

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 or to assess responsiveness to antiplatelet drugs in patients suspected of having platelets functional disorders or on antiplatelet therapy. 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 :³⁻⁸

The platelets ability or inability to respond to particular agonist is the basis for differentiating platelet dysfunctions, congenital (e.g.: Glanzmann thrombasthenia, Bernard-Soulier syndrome, gray platelet syndrome, etc.) or acquired (e.g.: medications, procedures, medical conditions, hematologic disease). When required, to assess response to antiplatelet therapy such as Acetylsalicylic acid (ASA, aspirin), P₂Y₁₂ receptor inhibitors, Glycoprotein IIb/IIIa inhibitors.

PRINCIPLE:

When adenosine 5'-diphosphate (ADP) is added to the platelet-rich plasma (PRP) from a healthy subject, it binds to P₂Y₁ and P₂Y₁₂ receptors present on platelets and induces platelet aggregation in two phases. ADP initially induces a primary wave of aggregation. If the stimulus is sufficiently strong, a secondary wave of aggregation arises when platelet granules release their contents, such as serotonin, fibrinogen, and endogenous ADP^{7,8}.

REAGENTS:

R Adenosine-5'-diphosphate (ADP) at approximately 0.1 µmol, 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** (200 µM).

Shake vigorously until complete dissolution. Allow the reagent to stabilize for 30 min. at room temperature (18-25 °C), shaking occasionally.

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

For final concentration in the test (µM)	10	5	2
Prepare following 10X solutions:			
"10X" ADP preparation (µM)	100	50	20
ADP 200 µM (µL)	500	250	100
Physiological Saline (µL)	500	750	900

R For analyzer:

Reconstitute the contents of each vial with **exactly 0.625 mL of distilled water** (160 µM).

Shake vigorously until complete dissolution. Allow the reagent to stabilize for 30 min. at room temperature (18-25 °C), shaking occasionally.

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

For final concentration in the test (µM)	10	5	2
Prepare following 8X solutions:			
"8X" ADP preparation (µM)	80	40	16
ADP 160 µM (µL)	500	250	100
Physiological Saline (µL)	500	750	900

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^{3,11}.

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^{3,11}: studies should be completed on fresh sample within a maximum of 4 h after blood collection.

PROCEDURE:

Platelet agonist should be used at 2 µM. If the platelet aggregation is abnormal, higher concentrations of ADP should be tested (e.g. 5 or 10 µM)^{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:

- Place a stirrer in each cuvette.
- Establish the 100% aggregation point with a cuvette containing 360 µL PPP.
- Pipette 360 µL PRP into a second cuvette. Incubate for 2 minutes at 37 °C. Establish the 0% aggregation point with the PRP.
- Add 40 µL of 10X ADP solution directly into the PRP using a long and fine pipette tip. Do not inject against the walls of the cuvette.
- 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 who has not taken any antiplatelet medication and 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.^{12,13}
- 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 limp 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 $150 \times 10^9/L$ or higher than $600 \times 10^9/L$, test results may be affected. The platelet count of PRP samples should not be adjusted to a standardized value with autologous PPP³.

EXPECTED VALUES:

The reference interval established, in internal study, on healthy adult subjects with 2 μM ADP on aggregometer (n=51), on CS-series (n=50) and on CN-series (n=82), was measured between 56 and 98%, between 58 and 96% and between 57 and 96% respectively (Central 90%, 95th percentile)¹⁴. However, each laboratory has to determine its own normal aggregation parameters^{3,11,15}

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 ADP. 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.

Sample	Repeatability	
	% Max Aggregation	CV%
Normal	71%	9.7%
Abnormal	42%	10.0%

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	360 mg/dL	250 mg/dL

Clinical performances

Agonist	Agreement	
	Reference method	Agreement (n = 109)
ADP (2μM)	Helena reagent	99%

Agonist	n	Sensitivity/Specificity			
		Sensitivity	Specificity	Area under the curve (ROC)	
ADP	109	100%	98%	0.993	
Agonist	n	PPV	NPV	LR+	LR-
ADP	109	98%	100%	58	0

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.

Sample	Repeatability	
	% Max Aggregation	CV / SD
Normal	88%	CV = 4.4%
Abnormal	0.51%	SD = 0.62%
CS-series	Repeatability	
Sample	% Max Aggregation	CV%
Normal	88%	6.9%
Abnormal	28%	10.7%

Interfering substances

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

Clinical performances

Agonist	Agreement	
	Reference method (aggregometer)	Agreement (n = 108) (CS-series)
ADP	Helena reagent	95%

Agonist	n	Sensitivity/Specificity			
		Sensitivity	Specificity	Area under the curve (ROC)	
ADP	108	100%	91%	0.974	
Agonist	n	PPV	NPV	LR+	LR-
ADP	108	91%	100%	11.6	0

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 ADP 2μM for antiplatelet drugs and normal samples, and confirmed at 10μM on bleeding syndrome, dual antiplatelet therapy, 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:

REF	Catalogue number	LOT	Batch code	IVD	In-vitro diagnostic medical device
Rx	Numerical < x > identification of reagent	i	See instructions for use	WHO STD	WHO standard code
CE	Temperature limitation	MA	Manufacturer	YYYY-MM-DD	Use by
XXXX	CE marking of conformity with notified body ID number.	→	Reconstitution volume	CONTENTS	Contents
Cx	Numerical < x > identification of control	i-MA	See instructions in Method Application guide	CONTAINS	Contains
EXP	Expiration date	Σ	Contains sufficient for <n> tests	UNIT	Measurement unit
TARGET VALUE	Target Value	☀	Keep away from sunlight and heat	CALx	Numerical < x > identification of calibrator
UDI	Unique Device Identifier	BIO	Contains biological material of animal origin	🩸	Contains human blood or plasma derivatives
UK CA	UKCA marking of conformity	🦠	Biological risks	ACCEPTANCE RANGE	Acceptance range