White Paper

Guideline for integrated platelet aggregometry

For hemostasis systems from Siemens Healthineers

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Platelets: Crucial for primary hemostasis

In physiological hemostasis, the accumulation of platelets on the damaged vessel helps to stop bleeding by forming a platelet plug. There exist two types of platelet abnormalities that promote bleeding: qualitative and quantitative platelet disorders.

Platelets are formed by megakaryocytes in bone marrow and are essential for primary hemostasis. In the secondary phase of hemostasis, platelets act as a reactive surface for clotting factors and increase their activity, by which a stable fibrin clot is formed.

Platelets are small discoid cells without a nucleus. Although they do not contain DNA, platelets can synthesize various proteins using their RNA. In addition, they contain granules loaded with various metabolites, signaling molecules, and proteins, which are released when the platelets are activated.

Primary hemostasis is fundamentally important in the hospital. About 7% of acutely hospitalized patients show defects in primary hemostasis, which significantly increases the risk of bleeding.

Platelets: Activation, adhesion, and aggregation

When a vascular injury occurs, platelets bind via von Willebrand factor (VWF) to the collagen fibers of the now-accessible subendothelial matrix.

Platelets' main receptor for VWF is the glycoprotein (GP) Ib-IX-V complex. Under high shear stress, VWF first binds to collagen via the A3 domain and, in the next step, via the accessible A1 domain to the thrombocytic GPIb-IX-V-receptor (platelet adhesion).

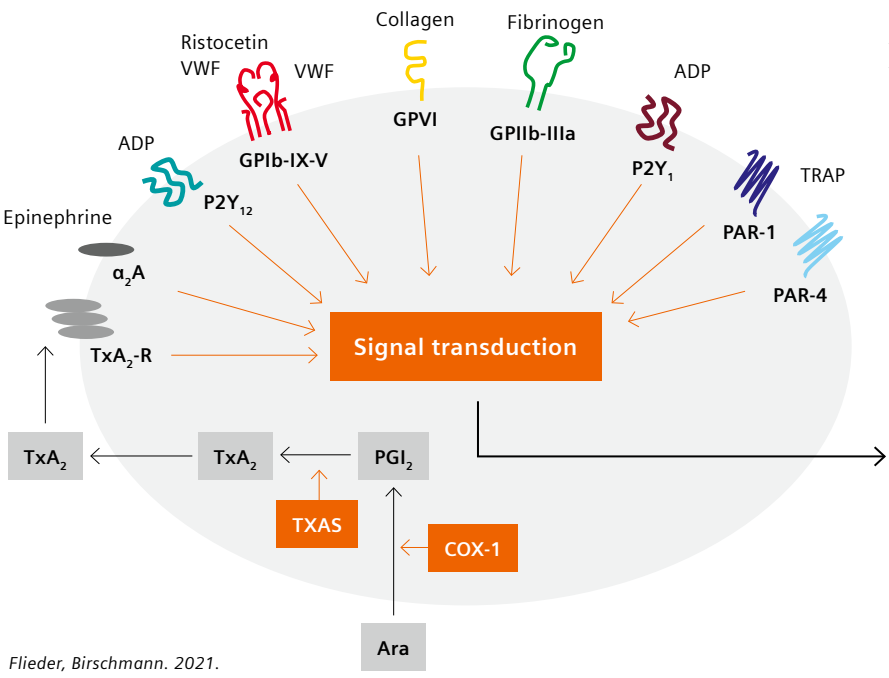
After platelets have adhered to the injured tissue, they are activated by binding collagen to the platelet GPVI receptor.

This leads to a shape change and increased activation of the adherent platelets, with subsequent secretion of granular substances and consecutive aggregate formation.

The released granule ingredients (e.g., ADP, serotonin) increase platelet activation. This leads to recruitment of platelets from circulation, which in turn causes a further buildup of the platelet aggregate.

This is followed by the release of arachidonic acid and the formation of thromboxane A_2 (TxA_2), which enhances activation by binding to a specific thromboxane receptor. TxA_2 also has a vasoconstrictor effect. Within the activation the GPIIb-IIIa receptor changes its conformation to an activated form, so that it is able to bind fibrinogen.

