Laboratory Diagnosis of Platelet Disorders

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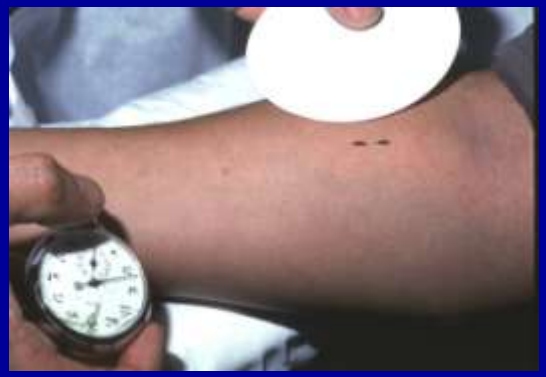
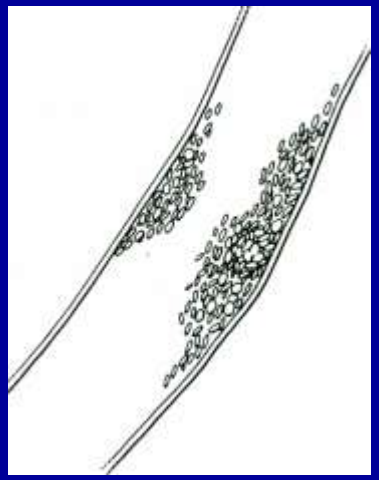




