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Andreas Calatzis is a coworker of Michael Spannagl at Munich University Clinic. He is a coinventor of the Multiplate analyzer.

The clinical data and algorithms presented in this compendium are based upon the experience of the authors and on the discussion with centres that use the Multiplate system in clinical routine.
However this data still requires prospective validation with respect to its clinical predictivity. Therapeutic interventions based on Multiplate analyses are the sole responsibility of the treating physician.

The authors thank Alby Pattison of Hart Biologicals for valuable discussions on this compendium.

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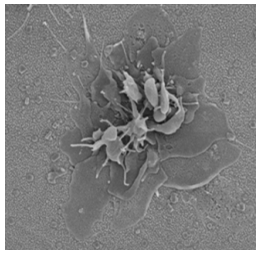
Multiplate® platelet function analysis - application and interpretation



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Introduction

This compendium is intended to give an overview over the device, its applications and limitations.

The Multiplate analyzer is a new platelet function analyzer utilising the analysis of whole blood.

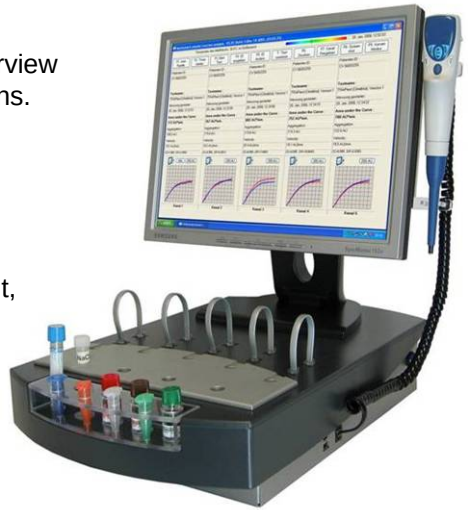
Whole blood is the physiological environment, where platelet function takes place in vivo, and the use of whole blood for in-vitro testing eliminates the need for time-consuming centrifugation steps.

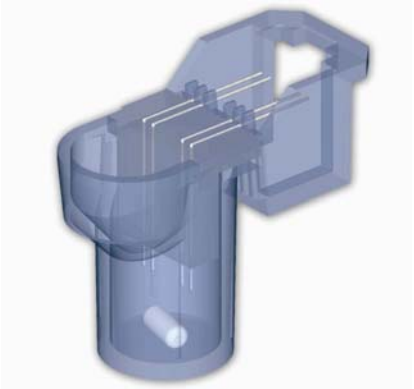
Several test reagents are available to allow triggering of different receptors / signal transduction pathways of the platelet in order to detect its function or drug effects.

The Multiplate detects the effects of the platelet inhibitors Aspirin®, clopidogrel and GpIIb/IIIa antagonists. Also the sensitivity for direct ADP receptor antagonists has been shown.

The use of a small amount of whole blood (0.3 ml per test) allows the differentiated assessment of platelet function without drawing large amounts of blood (as required for analysis of Born aggregation) and facilitates the analysis of blood from children and also in experimental settings (animals).

The term Multiplate is derived from the phrase "multiple platelet function analyzer". *Multiple* stands for the multiple electrodes in the disposable test cell (4 electrodes form 2 independent sensor units), multiple channels of the instrument (5) as well as multiple test procedures available for a comprehensive assessment of platelet function.





The instrument

The Multiplate instrument is a compact device with 5 channels for parallel tests and an internal Windows XP based computer system. User inputs are performed with the mouse and keyboard. Using the optional electronic pipette the application of the device can be simplified using computer-assisted operation procedures.

Single use test cell

Multiplate analysis takes place in a single use test cell, which incorporates a dual sensor unit and a teflon-coated stirring magnet.

The principle of Multiplate analysis is based on the fact that platelets get sticky upon activation, and therefore have a tendency to adhere and aggregate on metal sensor wires in the Multiplate test cell.

The test cell has a pipetting inlet, a cup portion with the sensor wires, which protrude into the blood and a jack portion, which allows to connect the test cell to the instrument in order to record the electrical resistance between the sensor wires during the test.

The sensor wires are made of highly conductive copper, which is silver-coated. When activated platelets adhere onto the sensor wires the electrical resistance between the wires rises, which is continuously registered.

Multiplate® detection principle

Impedance aggregometry was developed by Cardinal and Flower[1] and has been used since the 1980's for the assessment of platelet function in whole blood.

Impedance aggregometry is based on the principle that blood platelets are non-thrombogenic in their resting state, but expose receptors on their surface when they get activated which allow them to attach on vascular injuries and artificial surfaces.

When platelets stick on the Multiplate sensor wires, they enhance the electrical resistance between them, which is continuously recorded. In order to enhance the resistance on the sensor wires a tight attachment of the platelets is required. On the scanning electron microscopy shown on this page a typical platelet aggregate on the Multiplate sensor wire is shown (picture by Armin Reininger of Munich University Clinic).
Various states of platelet activation are visible, such as spreading of platelets on the surface as well as platelets aggregated with each other.

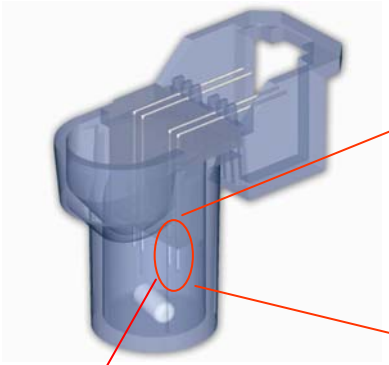
The fact that aggregation in Multiplate takes place on surfaces is a major difference compared to methods such as Born aggregometry and single platelet counting.

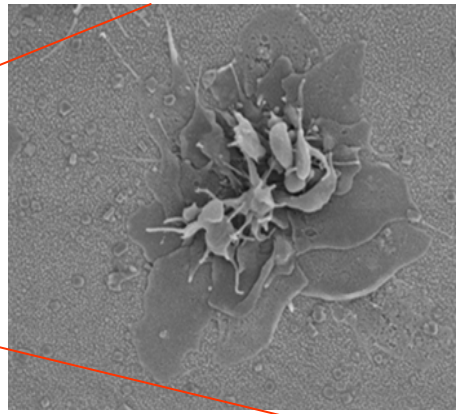
In Born aggregometry and single platelet counting methods, platelets aggregate with each other in the liquid phase. This presumably happens only in severely ill patients (e.g. during HIT type II and DIC), as coagulation and platelet aggregation in-vivo usually only take place on surfaces (vascular injuries / inflamed vessels / atheromatous plaques).

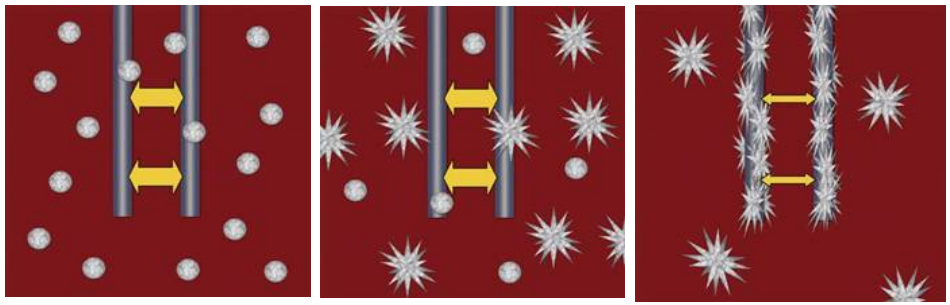
[1] Cardinal DC, Flower RJ. The electronic aggregometer: a novel device for assessing platelet behavior in blood. J Pharmacol Methods. 1980 Feb;3(2):135-58.