Activated platelets are primarily linked by fibrinogen bridges through the GPIIb-IIIa receptor, VWF, and the GPIb-IX-V receptors. At the end of secondary hemostasis, the platelet aggregate is stabilized with cross-linked fibrin by factor XIII.[7]



α_2A: *Adrenoreceptor*
ADP: *Adenosine diphosphate*
Ara: *Arachidonic acid*
COX-1: *Cyclooxygenase 1*
GPIb-IX-V: *Glycoprotein Ib-IX-V-complex*
GPIIb-IIIa: *Glycoprotein IIb-IIIa-complex*
GPVI: *Glycoprotein VI*
PAR -1/-4: *Protease-activated receptor-1/-4*
PGI_2: *Prostaglandin I_2*
TRAP: *Thrombin receptor-activating peptide*
TxA_2: *Thromboxan A_2*
TxA_2-R: *Thromboxan A_2-receptor*
TXAS: *Thromboxane synthase*
VWF: *von Willebrand factor*

Flieder, Birschmann. 2021.

Thrombocytopathy: Dysfunction of the platelets

Thrombocytopathy is a platelet dysfunction that can occur on several levels. These disorders are numerous and range from reduced secretion/synthesis of granules to defects in surface receptors. Since many signaling pathways that follow activation of the numerous surface receptors are not yet or only incompletely known, clear classification of many thrombocytopathies is often difficult.

The most frequent thrombocytopathy is drug-induced platelet dysfunction.[8,9]

Inherited thrombocytopathy

- Much less frequent
- Can lead to severe bleeding
- Often not diagnosed
- Examples: Glanzmann thrombasthenia, Bernard-Soulier syndrome, delta (δ)-storage pool disease (Hermansky-Pudlak syndrome), α-storage pool disease (gray platelet syndrome)

Acquired thrombocytopathy

- Frequent
- Drug-induced, e.g., by nonsteroidal anti-inflammatory drugs (NSAIDs), acetylsalicylic acid, and antiplatelet drugs
- Diseases, e.g., liver cirrhosis, kidney disease, myeloproliferative syndrome (MPS), multiple myeloma

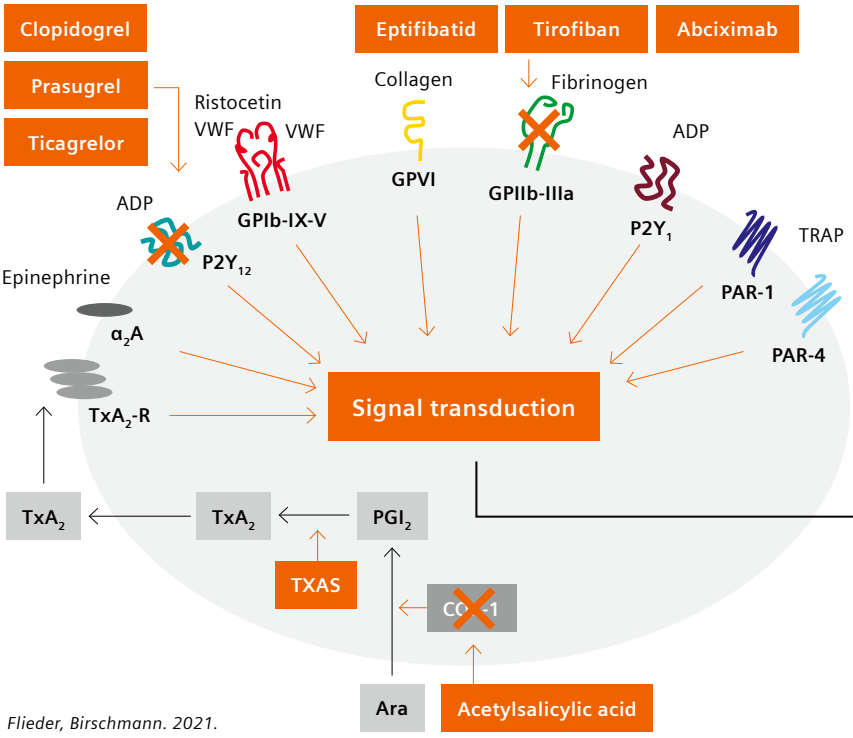
Classification of platelet dysfunction[7]

