Berichrom® α_2-Antiplasmin
Berichrom ANTIPLASMIN

Revision bar indicates update to previous version.

CE 0197

Reagents for the determination of α_2-antiplasmin

Intended Use

Berichrom ANTIPLASMIN is an in vitro diagnostic reagent for the quantitative determination of α2-antiplasmin activity as aid to diagnosis and monitoring of congenital or acquired α2-antiplasmin deficiencies in patients at risk for or suspected to have disorders of the fibrinolysis system in human sodium citrated plasma by means of automated chromogenic methods.

For determination of α2-antiplasmin, no international reference preparation or method is available.

Summary and Explanation

α2-Antiplasmin, a 51 kDa glycoprotein built in the liver, is the primary inhibitor of plasmin, regulating fibrinolytic activity. In addition, by binding to fibrin, together with Factor XIIIa, it makes the clot more difficult to lyse. Absence or very low levels of α2-antiplasmin result in uncontrolled plasmin-mediated breakdown of the fibrin clot and is associated with increased risk of bleeding[1].

Deficiency of α2-antiplasmin may be inherited or acquired. Homozygous congenital α2-antiplasmin deficiency is a rare bleeding disorder; heterozygotes are usually asymptomatic. Acquired α2-antiplasmin deficiency may result from diminished synthesis (liver disease) or increased consumption, Determination of α2-antiplasmin helps in a more complete assessment of hyperfibrinolysis (primary fibrinolysis), fibrinolytic activity in disseminated intravascular coagulation (DIC), or assessment of thrombolytic therapy[1,2].

Principles of the Procedure

The α_2-antiplasmin in the sample deactivates the plasmin present. The residual plasmin content is determined in a kinetic test measuring the increase in absorbance at 405 nm according to the following reaction scheme:

$$\alpha_2\text{-antiplasmin}_{\text{ sample}} + \text{plasmin}_{\text{ excess}} \longrightarrow [\alpha_2\text{-antiplasmin-plasmin}] + \text{plasmin}_{\text{ remainder}}$$

$$\text{HD-Nva-CHA-Lys-pNA} \xrightarrow{\text{plasmin}_{\text{ remainder}}} \text{HD-Nva-CHA-Lys-OH} + \text{p-Nitroaniline}$$

Reagents

Note: Berichrom ANTIPLASMIN 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
Berichrom® α_2-Antiplasmin Berichrom ANTIPLASMIN			
REAGENT P	Lyophilized reagent containing: • CTA (reconstituted: 0.1 U/mL)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: reconstituted, 3 hours; 15 °C: reconstituted, 2 days; 2–8 °C: reconstituted, 1 week; ≤ –20 °C: reconstituted, 1 month
PLASMIN SUBSTRATE	Lyophilized reagent containing: • HD-Nva-CHA-Lys-pNA (reconstituted: 3 mmol/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: reconstituted, 1 week; 15 °C: reconstituted, 2 weeks; 2–8 °C: reconstituted, 6 weeks; ≤ –20 °C: reconstituted, 6 months
REAGENT P DILUENT	Ready to use liquid containing: • Potassium dihydrogen phosphate • Sodium chloride • glycerol • Preservative: • Sodium azide (< 1 g/L) pH 7.5	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, 6 months

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 BIOHAZARD
Berichrom ANTIPLASMIN REAGENT P
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
Berichrom ANTIPLASMIN REAGENT P
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

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.

Preparing Reagents

REAGENT P: Reconstitute with the amount of REAGENT P DILUENT indicated on the label and incubate for 15 minutes at 15 to 25 °C.

PLASMIN SUBSTRATE: Dissolve the contents of the vial with the amount of distilled or deionized water indicated on the label.

Mix reagents carefully before each use.

Specimen Collection and Handling

Collecting the Specimen

To obtain the plasma, carefully mix 1 part sodium citrate solution (0.11 mol/L (3.2 %)) with 9 parts venous blood, avoiding the formation of foam. Centrifuge immediately at no less than 1500 × g for at least 10 minutes.

Storing the Specimen

Stability of the samples:

≤ −20 °C	2 weeks
2 to 8 °C	2 days
15 to 25 °C	4 hours

Thaw plasma stored at ≤ −20 °C within 10 minutes at 37 °C and perform the determination within 2 hours. Patient plasma is used undiluted in the chosen testing system.

Procedure

Materials Provided

REF	Contents		
OUBU15	Berichrom® α_2-Antiplasmin Berichrom ANTIPLASMIN		
	Plasmin Berichrom ANTIPLASMIN REAGENT P	3 × →	5 mL
	Plasmin Substrate PLASMIN SUBSTRATE	3 × →	2 mL
	Buffer Solution Berichrom ANTIPLASMIN REAGENT P DILUENT	1 ×	15 mL

Materials Required but not Provided

Item	Description
REF ORKL17	STANDARD PLASMA, Standard Human Plasma
REF ORKE41	CONTROL N, Control Plasma N
REF OUPZ17	CONTROL P, Control Plasma P
REF B4234-25 REF B4265-37 –	OV BUFFER, Dade® Owren's Veronal Buffer, or CA SYSTEM BUFFER, Dade® CA System Buffer, or isotonic saline solution (0.9 %)
–	Distilled or deionized water without preservatives
Coagulation analyzers[a], such as:	• AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) • AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System)

[a] 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.