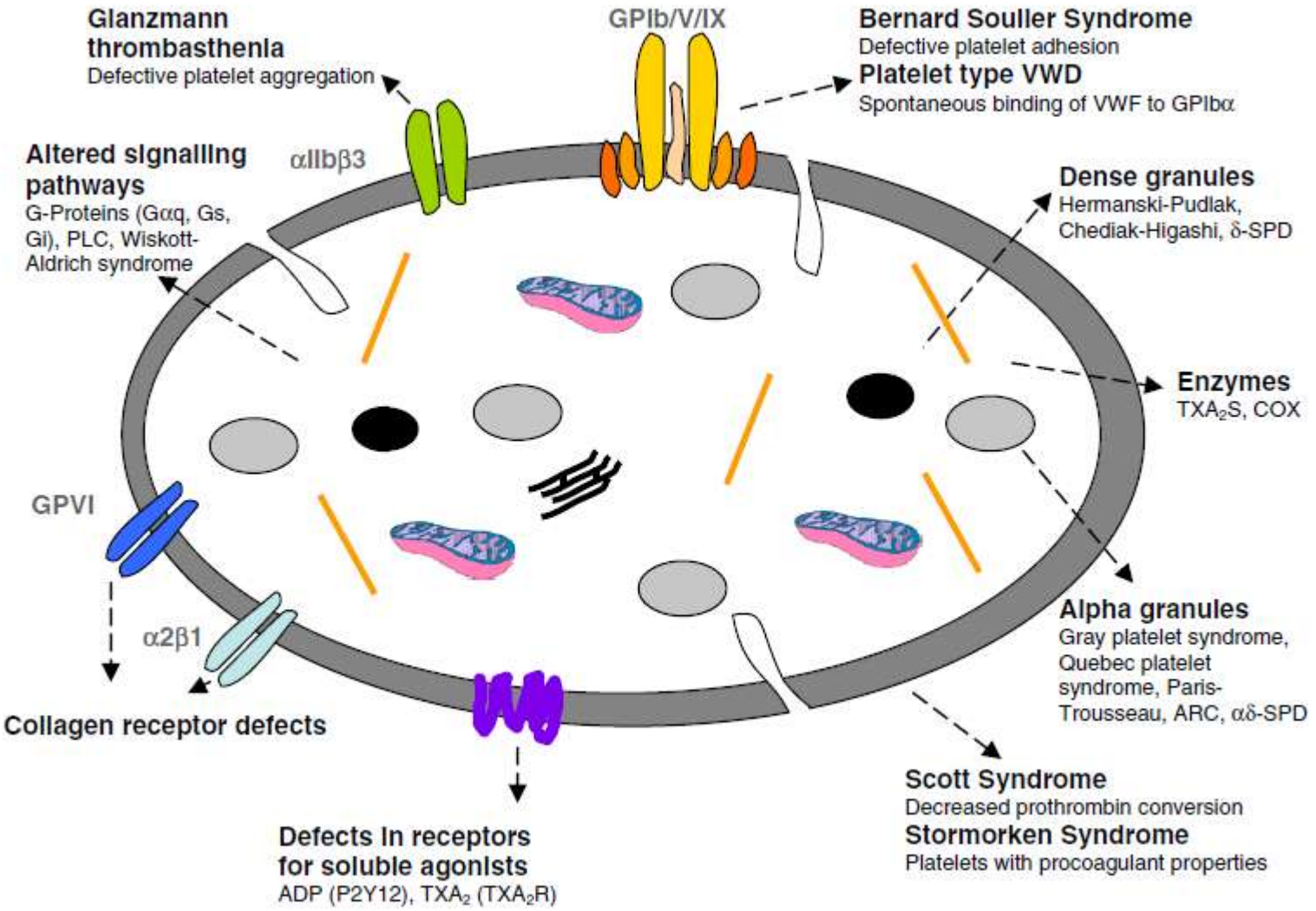
ECAT Participants Meeting 2010

Leiden, The Netherlands

12th November,

14:00-14:30

The Diversity of Platelet Defects



Salles II, Feys HB, Iserbyt BF, De Meyer SF, Vanhoorelbeke K, Deckmyn H
Inherited traits affecting platelet function.
Blood Rev 2008; 22, 155–172

Table I. A suggested classification of the heritable platelet disorders.

	OMIM number*	Site of gene defect†	Estimated no. cases UK	Estimated no. cases Worldwide
Disorders of platelet number				
Severe disorders of platelet function				
Wiskott–Aldrich syndrome	302000	*WAS*	<100	<1000
Glanzmann thrombasthenia	273800	*ITGA2B, ITGB3*	<100	<1000
Bernard–Soulier syndrome	231200	*GP1BA, GPIBB, GP9*	<100	<1000
Disorders of receptors and signal transduction				
Platelet cyclo-oxygenase deficiency	605735	Unknown	<10	<100
Thromboxane synthase deficiency	274180	Unknown	<10	<100
Thromboxane A2 receptor defect	188070	*TBXA2R*	<10	<100
ADP receptor defect ($P2Y_{12}$)	600515	*P2RY12*	<10	<100
Disorders of the platelet granules				
Idiopathic dense-granule disorder (δ-storage pool disease)	185050	Unknown	<100	<1000
Hermansky–Pudlak syndrome	203300	*HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, HPS8*	<100	>1000
Chediak–Higashi syndrome	214500	*LYST*	<100	<1000
Grey platelet syndrome	139090	Unknown	<10	<100
Paris–Trousseau/Jacobsen syndrome	188025 and 147791	*11q23 deletion (FLI1)*	<10	<100
Idiopathic α- and dense-granule storage pool disease	185050	Unknown	<100	<1000
Disorders of phospholipid exposure				
Scott syndrome	262890	*ABCA1*	<10	<10

PHB Bolton-Maggs, EA Chalmers, PW Collins, P Harrison, S Kitchen, R Liesner, A Minford, AD Mumford, LA Parapia, DJ Perry, SP Watson, JT Wilde, MD Williams (2006)
A review of inherited platelet disorders with guidelines for their management on behalf of the UKHCDO
British Journal of Haematology 135 (5), 603–633.

J Clin Pathol 1988;**41**:1322–1330

Laboratory techniques

Guidelines on platelet function testing

THE BRITISH SOCIETY FOR HAEMATOLOGY
BCSH HAEMOSTASIS AND THROMBOSIS TASK FORCE

Following a questionnaire from the Haemostasis and Thrombosis Task Force of the British Society for Haematology in 1985, there was obviously considerable variability and confusion as to how haematologists in the United Kingdom investigated platelet function. This document outlines a standardised approach which could be followed by most routine laboratories for the investigation of bleeding disorders. Platelet release studies are included for interest; these are not recommended for routine laboratories. Platelet function studies used specifically to investigate thrombotic disorders or an assumed hypercoaguable state will not be discussed in these guidelines. Tests which are primarily of a research nature or are in use only at a highly specialised referral unit are also not discussed. It is essential to have a working knowledge of platelet physiology so that the relevant platelet function tests can be performed in an orderly sequence and interpreted correctly.[1,2]. For this reason we have supplied some basic details on platelet biochemistry and structure.