Disease	Molecular Pathogens	Aggregometry
Receptor deficiencies		
Glanzmann thrombasthenia	Lack of expression or dysfunction of the GPIIb-IIIa receptor	Absent agonist-induced aggregation except ristocetin
Bernard-Soulier syndrome	Decreased expression or dysfunction of the GPIb-IX-V receptor	Absent or markedly reduced ristocetin-induced aggregation
von Willebrand syndrome Type 2B	Increased affinity of the GPIb-IX-V receptor to von Willebrand factor	Increased agglutination with low concentration of ristocetin
Defect of collagen receptor	Lack of expression or function of the GPIa-IIa or GPVI receptor	Absent or limited collagen-induced aggregation
Granular defects		
α-granular defects (e.g., gray platelet syndrome)	Absence of α-granules, disrupted release of adhesion proteins (e.g., fibrinogen, VWF) and membrane proteins (e.g., P-selectin, GPVI)	Reduced ristocetin- and collagen-induced aggregation
δ-granular defects (e.g., Hermansky-Pudlak syndrome)	Absence of δ-granules	Decreased ADP- and epinephrine-induced aggregation, absent or delayed second phase, disaggregation; reduced arachidonic acid- and collagen-induced aggregation
Signal transduction defects		
Aspirin-like defect	Disruption of the cyclooxygenase pathway (disorders of COX-1 enzyme or thromboxane synthetase)	Absent arachidonic acid-induced aggregation Failure of second phase of epinephrine-induced aggregation

Influences of drugs on platelet function

Most common are platelet dysfunctions as a result of taking medication, e.g., platelet aggregation inhibitors such as acetylsalicylic acid, NSAIDs, $P2Y_{12}$ receptor antagonists (clopidogrel, prasugrel, ticagrelor, cangrelor), or GPIIb-IIIa antagonists (abciximab, eptifibatide, tirofiban).[6]

Acetylsalicylic acid-induced platelet inhibition	$P2Y_{12}$ receptor antagonist-induced platelet inhibition	GPIIb-IIIa antagonist-induced platelet inhibition
Acetylsalicylic acid is the most often-used substance to inhibit platelet aggregation. It is established for the long-term prophylaxis of cardiovascular events. It selectively and irreversibly inhibits cyclooxygenase 1 (COX-1) and thus the formation of thromboxane from arachidonic acid. Activation of thrombocytes via this pathway is thereby prevented.	ADP receptor antagonists inhibit the $P2Y_{12}$ receptor (reversibly or irreversibly, depending on the mechanism of action). Because of this inhibition, the intracellular cAMP cycle can no longer be influenced via the ADP-dependent activation of the $P2Y_{12}$ receptor.	GPIIb-IIIa receptor antagonists are a category of drugs that bind to the activated GPIIb-IIIa receptor and, as a result, inhibit platelet aggregation. They are frequently used in percutaneous coronary interventions (intravenous only).



