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IVD

Ristocetin

REF AG004K

R 3 vials x 7.5 mg

English, revision: 11-2023

INTENDED USE:

Platelet agonist for light transmission aggregometry (LTA) method for the *in vitro* quantitative determination of platelet aggregation, in human citrated plasma, using an automated or semi-automated method. This method is used in aid to diagnosis of platelet function disorders in patients suspected of having platelets functional disorders. This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

SUMMARY AND EXPLANATION:

Technical :[1-3]

Platelet function is assessed by light transmission aggregometry (LTA).
LTA measures the transmission of light through a sample of platelet-rich plasma (PRP) in response to a panel of platelet agonists. Light transmittance through PRP is measured relative to a reference cuvette containing platelet poor plasma (PPP). Light transmission is set at 100% in the PPP and 0% in the PRP. When a platelet agonist is added to the stirred PRP, platelets then start to aggregate, and the light transmission of PRP increases.

Clinical :[3-9]

Ristocetin reagent is available for use in Ristocetin-induced platelet aggregation (RIPA) tests. It is used to detect von Willebrand disease, more specifically to highlight an increased affinity in von Willebrand factor (vWF) for GPIb α in type 2B and to identify Bernard-Soulier syndrome.

PRINCIPLE:

When ristocetin is added to the platelet rich plasma (PRP) from a healthy subject, it promotes the interaction between von Willebrand factor (vWF) and the platelet membrane glycoprotein GPIb α which is the vWF receptor on platelets. Ristocetin-induced platelet aggregation (RIPA) is used to measure the patient's PRP aggregation in the presence of various ristocetin concentrations. Platelet aggregation is observed using 1.2 mg/mL of ristocetin if platelet function and plasma vWF are normal. However, it is not observed in the presence of quantitative and qualitative defects of vWF (except type 2B vWF) or if GPIb α function is abnormal[4-6].

REAGENTS:

R **Ristocetin** at approximately 7.5 mg, lyophilized. Contains stabilizers.

The product is classified as non-hazardous and is not subject to labeling according to EC Regulation No. 1272/2008 [CLP].

WARNINGS AND PRECAUTIONS:

- Waste should be disposed of in accordance with applicable local regulations.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
- 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 or on request to HYPHEN BioMed).
- To ensure optimal test results, testing the specimens and controls in succession and without interruption is recommended.

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R **For aggregometer:**

Reconstitute the contents of each vial with **exactly 0.5 mL of distilled water** (15 mg/mL).
Shake vigorously until complete dissolution. Allow the reagent to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.
Dilute the reconstituted Ristocetin as follows (example for 1 mL):

For final concentration in test (mg/mL)	**1.2**	0.5

Prepare following 10X solutions:

"10X" Ristocetin preparation (mg/mL)	**12**	5
Ristocetin 15 mg/mL (µL)	**800**	333
Physiological Saline (µL)	**200**	667

For testing at 2 mg/mL, reconstitute vial with exactly 0.375 mL of distilled water (20 mg/mL). Shake vigorously until complete dissolution. Allow the reagent to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally and use immediately.

R **For analyzer:**

Reconstitute the contents of each vial with **exactly 0.625 mL of distilled water** (12 mg/mL).
Shake vigorously until complete dissolution. Allow the reagent to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.

Dilute the reconstituted Ristocetin as follows (example for 1 mL):

For final concentration in test (mg/mL)	**1.2**	0.5

Prepare following 8X solutions:

"8X" Ristocetin preparation (mg/mL)	**9.6**	4
Ristocetin 12 mg/mL (µL)	**800**	333
Physiological Saline (µL)	**200**	667

For testing at 2 mg/mL, reconstitute vial with exactly 0.470 mL of distilled water (16 mg/mL). Shake vigorously until complete dissolution. Allow the reagent to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally and use immediately.