The bleeding time

BACKGROUND

When investigating patients suspected of having a bleeding disorder, it is essential to obtain a detailed clinical history before embarking on tests of haemostatic function.

Prepared in 1988 on behalf of the BSCH Haemostasis and Thrombosis Task Force of the British Society for Haematology in collaboration with the UK Haemophilia Reference Centre Directors

These guidelines were prepared by SJ Machin and E Preston in consultation with the members of the Task Force.
During the preparation of this report the membership of the Task Force was:
Dr J F Davidson (Chairman)
Dr B T Colvin (Secretary)
Members
Dr T W Barrowcliffe
Dr D W Dawson
Dr S J Machin
Dr L Poller
Professor F E Preston
Dr B Roberts

The UK Haemophilia Reference Centre Directors were also consulted about this document and comments were received from Professor A L Bloom, Dr C Rizza, and Dr G F Savidge.

Accepted for publication 19 May 1988

The peripheral platelet count, blood film examination, and the skin bleeding time are the first line basic laboratory tests of platelet function. If these tests are within normal limits it is unlikely that a clinically important platelet defect is responsible for excessive clinical bleeding.

A drug history is particularly important, and as far as possible the use of drugs should be avoided when platelet function is assessed. This applies particularly to patients with congenital platelet disorders. In acquired bleeding states the drugs that patients are receiving may themselves be directly responsible for the haemostatic defect. When this is suspected platelet function should be assessed when the patient is both off and on the drug. Recent aspirin ingestion is of particular importance as a single dose may exert its effect for up to 10 days. Other drugs which affect the bleeding time include non-steroidal anti-inflammatory agents, ticlopidine, heparin, penicillin (in high doses) and the antibiotics carbenicillin and ticarcillin (table 1).

The bleeding time is arguably the most useful test of platelet function in that it provides clinically relevant information about the contribution of platelets to primary haemostasis.

How long it takes for bleeding to stop after a skin incision is largely influenced by rapid accumulation of metabolically active platelets at the site of the wound and the formation of a haemostatically effective platelet plug. The bleeding time reflects this process and if it is performed in a standardised manner is sensitive to changes in platelet function and platelet number.

Several attempts have been made to improve the sensitivity and reproducibility of the bleeding time since its introduction in 1910 by Duke. In the original method the ear lobe was punctured by a needle. Later, between 1935–41, Ivy described a method which consisted of three puncture wounds in the forearm. Some improvement in sensitivity was achieved by the application of a sphygmomanometer cuff, inflated to

Why do we need new guidelines?

- Last guideline published in 1988, few other guidelines
- Many new tests available now e.g. flow cytometry and PFA-100 etc
- BT shown to be a poor screening test
- Our knowledge of platelet biology and disorders has increased
- Newer agonists available e.g. TRAP peptides , CRP
- Recent Surveys – CAP, ISTH and UKNEQAS have all demonstrated that platelet function testing and LTA are all poorly standardised

New Guidelines

1) CLSI

LTA, WBA & PFA-100 – now published

2) ISTH platelet physiology SSC

LTA – completed at Cairo SSC, 2010 –

to be published

3) New BCSH guidelines

Platelet Function Testing – In Prep


ENGLAND
WE ARE ENGLAND

GERMANY 4 ENGLAND 1 (2?)

VIDEO TECHNOLOGY CONFIRMS ENGLAND ARE VERY BAD AT FOOTBALL

ENGLAND were knocked out of the World Cup today after state-of-the-art video technology showed the ball crossing their goal line many, many times.

Diagnostic Approach?