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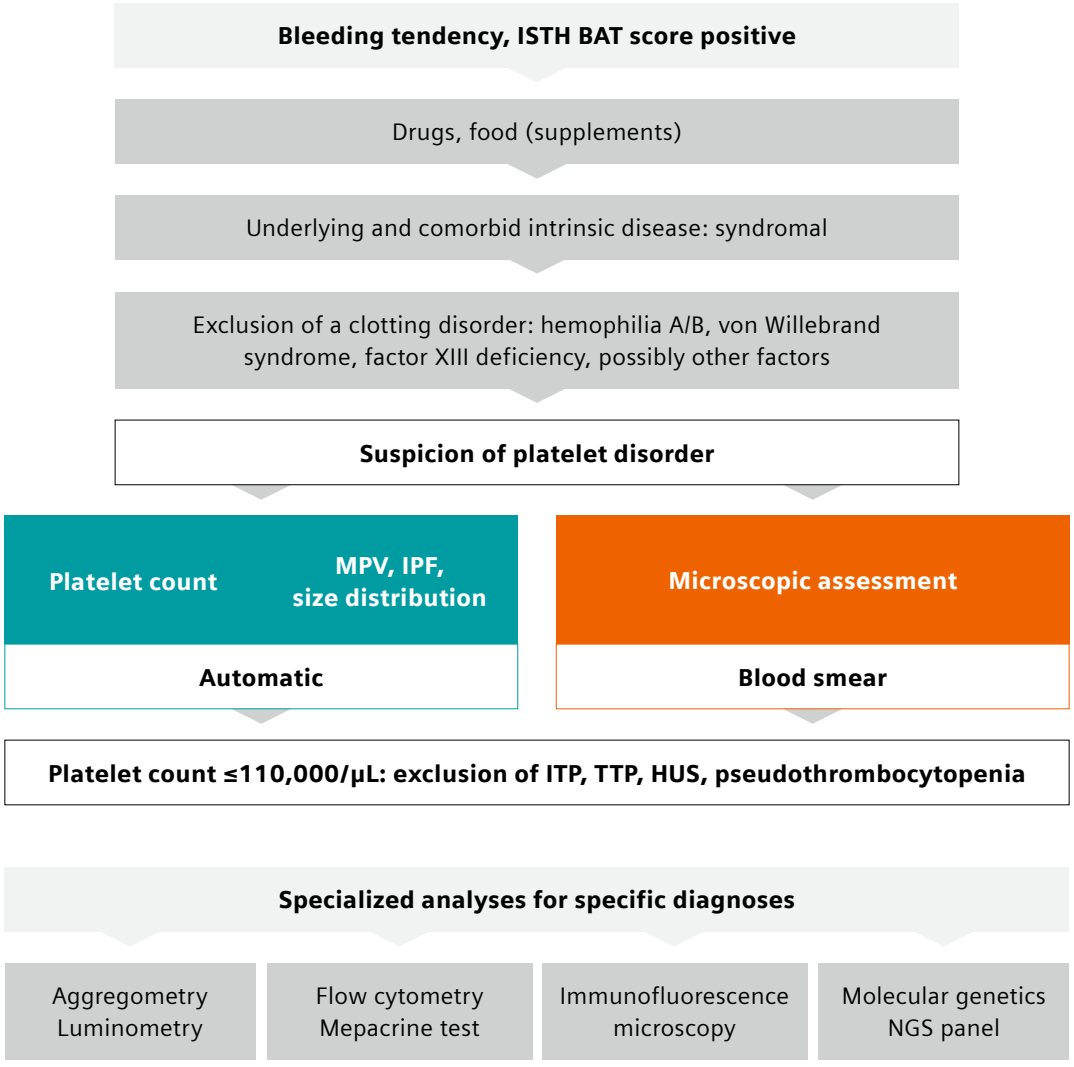
TXAS: *Thromboxane synthase*
VWF: *von Willebrand factor*

Diagnostic strategy for coagulation disorders

The recommended strategy is to support the diagnosis of inherited platelet disorders. The diagnostic steps depend on the patient's own and family history.[4]

AWMF-recommended diagnostic strategy



AWMF: *Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften*
ISTH: *International Society on Thrombosis and Haemostasis*
BAT: *Bleeding Assessment Tool*
MPV: *Mean platelet volume*
IPF: *Immature platelet fraction (immature platelets)*
ITP: *Immune thrombocytopenia*
TTP: *Thrombotic thrombocytopenic purpura*
HUS: *Hemolytic-uremic syndrome*
NGS: *Next-generation sequencing*

Pre-analytics: Sample collection and preparation

Pre-analytics plays a particularly important role in platelet aggregation testing.

Venous blood draw

- Draw blood carefully and gently to avoid contamination with tissue factor and hemolysis. Avoid activation of the platelets by shear stress.
- Minimize use of tourniquets.
- Preferred needle size is 21 gauge or larger.
- Use only plastic or siliconized sample tubes with a citrate anticoagulant (3.2% or 105–109 mMol).
- Do not use vacuum tubes if possible.
- Fill the sample tube completely.
- After collection, mix sample tube gently.

Sample handling issues

- **Poorly mixed samples:** Can lead to activation of coagulation factors and formation of small amounts of thrombin that can preactivate platelets.
- **Too-strongly shaken samples:** Due to increased mechanical stress, preactivation of the platelets may occur.
- **Too-low transport temperature:** Can lead to cryoactivation of the platelets.
- **Too-high transport temperature:** Can lead to reduced platelet and sample stability.