Homogenize the reagent prior to each use.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **7 days** at 2-8°C.
- **24 hours** at room temperature (18-25°C).
- **2 months** frozen at -20°C or less*
- **Stability on board of the analyzer: see the specific Application Guide.**

*Thaw only once at room temperature (18-25°C) and use immediately.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

- Laboratory material.
- Physiological Saline (0.9% NaCl).
- SB Cuvette (064-1041-9) and SB Set tool (063-4151-5) for CS- and CN-series.
- Automatic analyzer such as: CS-series, CN-series.
- Light transmission Aggregometer.

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 is not modified.

SPECIMEN COLLECTION AND PREPARATION:

Collection, preparation and storage of fresh samples (Platelet-rich Plasma (PRP) and Platelet-poor Plasma (PPP)) should be made according to laboratory or other validated methods.[3,10]
The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture.
CLSI H58-A and studies[3,10]: studies should be completed on fresh sample within a maximum of 4 h after blood collection.

PROCEDURE:

Platelet agonist should be used at 1.2 mg/mL. If the platelet agglutination is normal, lower concentration of Ristocetin should be tested (ie. 0.5-0.7 mg/mL). If platelet agglutination is abnormal or absent, testing should be repeated using 2 mg/mL of Ristocetin.[1,3]

HYPHEN BioMed provides Application Guides for defined coagulation analyzer families. The Application Guides contain analyzer/assay specific handling and performance information and complement the information in these Instructions for Use.

Protocol on Aggregometer:

1. Place a stirrer in each cuvette.
2. Establish the 100% aggregation point with a cuvette containing 360 µL PPP.
3. Pipette 360 µL PRP into a second cuvette.
   Incubate for 2 minutes at 37 °C. Establish the 0% aggregation point with the PRP.
4. Add 40 µL of 10X Ristocetin solution directly into the PRP using a long and fine pipette tip. Do not inject against the walls of the cuvette.
5. Allow the aggregation profile to develop for 5 to 10 minutes.

If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance.

QUALITY CONTROL:

Commercial controls are not available.
The control may consist of fresh sample collected from a normal donor with a history of normal platelet function. Include control samples preferably for each test series, and at least for each new reagent batch, or after instrument maintenance.

RESULTS:

- Results are evaluated by examining the aggregation curve and the maximal aggregation (%). These parameters vary depending on instrument type, and specific normal values should be determined by each laboratory.
- Results should be interpreted on the basis of a patient's clinical condition, platelet count, potential medication influences, lifestyle, nutrition, and pre-analytical conditions.[11,12]
- In healthy individuals, aggregation should occur at least at 1.2 mg/mL, but not at 0.5 mg/mL. In Bernard-Soulier syndrome and type 3 von Willebrand disease, aggregation is expected to be very weak or non-existent at all concentrations. In pseudo von Willebrand disease and type 2B von Willebrand disease, the platelets are hyper-sensitive to Ristocetin, leading to aggregation even at low concentrations (i.e. 0.5-0.7 mg/mL)[3].
- Abnormal curves should be confirmed via a retest.
- Lot to lot variability measured on 3 lots is %CV ≤ 10% (normal sample).

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting no limpid appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- User defined modifications are not supported by HYPHEN BioMed 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 HYPHEN BioMed Application Guides or these Instructions for Use.
- If the number of platelets is lower than 150 × 10^9/L or higher than 600 × 10^9/L, test results may be affected. The platelet count of PRP samples should not be adjusted to a standardized value with autologous PPP[3].

EXPECTED VALUES:

The reference interval established, in internal study, on healthy adult subjects with 1.2 mg/mL of Ristocetin on aggregometer (n=61), on CS-series (n=63) and on CN-series (n=71), was measured between 63 and 105%, between 71 and 92% and between 51 and 93% respectively (Central 90%, 95th percentile)[13].
However, each laboratory has to determine its own normal aggregation parameters[3,10, 14]

PERFORMANCES:

Performances studies were conducted as described in CLSI guidelines.
The following performance data represent typical results and are not to be regarded as specifications for Ristocetin.
Mathematical analyses are performed using a validated statistical software built in accordance with CLSI guidelines.
For automated assays, performances are documented in the respective Application Guides of the analyzers.