1) Clinical Evaluation

2) Primary Screening Tests

Full Blood Count, Blood Film, Bleeding Time, PFA-100, Clotting screen, VWF panel

3) Secondary Screening Tests

LTA, WBA, Stored and Released Nucleotides

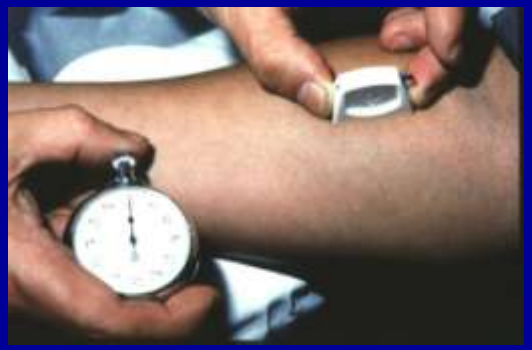
4) Specialised Tests

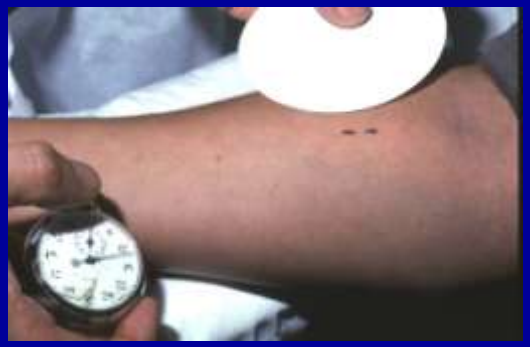
Flow Cytometry, Electron Microscopy, Molecular Biology

5) Research & New Tests

e.g. Real Time Thrombus Formation

Bleeding Time





Normal Range 2 - 10 minutes

Bleeding Time is prolonged in classical platelet disorders, thrombocytopenia, severe anaemia, acquired disorders, and by some antiplatelet drugs.

Great Variability in results

Unreliable test, invasive, insensitive, unpopular with patients

Not suitable for repeat or consecutive analysis

Now Less popular – recent UKNEQAS survey Revealed many centres still use the BT

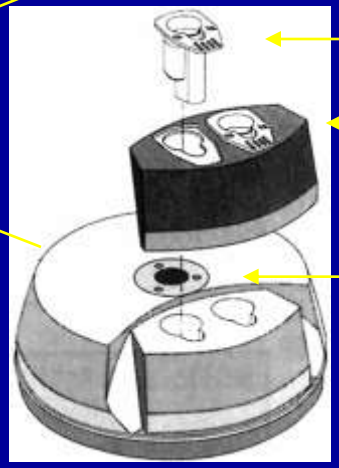
PFA-100® Platelet Function Analyzer

Built-In Printer

LCD screen

Soft keys

Trigger solution container



Test Cartridge

Cassette

Carousel

Journal of Thrombosis and Haemostasis, 4: 312–319

REVIEW ARTICLE

Platelet function analyzer (PFA)-100® closure time in the evaluation of platelet disorders and platelet function

C. P. M. HAYWARD,*† P. HARRISON,‡ M. CATTANEO,§ T. L. ORTEL¶ and A. K. RAO**†† ON BEHALF OF THE PLATELET PHYSIOLOGY SUBCOMMITTEE OF THE SCIENTIFIC AND STANDARDIZATION COMMITTEE OF THE INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS

*Chair, Working Group on the PFA-100®, ISTH-SSC Platelet Physiology Subcommittee; †McMaster University and the Hamilton Regional Laboratory Medicine Program, Hamilton, ON, Canada; ‡Oxford Haemophilia Centre and Thrombosis Unit, Churchill Hospital, Oxford, UK; §Unità di Ematologia e Trombosi, Ospedale San Paolo, DMCO, Università di Milano, Milan, Italy; ¶Duke University Medical Center, Durham, NC, USA; **Chairperson, ISTH-SSC Platelet Physiology Subcommittee, 2001–2004; and ††Sol Sherry Thrombosis Research Center, Temple University School of Medicine, Philadelphia, PA, USA

To cite this article: Hayward CPM, Harrison P, Cattaneo M, Ortel TL, Rao AK on behalf of the Platelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Platelet function analyzer (PFA)-100® closure time in the evaluation of platelet disorders and platelet function. *J Thromb Haemost* 2006; **4**: 312–9.