Interference 1

- **Hemolytic samples:** Release of ADP, calcium, and hemoglobin leads to preactivation of platelets and influences optical measurement.
- **Lipemic samples:** Increased optical density causes interference with optical measurement of platelet aggregation.
- **Icteric samples:** Do not interfere with optical measurement of platelet aggregation.
- Interferences can vary from system to system.

Interference 2

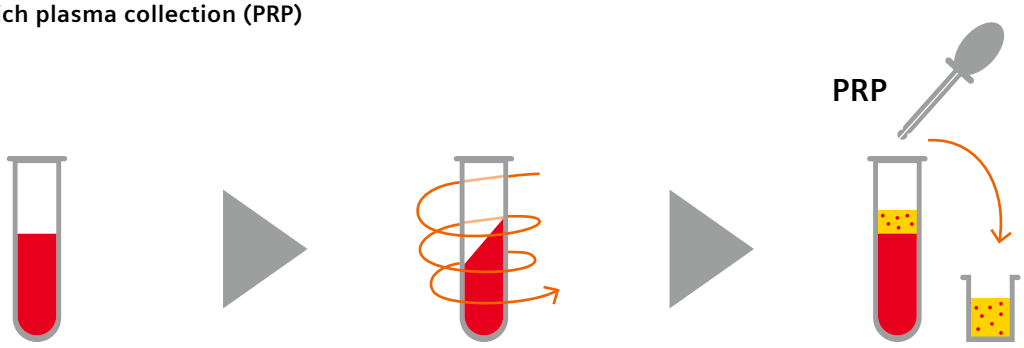
- Increased consumption of coffee and black tea as well as smoking influence platelet aggregation.
- Stress leads to the release of epinephrine, which can cause preactivation of platelets.
- Certain foods and food supplements can have an inhibitory or activating effect on platelet aggregation.[4]
- Antibiotic, psychotropic, and chemotherapeutic drugs influence platelet aggregation.[8,9]

Platelet aggregometry: Sample preparation

For proper performance of platelet aggregation testing according to Born, follow these instructions for sample handling:

- To exclude thrombocytopenia, platelets should be determined in citrated whole blood, not EDTA whole blood. Use the THROMBOEXACT tube to most accurately determine the platelet count.
- Platelets are enriched during preparation of platelet-rich plasma (PRP) from citrated whole blood.
- An increased platelet count in the PRP indicates that the preparation was successful. A decreased platelet count in the PRP may indicate a preactivated sample.
- Allow the PRP to rest for 15–45 minutes at room temperature before starting the analysis. Gently swirl the PRP and place it on the system. Do not vortex the sample.
- The platelet count should be included in the diagnosis. If the platelet count decreases <100 Gpt/L, there is an increased influence on aggregation.
- Measurement of platelet aggregation should be completed within 2–4 hours after collection.

Platelet-rich plasma collection (PRP)

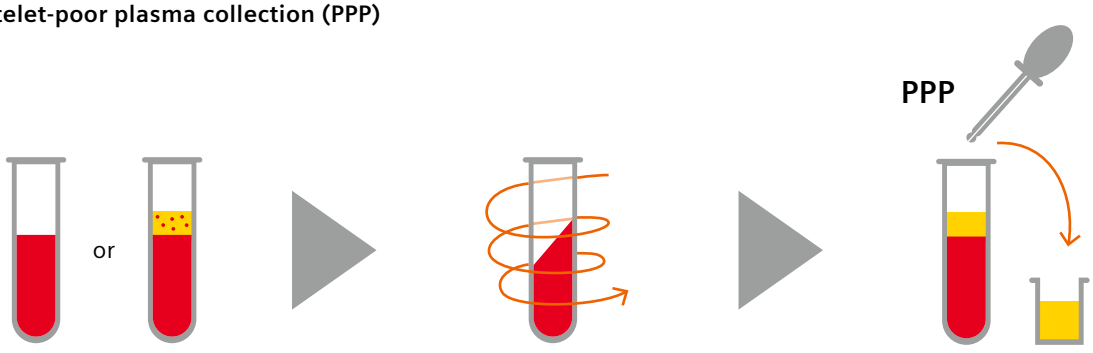


Citrate tube

Centrifugation: 200xg, 10 minutes at room temperature, without break

Carefully remove the PRP from the supernatant into a secondary vessel.