Dual sensor in Multiplate® analysis

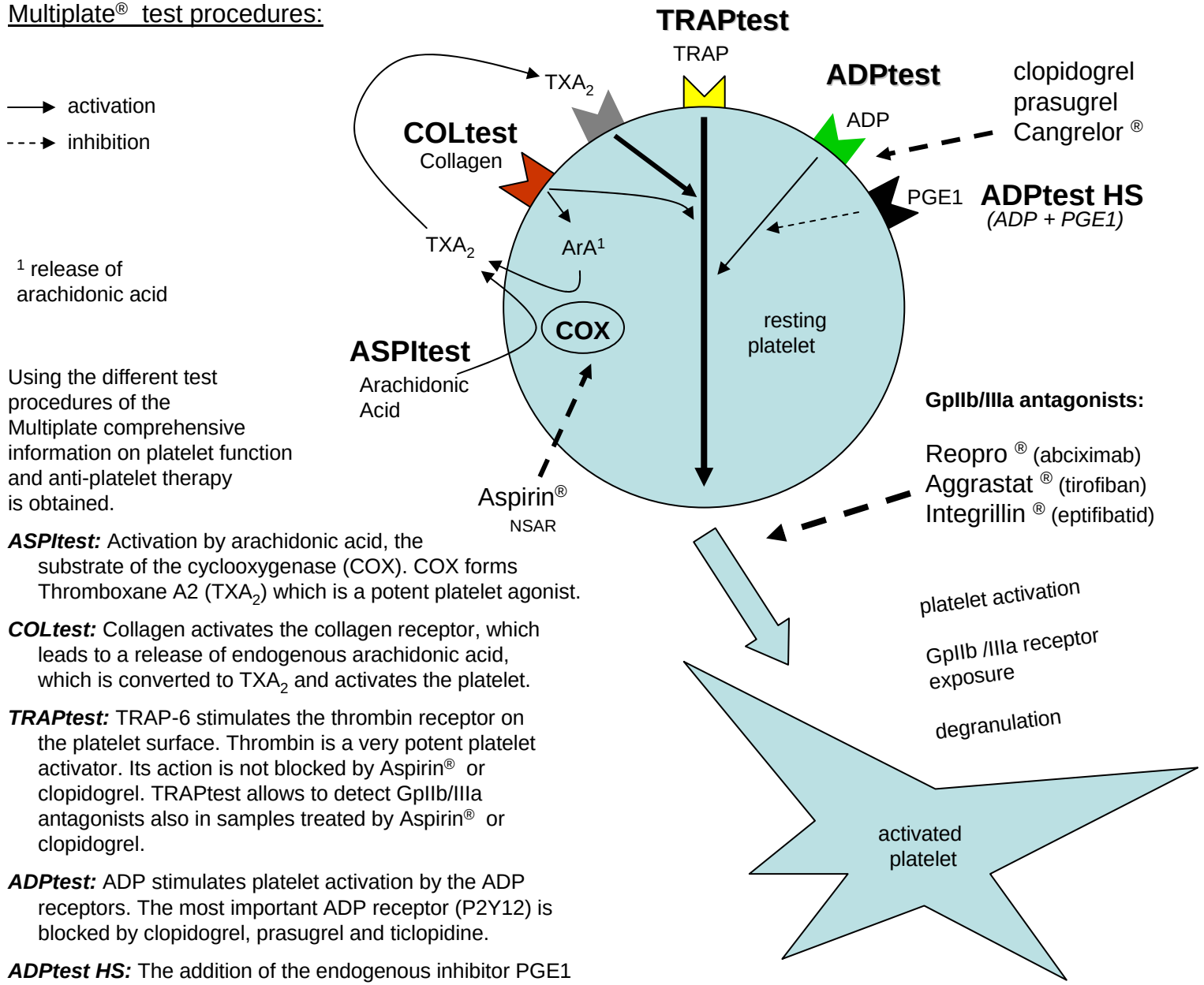
Every Multiplate test cell incorporates two independent sensor units, each consisting of 2 silver-coated highly conductive copper wires with a length of 3.2 mm. The instrument detects the impedance change of each sensor separately. The impedance change is expressed in arbitrary „Aggregation Units" (AU). Pearson´s correlation coefficient of the data points detected by each channels is calculated. If the correlation coefficient is lower than 0.98 a quality control flag is added to the measurement and the user is asked whether he wants to repeat the measurement. In addition the areas under the aggregation curve detected by each channels are compared and if the difference is higher than 20% (vs. the mean curve) again the user is prompted by the Multiplate software.







Multiplate® test procedures:



[1] release of arachidonic acid

Using the different test procedures of the Multiplate comprehensive information on platelet function and anti-platelet therapy is obtained.

ASPItest: Activation by arachidonic acid, the substrate of the cyclooxygenase (COX). COX forms Thromboxane A2 (TXA_2) which is a potent platelet agonist.

COLtest: Collagen activates the collagen receptor, which leads to a release of endogenous arachidonic acid, which is converted to TXA_2 and activates the platelet.

TRAPtest: TRAP-6 stimulates the thrombin receptor on the platelet surface. Thrombin is a very potent platelet activator. Its action is not blocked by Aspirin® or clopidogrel. TRAPtest allows to detect GpIIb/IIIa antagonists also in samples treated by Aspirin® or clopidogrel.

ADPtest: ADP stimulates platelet activation by the ADP receptors. The most important ADP receptor (P2Y12) is blocked by clopidogrel, prasugrel and ticlopidine.

ADPtest HS: The addition of the endogenous inhibitor PGE1 makes ADPtest HS more sensitive towards the effects of clopidogrel and related drugs compared to ADPtest.

Multiplate® test results:

Multiplate continuously records platelet aggregation. The increase of impedance by the attachment of Platelets onto the Multiplate sensors is transformed to arbitrary aggregation units (AU) and plotted against time.

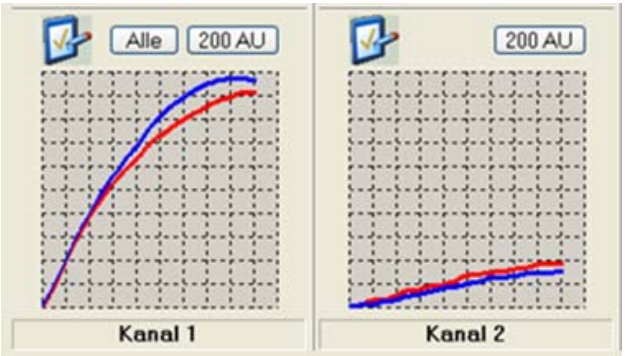
Three parameters are calculated: The most important parameter is the **area under the aggregation curve** (**AUC**). It is affected by the total height of the aggregation curve as well as by its slope and is best suited to express the overall platelet activity.

Two more parameters are calculated for research use: The **aggregation** is the height of the curve. The **velocity** is the maximum slope of the curve.

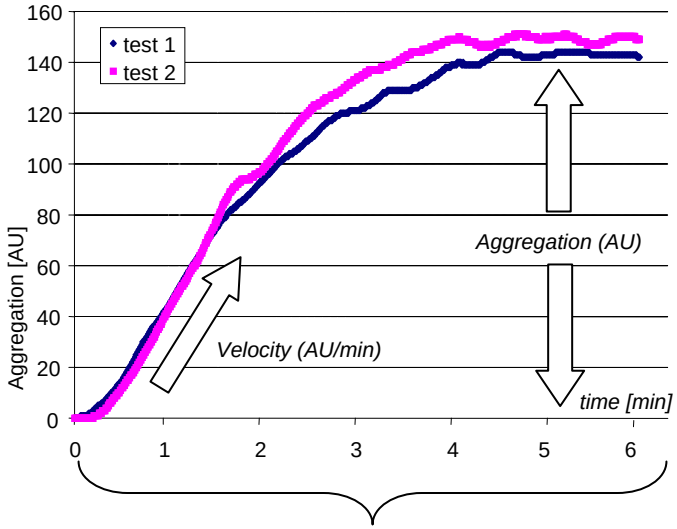
As seen on the diagram on the right, two curves are assessed using the two independent sensors in the test cell. The parameters calculated by the software are the mean values of the parameters determined with each curve.

When the option „print additional values“ in the service menu is selected, then the individual values of each curve are printed in addition to the mean values.

The scale of the y axis is shown both on the screen and on the printout. It can be set to 50, 75, 100 or 200AU.



ASPItest (normal sample) *ASPItest (patient on Aspirin®)*



Area under the curve = AUC (AU*min or U)
10 AU*min = 1 U

The unit of the AUC is AU * min (as the y-axis is the aggregation, expressed in Aggregation units (AU) and the x-axis is the time, expressed in minutes). Alternately The AUC can be expressed in U (1 U corresponds to 10 AU*min).