Summary. *Background:* Closure time (CT), measured by platelet function analyzer (PFA-100®) device, is now available to the clinical laboratory as a possible alternative or supplement to the bleeding time test. *Aim:* On behalf of the Platelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH-SSC), a working Group was formed to review and make recommendations on the use of the PFA-100 CT in the evaluation of platelet function within the clinical laboratory. *Methods:* The Medline database was searched to review the published information on the PFA-100 CT in the evaluation of platelet disorders and platelet function. This information, and expert opinion, was used to prepare a report and generate consensus recommendations. *Results:* Although the PFA-100 CT is abnormal in some forms of platelet disorders, the test does not have sufficient sensitivity or specificity to be used as a screening tool for platelet disorders. A role of the PFA-100 CT in therapeutic monitoring of platelet function remains to be established. *Conclusions:* The PFA-100 closure time should be considered optional in the evaluation of platelet disorders and function, and its use in therapeutic monitoring of platelet function is currently best restricted to research studies and prospective clinical trials.

Introduction

Closure time (CT), measured by platelet function analyzer (PFA-100®) device, is now available to the clinical laboratory as a possible alternative or supplement to the bleeding time [1–4]. On behalf of the Platelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH-SSC), this document reviews recent literature and provides consensus recommendations on using PFA-100 CT in the evaluation of platelet function within the clinical laboratory.

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Methodology

A working group of the Platelet Physiology Subcommittee of the ISTH-SSC on the PFA-100 CT was established to review information on the test and to make recommendations on the use of the PFA-100 CT in the evaluation of platelet function by clinical laboratories. Relevant articles were identified by searching the MEDLINE database for English papers on the PFA-100 CT, published before June 2005. Members of the working group reviewed and summarized the published literature, and provided expert opinions to establish concensus recommendations.

Results and discussion

Principles of the CT measured by the PFA-100 device

The PFA-100 CT was introduced to provide a simple, rapid assessment of high shear-dependent platelet function by a procedure that uses small amounts of citrated blood (0.8 mL/cartridge; maximal CT results: 300 s) [1,2,4,5]. Blood samples are aspirated at high shear rates (5000–6000 s^{-1}) through a capillary in the instrument cartridge and encounter a membrane coated with collagen and epinephrine (CEPI) or collagen and ADP (CADP) [1]. The membrane triggers platelet adhesion, activation and aggregate formation, leading to occlusion of the 150 μm central aperture and cessation of blood flow [1]. Results are reported as the CT in seconds for the

~300 papers published post Hayward et al.

Optional Screening Test for Platelet Disorders

What can the PFA-100 detect or do?

Hayward et al, ISTH SSC document, PFA-100 Closure Time in the Evaluation of Platelet Disorders and Platelet Function. 2006, JTH, 2006, 4, 312-319

- Improvement on the BT
- Good sensitivity for VWD (III, IIA & IIB) , GT and BS
- Variable sensitivity for Type I VWD (related to VWF level in plasma and platelets) – overall – 80-90%
- CEPI more sensitive than CADP for SPD and RD but overall sensitivity only 50%
- CEPI but not CADP detects ASA – can be bypassed as non specific
- Cannot detect Clopidogrel and related compounds on CADP/CEPI –

new INNOVANCE PFA P2Y cartridge now available

Screening Tests – BCSH Recommendations (in Prep)

- A full blood count is recommended on all samples
- Samples with abnormalities in platelet distribution and count should be checked on a blood film
- The BT is not recommended anymore
- The PFA-100 provides an optional screening test but test results should be interpreted with caution in conjunction with the clinical background as the test is not diagnostic or sensitive for mild platelet disorders and abnormal results can be frequent
- Consider performing Prothrombin Consumption Index

Light Transmission Aggregometry (LTA)

- Invented by Born in 1960's
- Regarded as the 'Gold Standard'
- Useful for diagnosis of a wide range of platelet defects
- Labour Intensive - reagent and sample preparation
- Non Physiological - PRP and Low Shear
- Poorly standardised – range of agonists and concentrations.