Platelet-poor plasma collection (PPP)



Citrate tubes or residual material from PRP extraction

Centrifugation: 1500xg, 15 minutes at room temperature

Carefully remove the PPP from the supernatant into a secondary vessel.

Platelet aggregometry: Light transmission aggregometry (LTA) according to Born

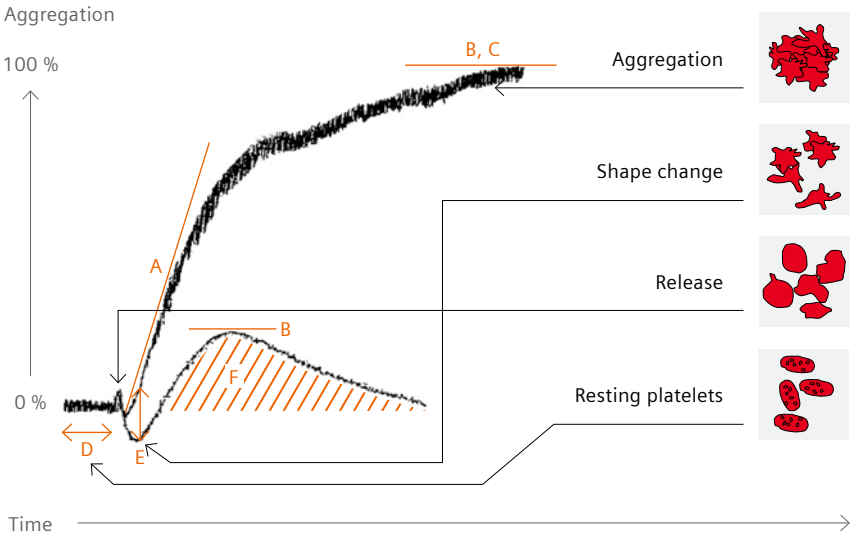
Light transmission aggregometry (LTA) according to Born[7] is considered the gold standard of platelet function diagnostics. It can be used to determine and characterize many functional properties of platelets. Determination, interpretation of the findings, and assessment to define a clinical picture are very challenging.

In LTA, the increase in light transmission is determined over time after the addition of a specific agonist to the initially turbid PRP.

With the aid of the platelet-poor plasma (PPP), light transmission can be converted to % of aggregation. To obtain a standardized aggregation result, it is important to constantly stir the PRP to simulate shear stress. The temperature during aggregation measurement must be 37°C.

Integrated platelet aggregation on a routine hemostasis analyzer helps make platelet aggregation more manageable, reliable, and accessible, with easy-to-use, standardized testing.

Available parameters to characterize the aggregation curve



Slope (A)

Maximum aggregation in percent (B)

End aggregation in percent (C)

Lag phase (D)

Shape change (E)

Area under the curve (AUC) (F)

Up to nine different evaluation parameters are available on CN-6000 and CN-3000 Systems* as well as CS-2500 and CS-5100 Systems.

**The products/features/applications mentioned here are not commercially available in all countries and are subject to local regulations. Their future availability cannot be guaranteed. Not available for sale in the U.S.*

Platelet aggregation agonists[7]

ADP	ADP activates the $P2Y_{12}$ receptor on the platelet surface and leads to inhibition of intracellular cAMP production, which results in a release of calcium. Furthermore, ADP activates the $P2Y_1$ receptor, initiating a platelet shape-change and a transient, rapidly reversible aggregation of the platelets. Activation of both receptors leads to a complete aggregation and release of contents from the platelet granules. Using a low ADP concentration, the aggregation may be biphasic or partially reversible.
Epinephrine	Epinephrine activates the platelets via the α_2-adrenoreceptor, and a biphasic aggregation curve occurs. Activation of the platelets by epinephrine leads to a platelet shape-change and a first wave of aggregation. As a result, fibrinogen receptors are exposed, and the activity of adenylatecyclases increases. A second wave of aggregation, quite different from the first, is caused by the release of ADP from the α-granules and the synthesis of thromboxane A_2.
Arachidonic acid	In the platelet, cyclooxygenase (COX-1) forms thromboxane A_2 from arachidonic acid. The TxA_2 leads to platelet activation over the thromboxane receptor. Platelet aggregation results in a simple aggregation wave.
Collagen	Collagen binds to the GPIa-IIa and GPVI receptors on the platelet surface. The GPVI receptor is partly responsible for platelet activation, which leads to the release of ADP, thromboxane A_2, and intracellular calcium. The aggregation curve typically shows a lag phase as a response to collagen, which is influenced by the collagen concentration. Platelet aggregation results in a simple aggregation wave.
Ristocetin	Ristocetin enables the interaction between von Willebrand factor and the glycoprotein GPIb-V-IX receptor. By using low-dose ristocetin concentrations during aggregation, VWF type 2B can be detected.
TRAP	TRAP activates the thrombin receptor PAR-1 independently of receptor cleavage and mimics the effect of thrombin. TRAP-induced aggregation is not or only slightly inhibited by acetylsalicylic acid or $P2Y_{12}$ antagonists. TRAP can therefore be used to check whether platelets respond at all to a strong stimulus. PAR-1 antagonists show a concentration-dependent inhibition of aggregation with TRAP.