On aggregometer:
Analytical performances
Precision
Precision studies were assessed using abnormal and normal samples, on 1 series and 10 repetitions.

	Repeatability	
Sample	**% Max Aggregation**	**CV%**
Normal	97%	4.7%
Abnormal	37%	15.0%

Interfering substances
No interference was observed with the molecules and up to following concentrations:

Bilirubin C	Bilirubin F	Intralipids	Hemoglobin
30 mg/dL	30 mg/dL	310 mg/dL	250 mg/dL

Clinical performances

Agreement

Agoniste	Reference method	Agreement (n = 121)
Ristocetin (1.2mg/mL)	Helena reagent	96%

Sensitivity/Specificity

Agonist	n	Sensitivity	Specificity	Area under the curve (ROC)	
Ristocetin	121	97%	95%	0.981	
Agonist	**n**	**PPV**	**NPV**	**LR+**	**LR-**
Ristocetin	121	96%	96%	18,09	0,03

PPV: Predictive value of a positive result LR+ : Likelihood Ratio +
NPV: Predictive value of a negative result LR- : Likelihood Ratio –

On CS-series / CN-series:
Analytical performances
Precision
Precision studies were assessed using abnormal and normal samples, on 1 series and 30 repetitions.

CS-series	Repeatability	
Sample	**% Max Aggregation**	**CV%**
Normal	84%	1.6%
Abnormal	28%	14.2%
CN-series	**Repetability**	
Sample	**% Max Aggregation**	**CV%**
Normal	79%	4.5%
Abnormal	45%	6.1%

Interfering substances
Interferences are defined by the analyzer system used and are documented in the respective Application Guides of the analyzers.

Clinical performances

Agreement

Agonist	Reference method (aggregometer)	Agreement (n = 119) (CS-series)
Ristocetin	Helena reagent	96%

Sensitivity/Specificity

Agonist	n	Sensitivity	Specificity	Area under the curve (ROC)	
Ristocetin	119	100%	91%	0.950	
Agonist	**n**	**PPV**	**NPV**	**LR+**	**LR-**
Ristocetin	119	93%	100%	10,80	0,00

PPV: Predictive value of a positive result LR+ : Likelihood Ratio +
NPV: Predictive value of a negative result LR- : Likelihood Ratio –

Clinical performance was defined at Ristocetin 1.2 mg/mL for abnormal (with less GPIbα) and normal samples.

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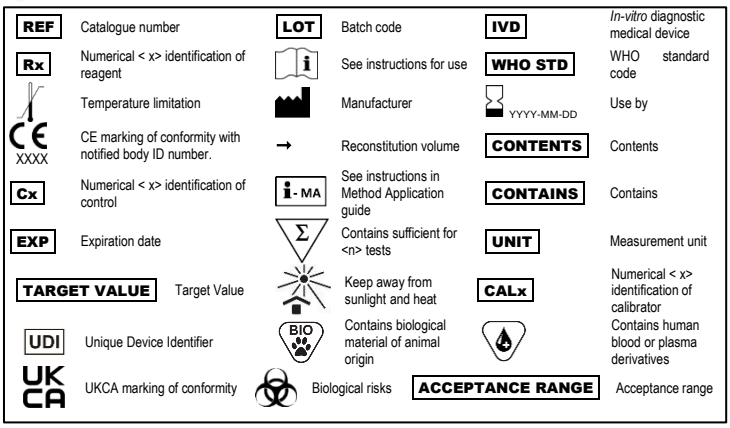
e-IFU (other languages) are available on www.hyphen-biomed.com.
For customer support and Application Guides, please contact your local provider or distributor (see www.hyphen-biomed.com).

Changes compared to the previous version.

The following symbols may appear on the product labeling:



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