Recent Surveys - (UKNEQAS, CAP and ISTH Platelet Physiology SSC)

- Mainly used within specialized laboratories
- Increasing number of agonists available e.g. TRAP peptides, CRP etc
- Increasingly recognised that PRP dilution with PPP introduces artifacts

Platelet Agonists used for LTA

Standard/Minimal Panel of agonists

- ADP (range 0.5 - 10 μM)
- Collagen (range 1 - 5 μg/ml),
- Epinephrine (range 0.5 - 10 μM)
- Arachidonic Acid (Single dose 0.5 -1.6 mM)
- Ristocetin – Low Dose (< 0.6 mg/ml) and High Dose (0.8-1.5 mg/ml)

Extended Panel of agonists

- Thrombin – α/GPRP or χ
- TRAP peptides (PAR-1 – SFLLRN or PAR-4- AYPGKF)
- Calcium ionophore
- U46619 – thromboxane analogue
- CRP – Collagen Related Peptide
- Serotonin, PAF and others

Typical Patterns of Aggregation Responses to Various Inherited and Acquired Platelet Defects

Disorder	ADP		AA	Epi	Collagen	Ristocetin
	Primary	Secondary				
VWD	N	N	N	N	N	A
Glanzmann thrombasthenia	A	A	A	A	A	N
Bernard Soulier syndrome	N	N	N	N	N	A
SPD	N	A	N	N, A	N, A	N
Secretion Defects	N	A	N,A	N,A	N,A	N
Scott syndrome	N	N	N	N	N	N
Aspirin	N	A	A	A	N, A	N
P2Y12 inhibitors	A	A	N	N	N	N
GpIIb/IIIa antagonists	A	A	A	A	A	N