Test Procedure

Cuvette: 10 mm thickness
Wavelength: 405 nm
Testing temperature: 37 °C

Prewarm REAGENT P, PLASMIN SUBSTRATE and the plastic cuvettes or tubes to the chosen testing temperature.

Pipetting scheme for the kinetic method

	Plasmin enzyme value (PEV)	Sample
Isotonic saline solution	20 µL	-
Plasma Sample	-	20 µL
REAGENT P	1 000 µL	1 000 µL
Mix and incubate for 1 minute at 37 °C.		
PLASMIN SUBSTRATE	100 µL	100 µL
Mix and determine $\Delta A_{405\ nm}$/min Photometer/Stopwatch: Read the optical density within 30 seconds and start the stopwatch at the same time. Repeat the reading after exactly 60 and 120 seconds, then calculate the respective ΔA/min by subtraction and then the mean value from both measured values.		

Evaluation, Kinetic Method

For each measurement series, at least 1 plasmin enzyme value ($\Delta A/min_{PEV}$) and 1 reference value is necessary. These must be determined like a sample using a reference plasma with target value specifications for α_2-antiplasmin (e.g., Standard Human Plasma). The laboratory internal factor F_L is determined as follows:

$$F_L = \frac{\text{Target value (\% of Norm)}_{\text{reference plasma}}}{\Delta A/min_{PEV} - \Delta A/min_{\text{reference plasma}}}$$

The α_2-antiplasmin content of the sample in % of Norm is calculated using the following formula:

$\alpha_2\text{-Antiplasmin}_{sample}$ (% of Norm) = F_L x ($\Delta A/min_{PEV}$ - $\Delta A/min_{sample}$)

Comments

1. Doubling or halving the volumes has no effect on the calculation and the laboratory internal factor.
2. If the α_2-antiplasmin content in the sample is less than 20 % of Norm, it is recommended that 40 µL of sample be used in the test. The result is then divided by 2.
3. The laboratory internal factor F_L or the reference curve must be redetermined for each change in instruments and for each new lot of Berichrom ANTIPLASMIN.

Automatic:

Sysmex provides Reference Guides (Application Sheets) for several coagulation analyzers.

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 8 hours on each day of testing. The controls should be treated like the samples. Each laboratory should determine 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 ranges determined in the laboratory. If the measured control value lies outside of the pre-determined range, then the reagents, the laboratory-internal factor F_L or the reference curve and coagulation analyzer should be checked.

Limitations

Plasmin inhibitors like aprotinin might lead to elevated results[3].

Because of the increased viscosity (glycerol content) of the reconstituted plasmin, it is recommended that the plasmin solution not be pipetted too quickly.

If the patient sample result exceeds 120 % of Norm, dilute the sample with isotonic saline and assay the dilution. Multiply the result by the dilution factor to compensate for the increased dilution. If the patient sample result is greater than the Plasmin Enzyme Level, complete depletion of patient α_2-antiplasmin is indicated.

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

80 to 120 % of Norm[4]

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 is from 10.0 to 150 % of Norm.

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

Precision

The within-series reproducibility, estimated by the coefficient of variation calculated from repetitive assay of samples in different laboratories, was 0.5 to 9.9 % for samples in the normal range and 3.1 to 8.4 % for pathological samples. The inter-assay coefficients of variation, measured similarly, were 3.2 to 8.4 % for normal range samples and 3.2 to 7.4 % for pathological samples.

Additionally, the reproducibility was assessed by the manufacturer for α2-antiplasmin / Berichrom α2-Antiplasmin based on publicly available proficiency testing information in 2020. The overall reproducibility median CV% was found to be <13 % including lot, instrument, laboratory and operator variability factors.

Method Comparison

At 4 European laboratories, the performance of the Berichrom ANTIPLASMIN test (y) was compared with a commercial test (x) which employs a similar synthetic substrate for measuring the analyte. Assays were performed on 108 plasma specimens throughout the measuring range of the method using the manual two point procedure at 37 °C. The calculated least squares regression equation was y = 1.01 x + 2.7 % with a correlation coefficient (r) of 0.946.

Technical Assistance

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

Current Version of Application Sheets

Berichrom ANTIPLASMIN 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 4 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

1. Carpenter SL, Mathew P. Alpha2-antiplasmin and its deficiency: fibrinolysis out of balance. Haemophilia 2008;14(6):1250-4.
2. Saes JL, Schols SEM, van Heerde WL, et al. Hemorrhagic disorders of fibrinolysis: a clinical review. J Thromb Haemost. 2018;16:1498-1509.
3. Johannessen M, Edsberg B, DeVries C, Nielsen F. A modified non-amidolytic assay non-affected by aprotinin for determination of α_2-antiplasmin in plasma from patients undergoing cardiopulmonary bypass (CBP). Thromb Haemost. 1993; 69: 1353 (Abstract).
4. Kraus M. α_2-antiplasmin. In: Thomas L, ed. Clinical Laboratory Diagnostics. Frankfurt: TH-Books Verlagsgesellschaft, 1998: 627-8.

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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