Aggregation: Integrated, automated, and standardized

On Siemens Healthineers mid- and high-volume hemostasis systems, many manual steps can be automated with the help of integrated platelet aggregation.* A high level of automation ensures reliable, reproducible, and safe processing of samples.

The following systems offer this integrated method:*

CN-6000 and CN-3000 Systems*

CS-5100 and CS-2500 Systems

To set up the method on your system, please contact your local Siemens Healthineers representative.

The example curves and explanations on the following pages assist in interpretation of test results.

CN-6000 and CN-3000 Systems*

CS-5100 System

CS-2500 System

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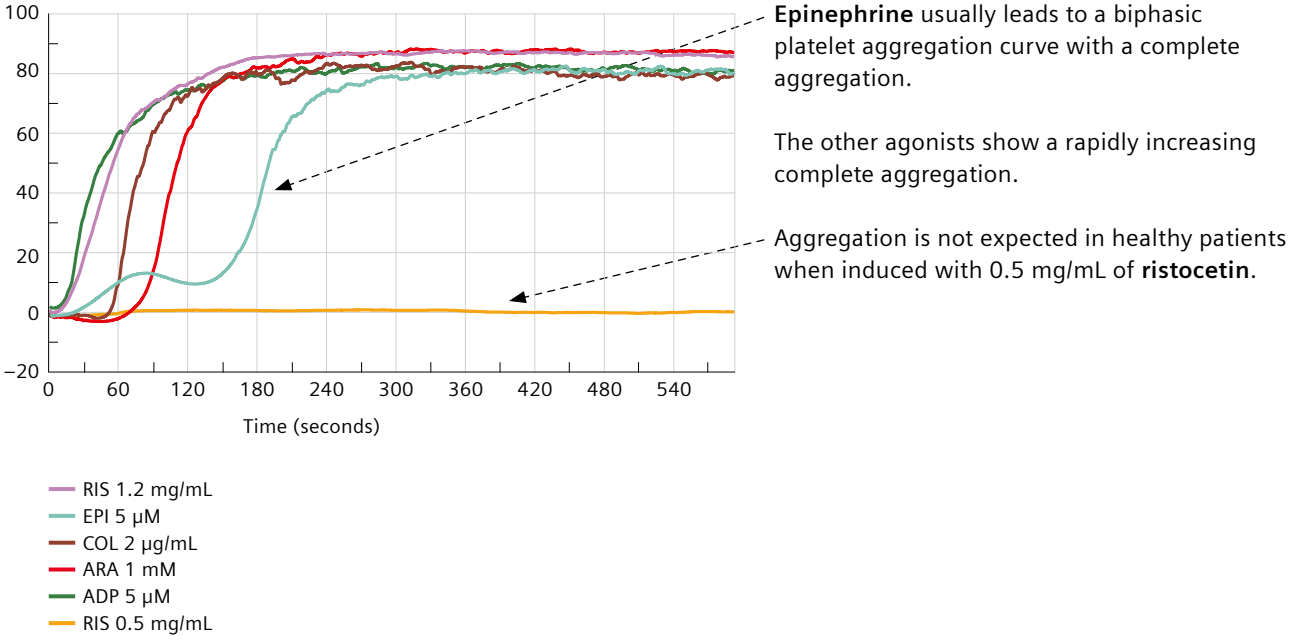
Platelet aggregation integrated on the coagulation analyzer (1/2)*