N= Normal, A = Abnormal

Insensitive to storage and release defects at standard agonist concentrations

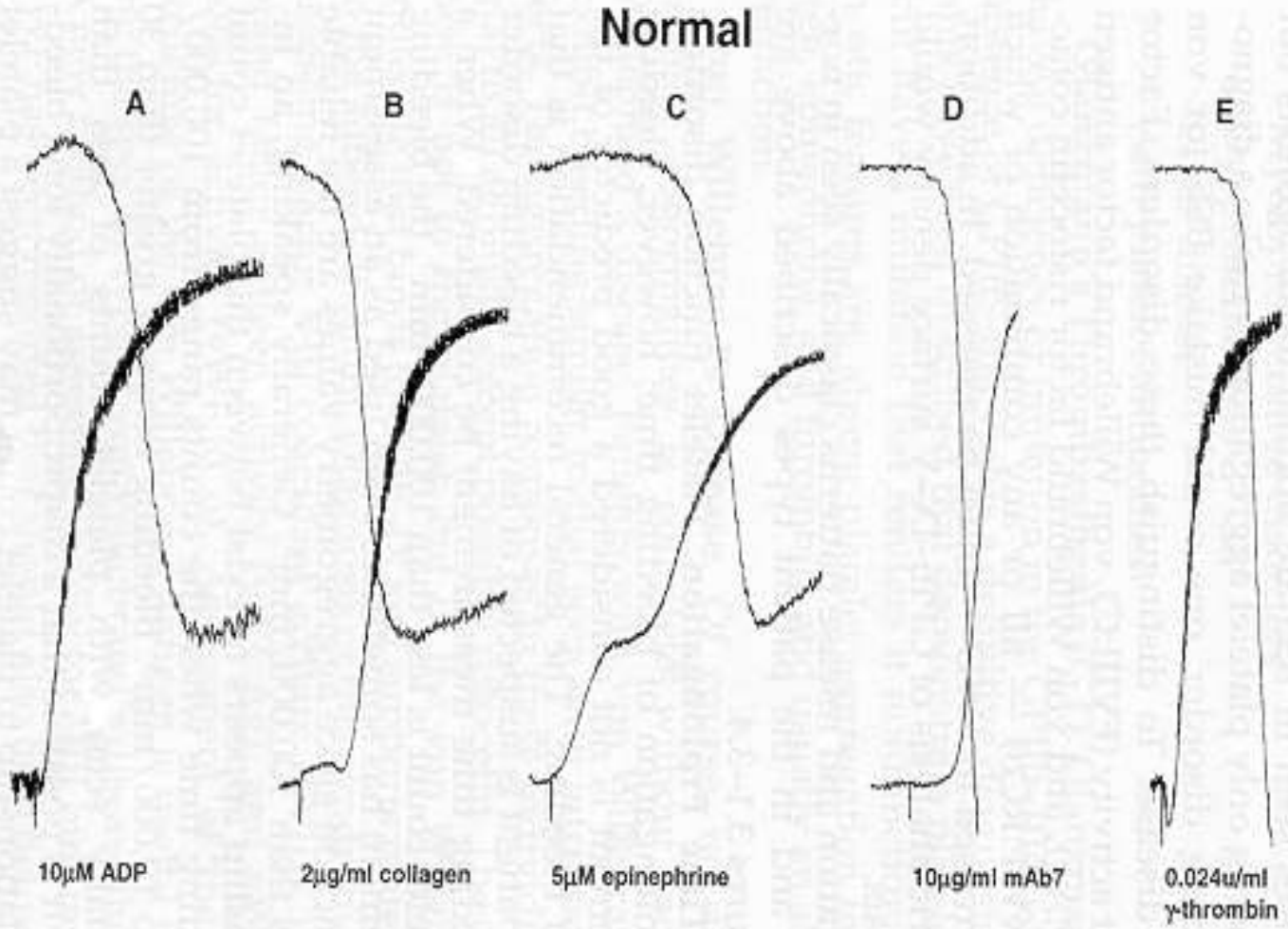


Figure 3.1. Aggregation and release tracings from a normal donor reflecting responses to typical agonists used in the laboratory. Deflection in baseline tracing indicates point at which agonist is added. Aggregation response is lower tracing; release response is upper tracing. Note classic biphasic response of epinephrine. ADP second wave is masked by strong response to exogenous agonist. Lumi-responses to both mAb7 and γ-thrombin are off the scale.