The aggregation and velocity are calculated for research use.

The correlation coefficient (cc) between the values of the 2 individual curves is determined. The analysis is accepted when the cc is at least 0.98.

The difference from the mean curve (DIF) is calculated based on the AUC values of the 2 individual measured curves. The analysis is accepted when the difference is lower than 20% (vs. the mean value of the 2 curves).

new scale for the AUC → 10 AU*min = 1 U

Influence of the blood collection system

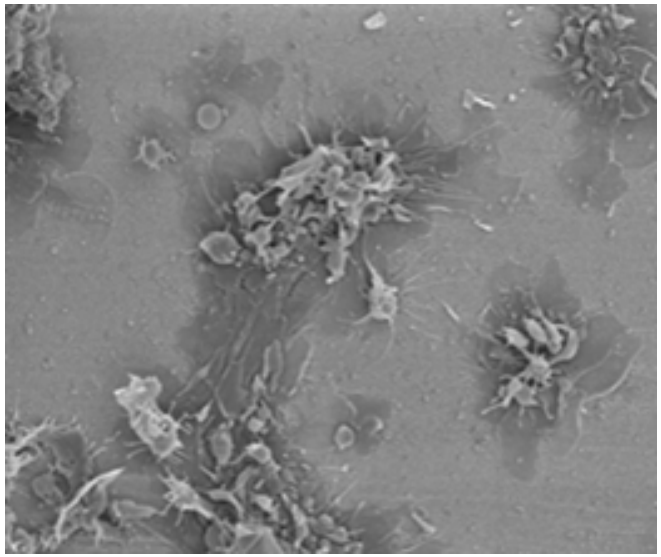
During the early phase of Multiplate use, citrated blood (either 3.2% or 3.8%) was mainly used for the sample collection. During this phase it was noticed that some patients and healthy subjects did not aggregate properly, particularly with ADP as agonist without any history of coagulopathy or other reasons that could explain this finding. In addition large variations in aggregation response were observed using the same sample and repeated determinations. With several research projects the depletion of calcium in the citrated blood Was determined as the reason for this phenomenon.

Citrate complexes calcium and thereby prevents clotting of the sample. However calcium is also an important second messenger for platelet aggregation. For a full shape change and full exposure of receptors on the platelet surface the release of calcium from intracellular stores is necessary.

Most aggregation techniques do not require full platelet activation for their signal reaction:

- in Born aggregation a loose aggregate formation already leads to a loss of turbidity in the blood sample and therefore to an increase in the aggregation curve.
- in the PFA-100® system the response is strongly mediated by a binding of vWF to the platelet GpIb receptor, a receptor which is present on the platelet surface also in non-activated platelets.
- in the Accumetrics® Verifynow® system platelets aggregate with fibrinogen coated latex beads, so also for this test no full platelet aggregation including platelet spreading reaction on surfaces is required.

In contrast to this in Multiplate – as shown by the electron microscopy of the sensors after the measurement, platelets aggregate with each other on the artificial surfaces of the sensors.



A tight attachment of the platelets on the surfaces is required for the Multiplate signal reaction and this seems to be more dependent on physiological calcium levels than other platelet function tests. The best solution for this problem is to use an anticoagulant which does not affect the free calcium concentration in the sample, i.e. ideally hirudin (a direct thrombin inhibitor) or heparin. Heparin has some activating properties on platelets, therefore the use of hirudin blood is preferred. However the monitoring data using heparin for Aspirin® and clopidogrel shows also a very good sensitivity for these drugs.

To reduce the influence of calcium depletion by citrate, a partial recalcification using the NaCl-$CaCl_2$ diluent solution (system reagent by the manufacturer) is used. This reagent is used instead of the regular saline solution and contains 0.9% NaCl and 3 mM $CaCl_2$.

However, the partial recalcification leads to a disaggregation phenomenon in ASPItest and RSTOtest. For these tests, use regular saline when analyzing citrated blood.

Multiplate® tests: Sensitivity to anti-platelet agents

** For thrombin inhibitor blood. For heparin and citrated blood please inquire with the manufacturer. it is advised to determine locally own reference ranges.*

Test	Activation	Sensitivity			
		Aspirin®	Clopido-grel	GPIIbIIIa Ant.	Reference range (AUC [U]) *
TRAPtest	direct activation of the thrombin receptor using the peptide TRAP (thrombin receptor activating peptide)	-	-	+	**94-156**
ASPItest	activation via arachidonic acid. Arachidonic acid is the substrate of the cyclooxygenase (target enzyme of Aspirin®).	++	-	+	**74-136**
ADPtest	activation by ADP	-	+	+	**53-122**
ADPtest HS	activation by ADP with the addition of the inhibitor Prostaglandin E1 (PG).	-	++	+	**31-107**

The main application of the Multiplate system so far is the monitoring of platelet function inhibitors. Several tests are available which possess a different sensitivity towards the various anti-platelet agents.

TRAPtest, by triggering the thrombin receptor is not sensitive towards a blockade of cyclooxygenase by Aspirin® or the ADP receptor by clopidogrel (there might be some minor effect on TRAPtest, however normally aggregation is still in the normal range). All Multiplate tests are affected by a blockade of the GpIIb/IIIa receptor. This shows that the binding of platelets to the metal sensors is dependent on the GpIIb/IIIa receptor.

ASPItest is highly sensitive to Aspirin® but not towards a blockade of the ADP receptor by clopidogrel.

ADPtest again is sensitive towards the ADP receptor blockade, but not towards the inhibition of cyclooxygenase.

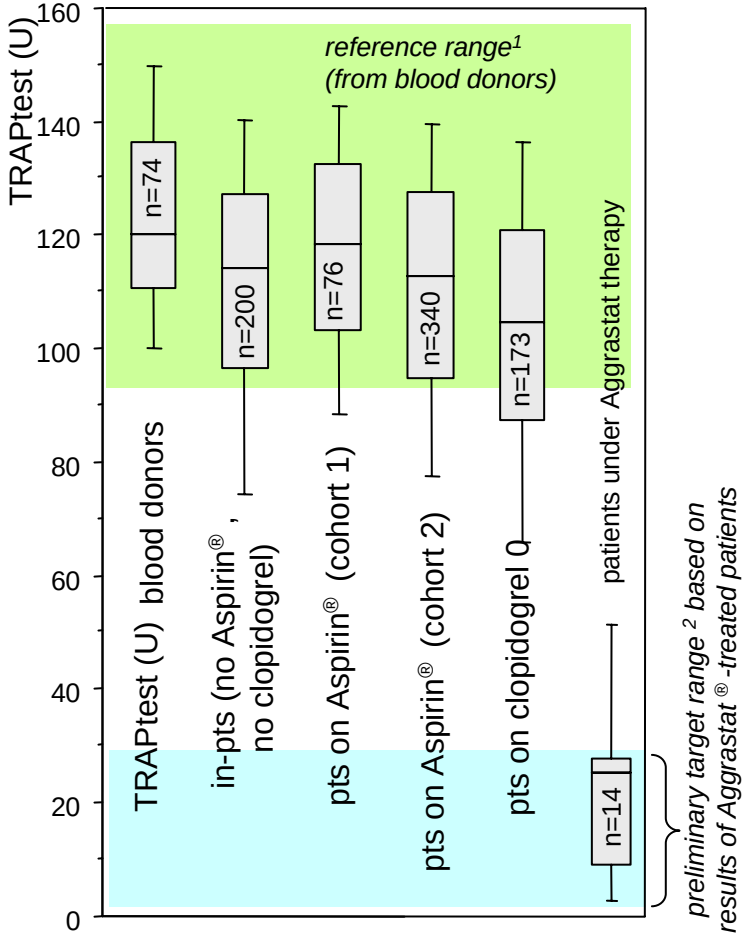
The addition of prostaglandin E1 to ADP enhances the sensitivity of the ADPtest towards clopidogrel, but leads also to an increased proportion of samples from non-clopidogrel treated individuals which show only a very weak aggregation (lower specificity compared to ADPtest).

