Use.

$CaCl_2$ SOLUTION 0.025 mol/L:

- Warm to 37 °C (not required for automated coagulation systems with heated reagent probes).

Specimen Collection and Handling

Collecting the Specimen

To obtain the plasma, carefully mix one part of 0.11 or 0.13 mol/L (3.2 % or 3.8 %) sodium citrate solution with nine parts of freshly collected patient blood (commercial blood collection systems), avoiding the formation of foam. Centrifuge the blood specimen at 1 500 × g at least 15 minutes at 15 to 25 °C[6]

Storing the Specimen

Stability of the samples:

15 to 25 °C	3 hours
−20 °C	2 weeks

Plasma stored at −20 °C is to be thawed in a water bath within 10 minutes at 37 °C, mixed gently and then tested immediately. If testing cannot be performed immediately after thawing, the specimen may be held for a maximum of 2 hours at 4 °C until tested (H21-A5)[6].

Procedure

Materials Provided

REF	Contents		
OTXW17	Coagulation Factor VIII Deficient Plasma FACTOR VIII DEFICIENT	8 × →	1 mL

Materials Required but not Provided

Item	Description
REF OQGS29, OQGS35	Pathromtin® SL, or
REF B4218-1, B4218-2	ACTIN, Dade® Actin® Activated Cephaloplastin Reagent, or
REF B4218-20, B4218-100	ACTIN FS, Dade® Actin® FS Activated PTT Reagent, or
REF B4219-1, B4219-2	ACTIN FSL, Dade® Actin® FSL Activated PTT Reagent
REF ORHO37	$CaCl_2$ SOLUTION, Calcium Chloride Solution
REF OQAA33 REF B4234-25 REF B4265-35, B4265-37 –	IMIDAZOLE BUFFER, Imidazole Buffer Solution, or OV BUFFER, Dade® Owren's Veronal Buffer, or CA SYSTEM BUFFER, Dade® CA System Buffer, or Physiological Saline Solution
REF ORKL17	STANDARD PLASMA, Standard Human Plasma
REF ORKE41	CONTROL N, Control Plasma N
REF OUPZ17	CONTROL P, Control Plasma P
Coagulation analyzers[c], such as:	• Automated Blood Coagulation Analyzer CA-600 series (CA-600 series) • AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) • AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System) • Automated Blood Coagulation Analyzer CN-3000/CN-6000 (CN-3000/CN-6000 System)

[c] Availability of analyzers may vary by country.

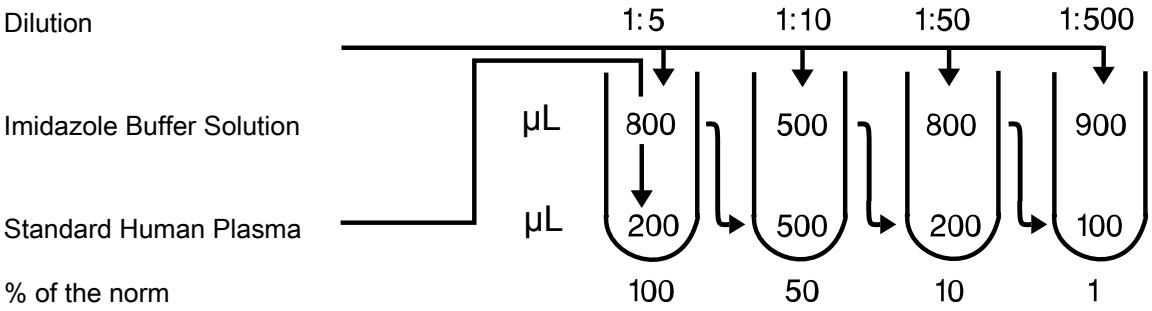
Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified.

Manual Testing

Pre-warm $CaCl_2$ SOLUTION at 37 °C	
Immediately before the measurement: Dilute plasma sample 1:5 in IMIDAZOLE BUFFER (equilibrated at 15–25 °C).	
Pipette into a test tube pre-warmed to 37 °C:	
FACTOR VIII DEFICIENT	100 µL
Plasma (diluted)	100 µL
APTT reagent	100 µL
	Mix well. Incubate at 37 °C for 2 minutes.
$CaCl_2$ SOLUTION (pre-warmed)	100 µL
	On addition of $CaCl_2$ SOLUTION start stop-watch or timer on the coagulation analyzer and determine the coagulation time.

Establishment of the Reference Curve

Use either STANDARD PLASMA or fresh citrated pooled plasma of at least 10 healthy donors. Using IMIDAZOLE BUFFER, prepare dilutions as shown in the following scheme and determine the coagulation times as described under "Manual Testing", page 4. A new reference curve must be generated if there is a change in the instrument or in the lot of reagent used, or if there is any change in the experimental conditions.