Expected aggregation curves in normal patients

CN-6000 and CN-3000 Systems*
CS-2500 and CS-5100 Systems



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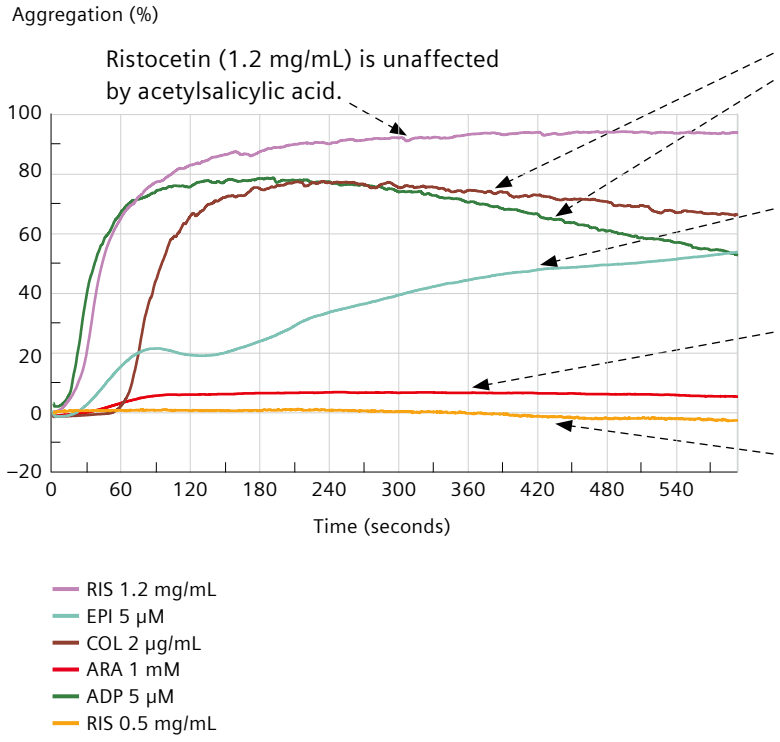
Platelet aggregation integrated on the coagulation analyzer (2/2)*

Expected aggregation curves in patients taking acetylsalicylic acid

CN-6000 and CN-3000 Systems*
CS-2500 and CS-5100 Systems



Moderate inhibition of **collagen**- and **ADP**-induced aggregation is visible. This is dependent on the concentration of the agonists and the dosage of acetylsalicylic acid.

Inhibition of **epinephrine**-induced aggregation and absence of the second wave are also typical.

Clear inhibition of **arachidonic acid**-induced aggregation is characteristic in patients taking acetylsalicylic acid.

Ristocetin (0.5 mg/mL) is unaffected by acetylsalicylic acid.

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Notice

Results of light transmission aggregation (LTA), in particular if platelet counts of the PRP are lower or higher than recommended, should always be evaluated in conjunction with clinical history, clinical presentation, and other laboratory findings (such as bleeding time, complete blood count [CBC], and coagulation parameters).

In cases where aggregation results from tests with standard agonist concentrations do not agree with the clinical assessment, additional aggregation tests with lower agonist concentration should be performed. When using lower agonist concentrations, it is recommended to measure a healthy patient in parallel to evaluate the patient result. Moreover, further platelet function tests, e.g., ATP release, serotonin release, or flow cytometric assays, might be necessary.

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We hope this guide helps you introduce integrated, automated, and standardized platelet aggregation testing to your daily laboratory routine.

Abbreviations

Ara	Arachidonic acid
ADP	Adenosine diphosphate
COL	Collagen
COX-1	Cyclooxygenase 1
DNA	Desoxyribonucleic acid
EPI	Epinephrine
GP	Glycoprotein
LTA	Light transmission aggregometry
MPS	Myeloproliferative syndrome
NSAID	Nonsteroidal anti-inflammatory drugs
PAR	Protease-activated receptor
PPP	Platelet-poor plasma
PRP	Platelet-rich plasma
RIS	Ristocetin
RNA	Ribonucleic acid
TRAP	Thrombin-receptor-activating peptide
TxA_2	Thromboxan A_2
UDM	User-defined method
VWF	von Willebrand factor

Guidelines and literature

Available guidelines

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