The typical workup for monitoring of Aspirin is ASPItest or ASPItest + TRAPtest. In case of the performance of ASPItest + TRAPtest the difference of the two tests can help to assume the inhibitory function of Aspirin® (aggregation is normally quite similar in TRAPtest and ASPItest in the non-Aspirin® treated individual, while aggregation is typically much weaker in ASPItest when the patient is on Aspirin® treatment).

For the monitoring of clopidogrel we recommend to perform ADPtest + ADPtest HS. Also for this indication the additional performance of TRAPtest can help to assess the baseline aggregability of platelets.

TRAPtest: Expected values

Values obtained with thrombin inhibitor blood.
For heparin or citrated blood inquire with the manufacturer.



TRAPtest: reference range [1] based on the results of healthy blood donors: 94-156

[1] It is advised to determine locally own reference ranges.

[2] Whether an increased TRAPtest under anti-GpIIb/IIIa treatment leads to increased thrombembolic risk has not yet been prospectively evaluated

In TRAPtest platelets are activated by TRAP-6, a peptide that mimics the activation of platelets by the action of thrombin. Thrombin is the most potent platelet activator, its action is not blocked by the anti-platelet agents Aspirin® or clopidogrel. However also thrombin- (and TRAP-6-) activated platelets are blocked by the action of GpIIb/IIIa antagonists[3] (i.e. ReoPro®, Aggrastat® and Integrillin®).

The box plots on the left show the distribution of TRAPtest values in several patient groups.

In healthy blood donors the median for TRAPtest is at 120 U and 90% of individuals have 100 U or more. A total of 5 patient groups are shown: in-patients without aspirin or clopidogrel treatment, 2 groups of patients on aspirin (75-300 mg qd) and one group of patients on clopidogrel 75 qd, and under i.v. treatment with Aggrastat® (Tirofiban).

Most patients (between 60-80%), depending on the cohort examined, have a TRAPtest of more than 100 U, while more than 90% of patients have a TRAPtest of more than 60 U. As seen by the comparison of groups with and without clopidogrel or Aspirin® these drugs have only a minor – if any - effect on TRAPtest.

This allows the assessment of the effect of GpIIb/IIIa antagonists also in patients treated with Aspirin® or clopidogrel.

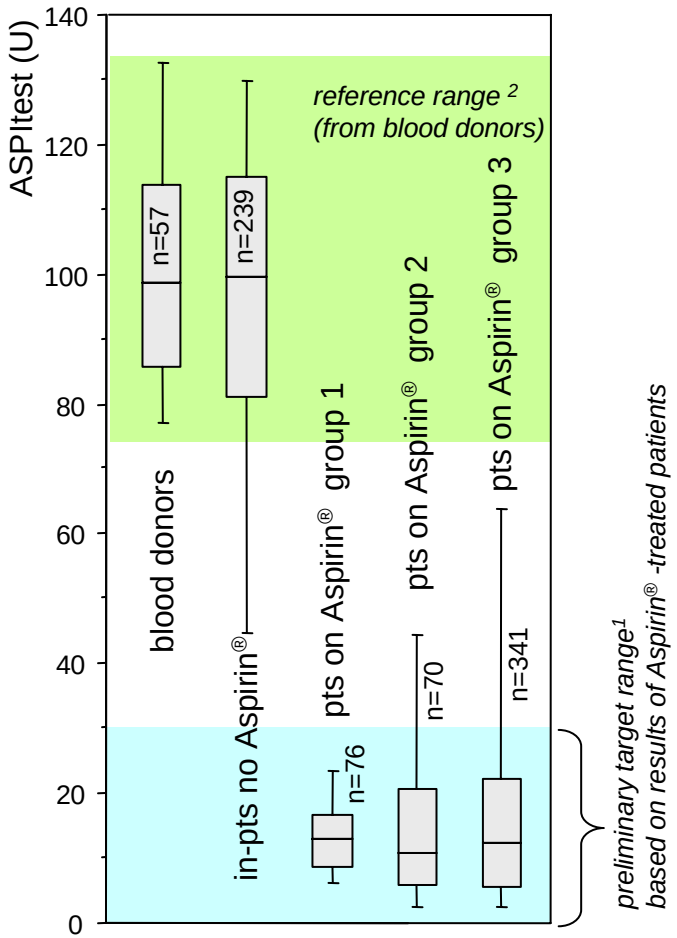
Further factors affecting platelet aggregation in TRAPtest are the platelet number, Glanzmann´s thrombasthenia[4] and severe platelet dysfunction as e.g. induced by prolonged cardiopulmonary bypass.

[3] The GpIIb/IIIa receptor is the platelet receptor that binds to fibrin and fibrinogen. This receptor is believed to be one of the most important receptors for platelet aggregation. The signal reaction of Multiplate is mainly mediated by GpIIb/IIIa.

[4] Glanzmann´s Thrombasthenia is a hereditary deficiency in GpIIb/IIIa receptors on the platelet surface.

ASPItest: Expected values

Values obtained with thrombin inhibitor blood.
For heparin or citrated blood inquire with the manufacturer.



ASPItest: reference range [2] based on the results of healthy blood donors: 74-136

[1] Whether an increased ASPItest under Aspirin® treatment leads to increased thrombembolic risk has not yet been prospectively evaluated.

[2]It is advised to determine locally own reference ranges.

In ASPItest platelets are activated by arachidonic acid, which is converted by the platelet cyclooxygenase (COX) to the potent platelet agonist Thromboxane A2. Arachidonic acid alone is not a platelet agonist. Therefore the platelet activation in ASPItest allows a very sensitive and specific detection of Aspirin action. Arachidonic acid is the physiological substrate of the platelet COX.

Aspirin and NSAID can block the platelet COX, resulting in a reduced aggregation in ASPItest. However, the potency of Aspirin is higher compared to NSAID.

ASPItest can also be reduced when other drugs interfering with platelet aggregation are taken, or in case of more global platelet disorders, e.g. in hematological disorders, thrombocytopenia, etc.

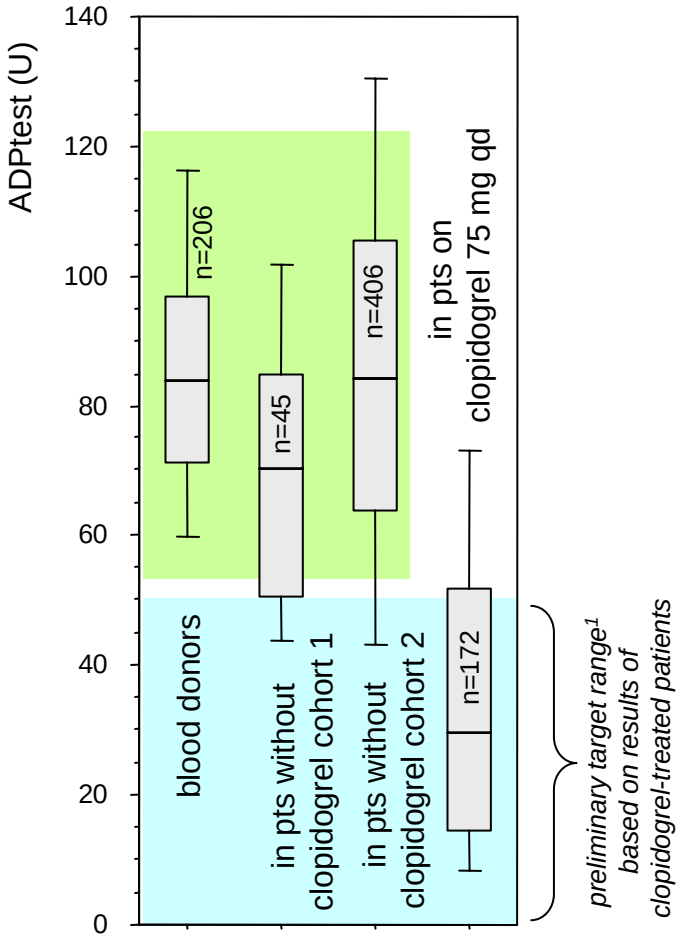
On the left the distribution of ASPItest in 5 cohorts is shown. Healthy blood donors showed a good aggregation in ASPItest with a median of almost 100 U. More than 90% had an aggregation of at least 75 U in ASPItest. In in-patients without aspirin treatment more than 75% of the patients had an ASPItest of at least 80 U. However some patients may also have a reduced platelet aggregation, due to other drugs interfering with COX activity or other comorbidities.