Additional dilutions can be prepared if necessary. Plot the measured coagulation times (ordinate) on log graph paper against the corresponding percentage factor activities (abscissa). The coagulation times are dependent on the measurement principle and thus also on the coagulation analyzer used. Therefore, each laboratory must establish its own reference curve.

Internal Quality Control

Normal range: CONTROL N
Pathological range: CONTROL P
Two levels of quality control material (normal and pathologic range) have to be measured at start of the test run, with each calibration, upon reagent vial changes and at least every eight hours on each day of testing.The controls should be processed like the samples. Each laboratory should establish its own quality control range, either by means of the target values and ranges provided by the manufacturer of the controls or by means of its own confidence ranges established in the laboratory. If the control values lie outside the range the reagent, calibration curve and coagulation analyzer should be checked. Patient results must not be released until the cause of the deviation is identified and corrected.

Results

Read the coagulation factor content from the reference curve in % of Norm. If the given nominal value of the STANDARD PLASMA is not 100 % of Norm, but e.g. only 95 % of Norm, multiply the result read from the curve by 0.95. In the case of coagulation times which correspond to a coagulation factor content of more than 100 % of Norm, further determination will be required using higher dilutions of specimen (e.g. 1:10). The percentage of norm value read from the reference curve for such a higher dilution must be multiplied by a correction factor corresponding to the dilution; e.g. for a dilution of 1:10 by a correction factor of 2.

Limitations

Therapeutic doses of hirudin or other direct thrombin inhibitors lead to an erroneously lower factor activity[7,8].

Specific inhibitors against plasmatic coagulation factors may also modify the real factor activity[9]. Partial activation of the coagulation factors due to incorrect sample handling can lead to falsely elevated single factor results. In single factor determination, lupus anticoagulant can affect the apparent factor activity. Non-parallelism upon dilution may occur when a lupus anticoagulant is present in the test sample[10].

The manufacturer has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified. User defined modifications are not supported by the manufacturer as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Application Sheets or these Instructions for Use.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Expected Values[11]

Factor VIII 70 to 150 % of Norm[11]

Reference intervals vary from laboratory to laboratory depending on the population served and the technique, method, equipment and reagent lot used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the aforementioned variables are changed.

Performance Characteristics

Measuring Range

The measuring range depends on the individual application of the assay due to instrument related conditions. Application specific performance data are listed in the respective Reference Guides of the instruments.

Precision

The results of all precision studies with applications of the assay Factor VIII showed a precision for controls (repeatability and within-device/lab precision) of lower or equal than 11 %.

Other system specific results are given in the respective Reference Guides (Application Sheets).

The reproducibility was assessed by the manufacturer for coagulation Factor VIII based on publicly available proficiency testing information in 2018/2019. The overall reproducibility median CV% was found to be <11 % including APTT reagent used, lot, instrument, laboratory and operator variability factors.

Method Comparison

In a study, the determination of FVIII on the Atellica® COAG 360 System was compared to the CS-2000i System.

The correlation resulted in the following:

	n	Slope	Intercept [% of Norm]	Correlation Coefficient
Factor VIII	118	0.98	+1.03	0.997

Standardization

The uncertainty of the Standard and the Controls to the reference material is included in the Certificates of Traceability (CoT). The CoTs are available upon request.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

Current Version of Application Sheets

FACTOR VIII DEFICIENT can be used in combination with various automated coagulation analyzers. Sysmex provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 3 under the dedicated link below:

sysmex-ifu.com/ag

As the manufacturer continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Sysmex ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via sysmex-ifu.com.

References

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5. Castellone DD, Adcock DM. Factor VIII Activity and Inhibitor Assays in the Diagnosis and Treatment of Hemophilia A. Semin Thromb Hemost. 2017;43:320-30.
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7. Boogen C, Niederau C, Reinauer H. Assessment of the influence of r-hirudin on coagulometric factor assays [Abstract]. Ann Hematol. 1999;78 Suppl:73.
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Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community	CH REP	Authorized representative in Switzerland
	Contains sufficient for <n> tests		Biological Risks
IVD	*In Vitro* Diagnostic Medical Device		Temperature Limitation
	Consult instruction for Use		Non-sterile
CE	CE marking of conformity	CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.
CONTENTS	Contents	→	Reconstitution volume
LEVEL	Level		Keep away from sunlight and heat
WARNING	Warning	DANGER	Danger
RxOnly	Prescription device (US only)	UDI	Device Identification (UDI) barcode
REACH xx/xx/xx	REACH Authorization Number		

Legal Information

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