In all the three cohorts examined under aspirin treatment, the majority of patients showed strongly decreased aggregations in ASPItest with values lower than 30 U, which we therefore regard as preliminary cut-off. Patients with higher ASPItest values show an incomplete or no platelet inhibition and may thus have an increased risk for arterial thromboembolism compared to patients with aggregation values below the cut-off [1].

By definition Aspirin therapy should block arachidonic acid induced aggregation. Potential strategies to treat an Aspirin non response as determined by this method [1] would be either an increase of the aspirin dose (most likely treating Aspirin non-response because of reduced aspirin absorption), an increase in frequency of Aspirin® administration (in case of suspected elevated turn-over of platelets in the body), or switching the medication to a different drug (e.g. clopidogrel).

ADPtest: Expected values

Values obtained with thrombin inhibitor blood.
For heparin blood inquire with the manufacturer.
We do not recommend the use of citrated blood for the monitoring of clopidogrel.



ADPtest: reference range[2] based on the results of healthy blood donors: **53-122**

In ADPtest platelets are activated by ADP, which triggers several receptors on the platelet surface. clopidogrel and related drugs block the P2Y12 ADP receptor, which is believed to be the most important receptor for ADP on the platelet surface.

ADPtest can also be reduced when other drugs interfering with platelet aggregation are taken, or in case of more global platelet disorders, e.g. in hematological disorders, thrombocytopenia, etc. An ingestion of Aspirin® leads typically to no or only a minor inhibition of aggregation in ADPtest.

On the left the distribution of ADPtest in 4 cohorts is shown. Healthy blood donors showed a good aggregation in ADPtest with a median of 85 U. 90% had an aggregation of at least 60 U in ADPtest. For in-patients without clopidogrel treatment more than 75% of the patients had an ADPtest of at least 50 U in cohort 1 and 60 U in cohort 2. However some patients may also have a reduced platelet aggregation, due to other drugs interfering with platelet activity or due to comorbidities.

In the patients tested who were on clopidogrel treatment, approx. 75% of patients showed decreased aggregation in ADPtest with values lower than 50 U, which we therefore regard as a preliminary cut-off.
Patients with higher ADPtest values show an incomplete or no platelet inhibition and may thus have an increased risk for arterial thromboembolism compared with patients with aggregation values below the cut-off [1].

Potential strategies to treat an clopidogrel non response as determined by this method [1] would be either an increase of the clopidogrel dose, an increase in frequency of clopidogrel administration, switching the medication to a different drug (e.g. Aspirin® , Aggrastat for short-term use) or adding a second drug to the treatment.

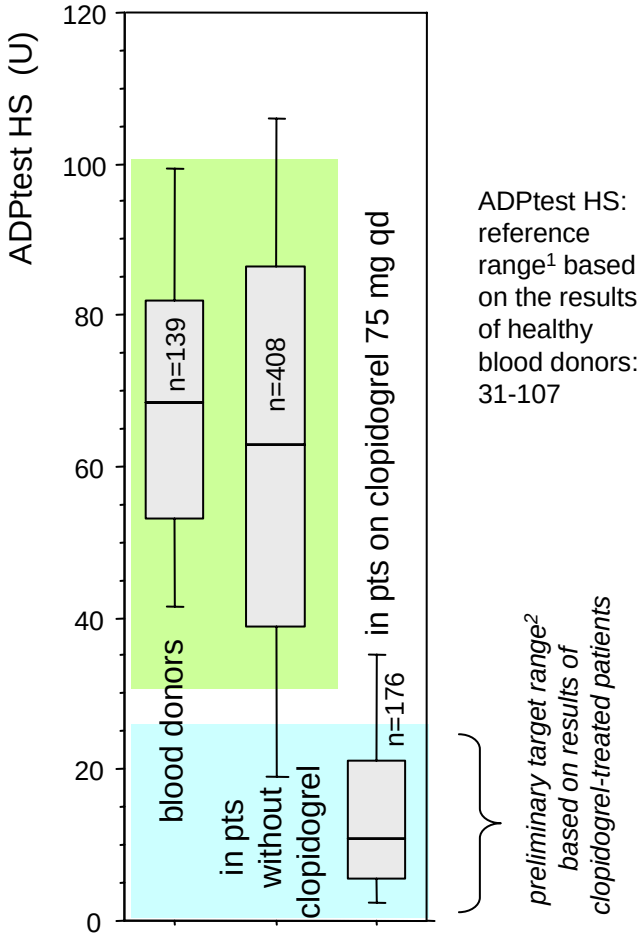
[1] Whether an increased ADPtest under clopidogrel treatment leads to increased thrombembolic risk has not yet been prospectively evaluated.

[2] It is advised to determine locally own reference ranges.

ADPtest HS (high sensitivity):
Expected values

NB: ADPtest HS was previously referred to as ADP+PG

Values obtained with thrombin inhibitor blood.



ADPtest HS: reference range[1] based on the results of healthy blood donors: 31-107

[1] It is advised to determine locally own reference ranges.

[2] Whether an increased ADPtest HS under clopidogrel treatment leads to increased thromboembolic risk has not yet been prospectively evaluated.

In ADPtest under clopidogrel therapy an incomplete blockade of aggregation is often seen.

This may be due to the fact that ADP not only triggers the P2Y12 ADP receptor on the platelet surface (i.e. the receptor that is blocked by clopidogrel), but also other ADP receptors (which are not affected by clopidogrel).

Heptinstal, Fox et al [3] have shown that a combination of ADP and a physiological platelet inhibitor (prostaglandin E1 = PGE1) can be more sensitive for the detection of the action of clopidogrel than the use of ADP alone.

The binding of ADP to the P2Y12 receptor reduces the level of cAMP in the platelet which in turn enhances the release of calcium from endogenous sources. The enhancement of intracellular calcium then leads to the activation and aggregation of the platelet.

PGE1 reduces the mobilisation of calcium and thus inhibits platelet aggregation. As clopidogrel also reduces the platelet activation by ADP, clopidogrel and PGE1 act synergistically.

On the left the distribution of ADPtest HS in 3 cohorts is shown. Healthy blood donors showed a good aggregation in ADPtest HS with a median of 68 U. 90% had an aggregation of more than 40 U in ADPtest HS. In in-patients without clopidogrel treatment almost 75% of the patients had an ADPtest HS of at least 40 U. However some patients may also have a reduced platelet aggregation, due other drugs interfering with platelet activity or other comorbidities.

In the patients examined under clopidogrel treatment, more than 75% of patients showed decreased aggregation in ADPtest with values lower than 25 U, which we therefore regard as a preliminary cut-off [2].

Patients with higher ADPtest HS values show an incomplete or no platelet inhibition and may thus have an increased risk for arterial thromboembolism compared with patients with aggregation values below the cut-off [2].

[3]Fox SC, Behan MW, Heptinstall S. Inhibition of ADP-induced intracellular Ca2+ responses and platelet aggregation by the P2Y12 receptor antagonists AR-C69931MX and clopidogrel is enhanced by prostaglandin E1. Cell Calcium. 2004 Jan;35(1):39-46.

Personalisation of anti-platelet treatment based on Multiplate® analysis

The data presented in this compendium shows that Multiplate detects systematically anti-platelet effects of the platelet function inhibitors Aspirin® , clopidogrel and GpIIb/IIIa antagonists.

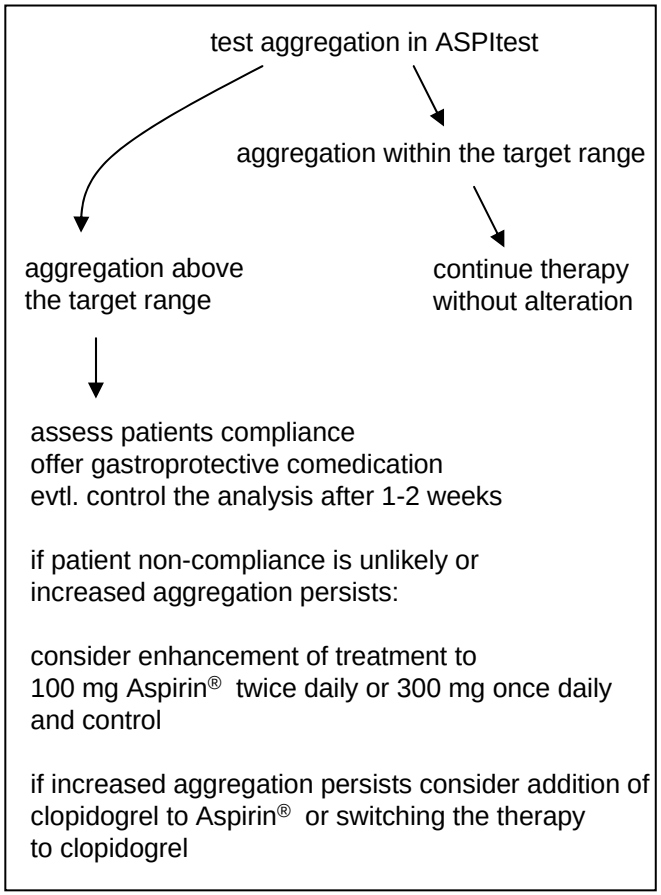
Controlling the effects of Aspirin® :
For the detection of Aspirin® arachidonic acid induced aggregation using ASPItest is typically used. Arachidonic acid is the direct substrate of cyclooxygenase, which is the enzyme which is blocked by Aspirin®. When a patient shows an increased aggregation in ASPItest (compared to the target range) this directly detects cyclooxygenase activity in the sample, and therefore shows that the therapeutic aim of the drug has not been reached.

We call this phenomenon an “Aspirin® non-response” and attempt to correct it by the following interventions:

- talk to the patient and try to assess the likelihood of compliance (i.e. assess whether the patient is taking the drug or not)
- if good compliance is rather unlikely explain to the patient the importance of the treatment and offer a gastro-protective comedication in case the patient reports gastric pain. Retest after 1-2 weeks.
- if good compliance is likely we try to improve platelet inhibition by a moderate increase of the frequency of Aspirin® application (i.e. switching from Aspirin® 100 mg once daily to twice daily) or intensity of treatment (i.e. switching from 100 mg once daily to 300 mg once daily). Control after 1-2 weeks.
- in the situation that enhanced aggregation persists in spite of education and dose increase of Aspirin® an addition of clopidogrel to the Aspirin® or a switch of the therapy to clopidogrel is evaluated. This is only performed, if data exists in literature that shows that the use of Aspirin® + clopidogrel or clopidogrel alone is effective and safe for prevention of thromboembolism in the clinical situation of the patient.

This means that based on the results of the aggregation test a moderate adaptation of the patient treatment is performed by moderately enhancing the Aspirin® dose or by switching to another effective and safe medication (i.e. clopidogrel or Aspirin® + clopidogrel).

Clinicians adopting such strategies should be aware that the effectiveness of such modifications in therapy are not proven, i.e. it has not been shown that fewer patients will experience thromboembolism on the modified therapy. Therefore it is very important that the changes to therapy are acceptable and safe, without making reference to the Multiplate data.



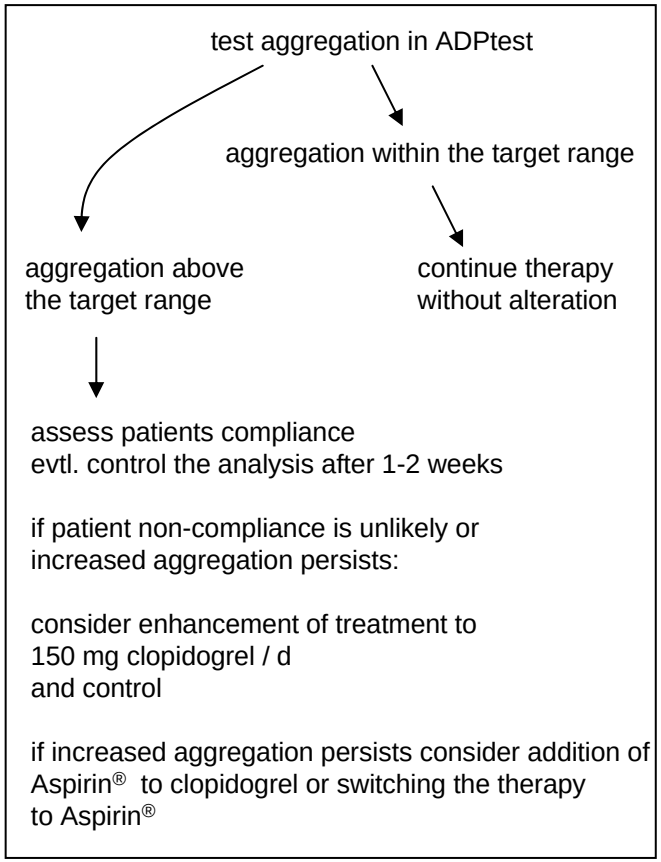
Controlling the effects of clopidogrel or other ADP receptor antagonists:

For detecting the effects of clopidogrel or ticlopidine, ADP induced aggregation is used. Typically the effect of the ADP receptor antagonist leads to a decreased response of platelets to the stimulation by ADP. If a higher than expected aggregation result is seen in ADPtest in spite of clopidogrel therapy (e.g. higher than our preliminary target range of 500 U) we attempt to increase the inhibition by the following protocol:

- talk to the patient and try to assess the likelihood of compliance (i.e. assess whether the patient is taking the drug or not)
- if good compliance is rather unlikely explain to the patient the importance of the treatment. Retest after 1-2 weeks.
- if good compliance is likely we try to improve platelet inhibition by a moderate increase of the intensity of treatment (i.e. switching from 75 mg once daily to 150 mg once daily or 75 mg twice daily). Control after 1-2 weeks.
- if the enhanced aggregation persists in spite of education and dose increase of clopidogrel an addition of Aspirin® to the clopidogrel or a switch of the therapy to Aspirin® is evaluated. This is only performed, if data exists in literature that shows that the use of Aspirin® + clopidogrel or Aspirin® alone is effective and safe for prevention of thromboembolism in the clinical situation of the patient.

This means that based on the results of the aggregation test a moderate adaptation of the patient treatment is undertaken by moderately enhancing the clopidogrel dose or by switching to another effective and safe medication (i.e. Aspirin® or Aspirin® + clopidogrel).

Again it is very important that the modified treatment is acceptable and safe, without reference to the Multiplate results.

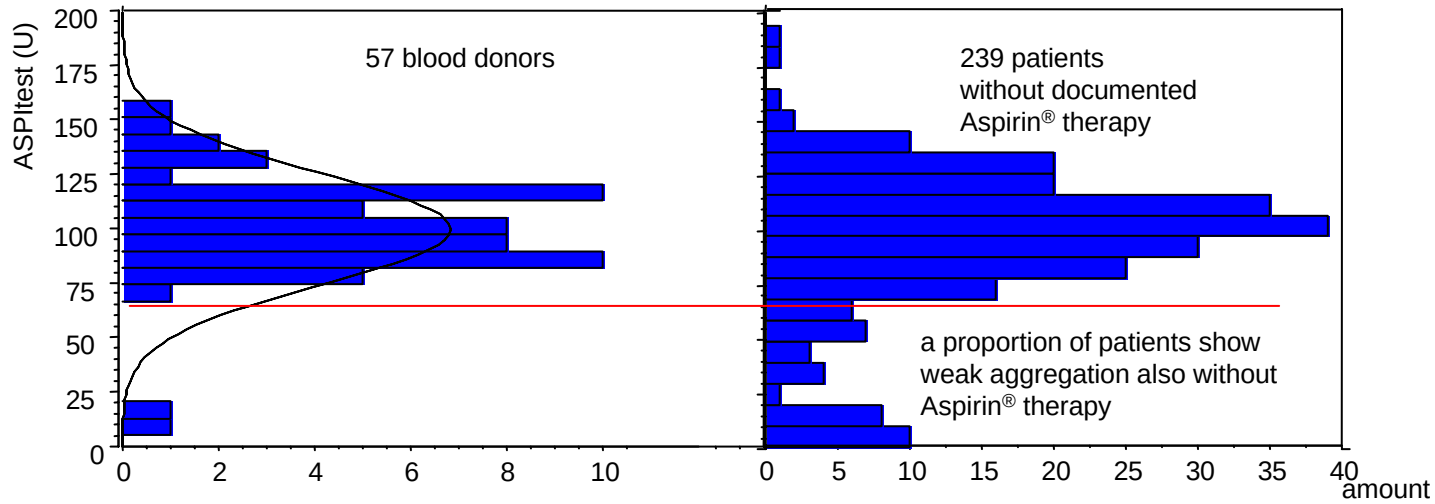


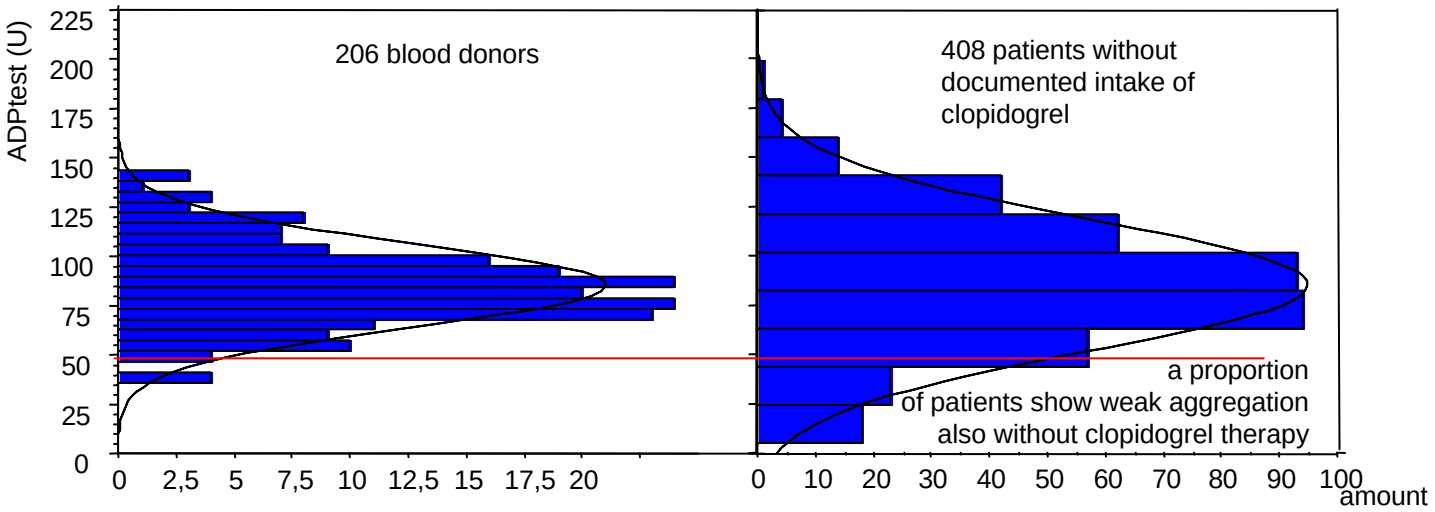
NB: Do not abstain from indicated anti-platelet therapy because of weak baseline aggregation in Multiplate®

Some patients show weak aggregation in ASPItest or ADPtest without the ingestion of Aspirin® or clopidogrel. Several drugs or comorbidites are known to impair platelet function and can explain this finding. However whether this also implies full inhibition against platelet-mediated thromboembolism is unclear.

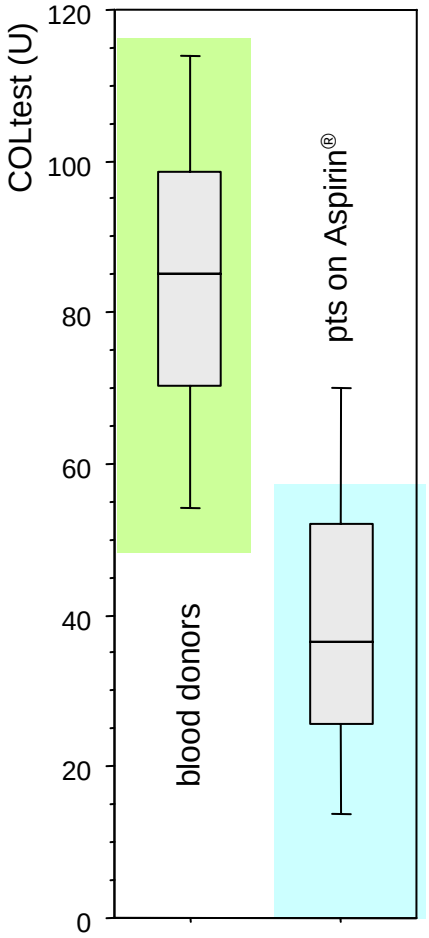
Therefore it is very important not to withhold indicated treatment in patients showing weak baseline in vitro aggregation in Multiplate.

In patients with high risk of bleeding or patients with clinical manifestations of bleeding a weak in vitro aggregation can support the clinical decision to reduce the antiplatelet treatment. However this decision must be acceptable without making reference to the Multiplate data.





COLtest: Expected values



COLtest: reference range[1] based on the results of healthy blood donors: 46-116

[1] It is advised to determine locally own reference ranges.

Whether an increased COLtest in patients under Aspirin® treatment leads to increased thromboembolic risk has not yet been prospectively evaluated.

COLtest leads to platelet activation via the platelet collagen receptor. For suitable platelet activation the release of endogenous arachidonic acid from the platelet phospholipids is necessary, which is then transformed to the platelet agonist thromboxane A2 by the enzyme cyclooxygenase. Cyclooxygenase is blocked by Aspirin® , and therefore COLtest is sensitive to Aspirin® action.

However this mechanism also explains why COLtest is less specific towards the action of Aspirin® compared to ASPItest. In ASPItest a defined amount of arachidonic acid is used as the activator, while in COLtest platelet are activated through endogenous TXA2 and other TXA2 independent mechanisms.

The distribution of COLtest values in the two groups shown on the left confirm the Aspirin® sensitivity of the assay. However the distinction between the two groups (blood donors and Aspirin® treated patients) is weaker than with the use of ASPItest. Therefore we prefer the use of ASPItest for the monitoring of Aspirin® responsiveness.

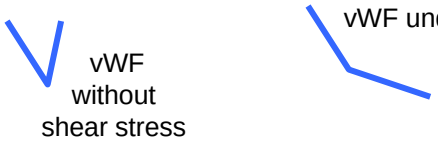
Collagen in aqueous solution has to be stored at 4-8°C and loses activity within few hours at room temperature. Appropriate storage of the reagent is therefore essential for the use in the test.

It is important to know that unlike arachidonic acid or ADP collagen is a less clearly defined activator and there are large differences between different commercially available collagen preparations when used for whole blood aggregometry on Multiplate.

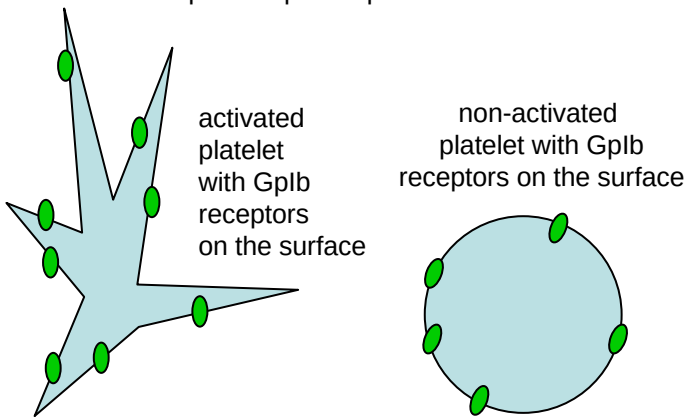
Other collagen preparations that work well on Multiplate are the collagen by Nycomed (Horm) or Chronolog. Using these collagens it is possible to receive a very high aggregation signal when higher concentrations of collagen (e.g. 10 µg/ml) are used. Then the platelet activation is not Aspirin® - sensitive.

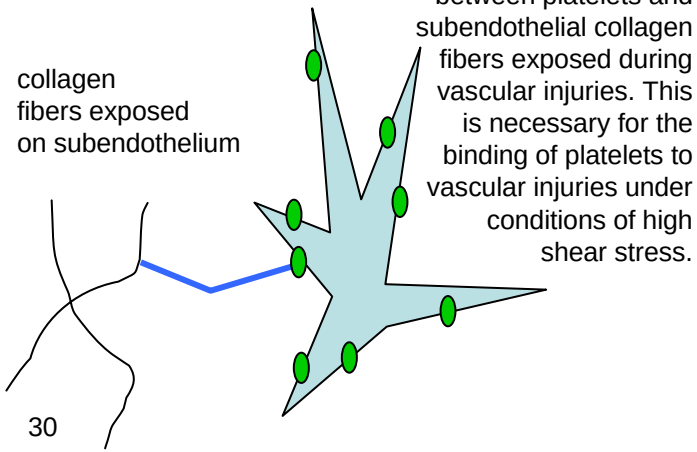
Natural action of vWF

vWF (von Willebrand factor) is a large adhesive molecule, which has an important role during adhesion of platelets to the subendothelium.



vWF changes its shape when under shear stress, which allows it to bind to the GpIb receptor of platelets.



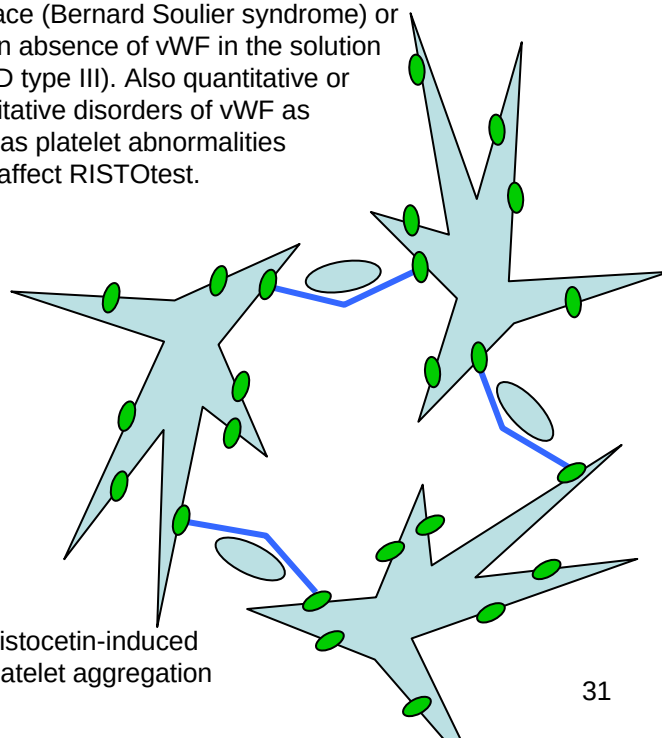


Mechanism of Ristocetin-induced platelet aggregation in RISTOtest)

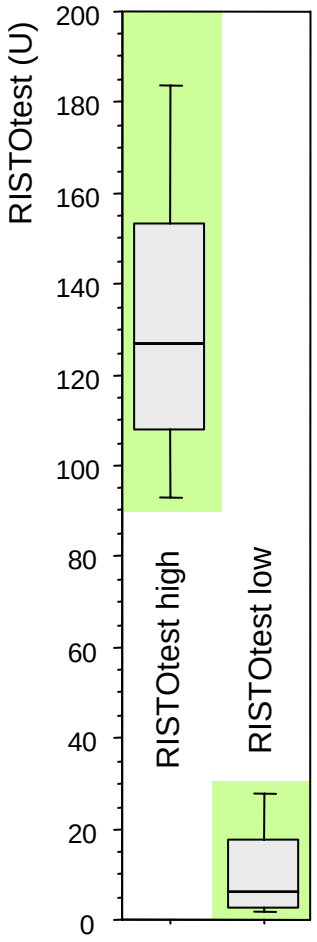
Ristocetin is an anti-microbial substance which foms complexes with vWF. In this complex vWF changes its conformation in a way that allows it to bind to platelets.

The binding of Ristocetin-vWF complexes leads to the aggregation of platelets and also to their activation. This activation relies on the binding of vWF to the GpIb receptor and is inhibited by Aspirin®. This explains the Aspirin® -sensitivity of RISTOtest.

Complete lack of aggregation in RISTOhigh can be caused by an absence of GpIb receptors on the platelet surface (Bernard Soulier syndrome) or by an absence of vWF in the solution (vWD type III). Also quantitative or qualitative disorders of vWF as well as platelet abnormalities can affect RISTOtest.



RISTOtest: Expected values



Ristocetin is used on Multiplate in 2 concentrations:

RISTOhigh: 0.77 mg/ml final concentration

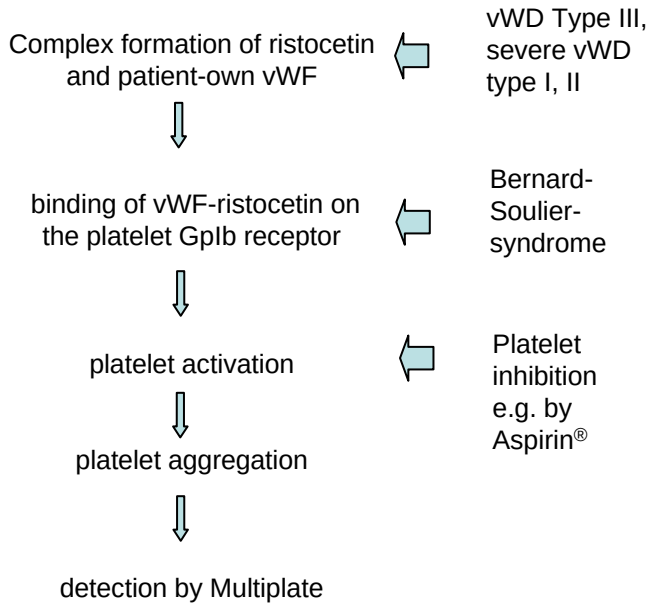
RISTOlow: 0.2 mg /ml final concentration

The concentrations are chosen so that typically a sample will not aggregate – or show only weak aggregation in RISTOtest low and will aggregate well in RISTOtest high.

This allows the detection of samples with **enhanced** tendency for Ristocetin-induced aggregation (especially vWD type IIB) and samples with an **absent or severely reduced response** to Ristocetin (Bernard Soulier-Syndrome, vWD Type III, severe vWD Type I or II).

reference ranges based on the results of healthy blood donors
RISTOhigh: 90-201
RISTOlow: 1-34

Series of events leading to platelet aggregation in Multiplate® using Ristocetin as a trigger:



It is important to keep in mind that the determination of vWF in the RISTOtest assay takes place under low shear stress conditions and the artificial addition of Ristocetin. Other methods are more specific and sensitive for the diagnosis of vWD type I or type II.

So far our experience has shown RISTOhigh to be sensitive for the detection of Bernard-Soulier-syndrome and RISTOlow to be sensitive for the determination of vWD Type IIB. The clinical value of Multiplate in the assessment of vWD when used in combination to other techniques is being investigated.

Ensuring correct test performance using the Multiplate®

Read the package inserts and documentation supplied by the manufacturer.

Ensure correct preanalytics: Avoid foam formation during blood collection. Mix blood carefully.

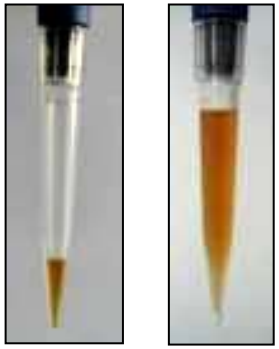
Ensure good reagent handling: Aliquot the reagents. Store the reagents at 4-8°C when not in use (NB: ASPItest has to be frozen for a stability > 24h).

Follow exactly the operating procedures of the tests.

Prewarm the saline solution.

Perform regularly quality controls by testing healthy subjects without the ingestion of platelet function inhibitors. You can run positive controls by adding platelet function inhibitors in vitro (available from the manufacturer).

When using the electronic pipette on the Multiplate: visually control the action of the pipette.



Control unexpected results and borderline results (i.e. samples that are between normal and abnormal or between response and non-response.