Storage Pool Disease

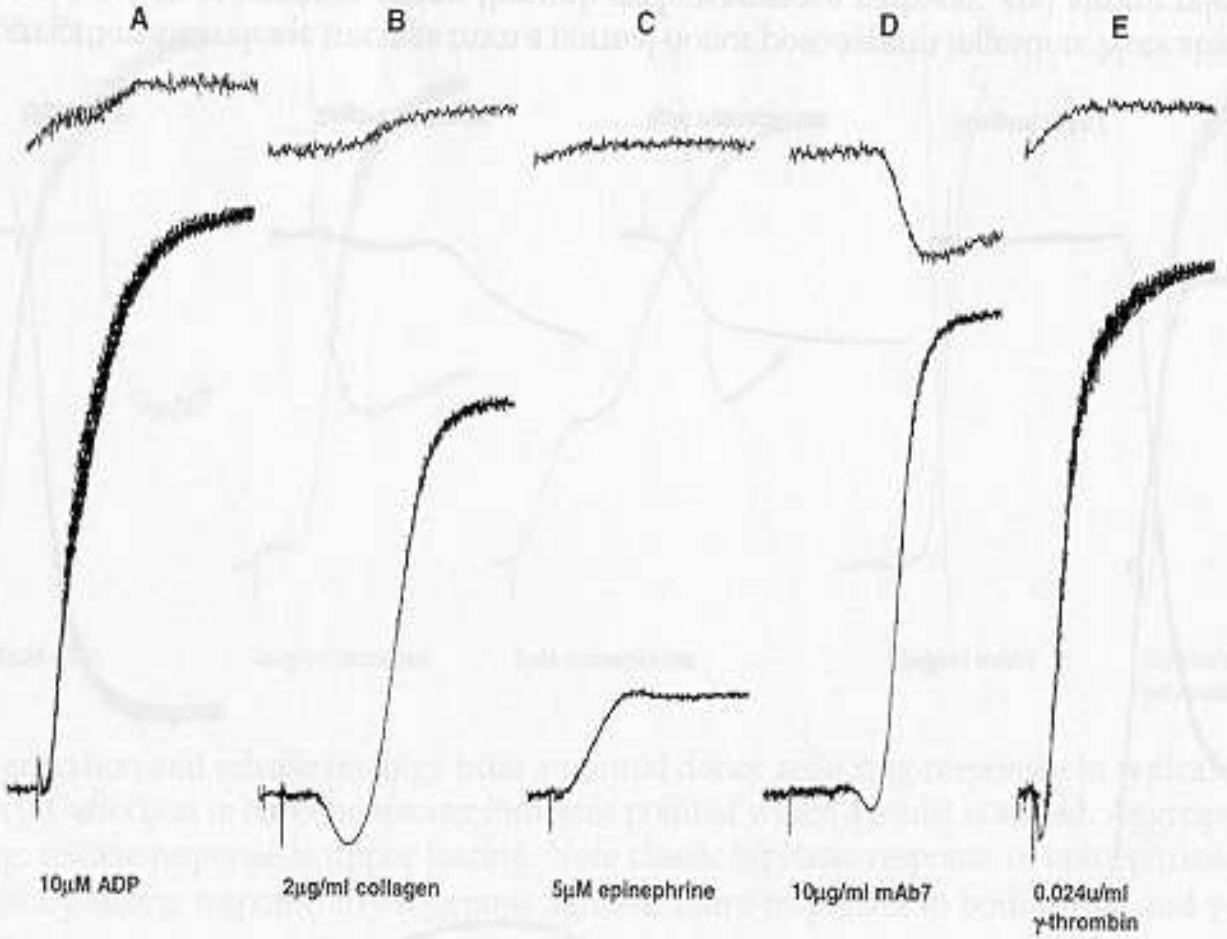


Figure 3.3. Aggregation and release tracings from a patient with storage pool disease (SPD). Note the relatively high response to ADP in the absence of release; this is due to the high concentration of ADP used that can mask release defects and SPD. The collagen response is high as well, indicating the potent aggregating power of this amount of collagen. Characteristic of SPD is the absent or significantly diminished release response.

ISTH – SSC METHODOLOGY - 1

- Clinical guidelines or recommendations are produced, based on the evidence emerging from the medical literature
- When the medical literature search does not provide definitive answers (due to absence of evidence, low quality and/or contradictory evidence), alternative methods are needed

ISTH - SSC METHODOLOGY - 2

- The RAND method – developed in the ’80s – is intended to obtain a formal consensus among expert groups about the appropriateness of health care interventions, particularly when scientific evidence is absent, scarce and/or heterogeneous

Brook RH, Chassin MR, Fink A, et al. A method for the detailed assessment of the appropriateness of medical technologies. *Int J Technol Assess Health Care 1986;2:53-63*

ISTH-SSC Working Party on Platelet Aggregation
Experts who accepted to participate in the consensus using the RAND method

1. M. Cattaneo, Milano, Italy
2. C.P.M. Hayward, Hamilton, Ont., Canada
3. P. Harrison, Oxford, UK
4. D. Kenny, Dublin, Ireland
5. A.D. Michelson, Worcester, MA, USA
6. D. Nugent, Orange, CA, USA
7. S. Watson, Birmingham, UK
8. C. Cerletti, Campobasso, Italy
9. P. Nurden, Pessac, France
10. A.K. Rao, Philadelphia, PA, USA
11. A.H. Schmaier, Cleveland, OH, USA *(Member, Standardization of Platelet Function Testing, Clinical and Laboratory Standards Institute)*

ISTH SSC – LTA consensus document

Pre-analytical Considerations :-

Blood samples for LTA should be collected from subjects who:

- refrain from smoking for at least 30 minutes
- abstain from caffeine for at least 2 hours
- rest for a short period

- A record of all drugs that the subject has taken in the week prior to testing should be collected

- Treatment with drugs known to reversibly inhibit platelet function (e.g. NSAIDs) should be stopped at least 3 days before sampling

Pre-analytical Considerations (2) :-

- Treatment with drugs known to irreversibly inhibit platelet function (e.g. aspirin, thienopyridines) should be stopped at least 10 days before sampling

- When treatment with drugs that inhibit platelet function cannot be stopped before sampling, drug-induced effects on platelet function should be considered when interpreting the LTA results

- It is uncertain whether blood samples for LTA should be collected from fasting patients, and whether treatment with any drug should be stopped before sampling

ISTH SSC – LTA consensus document

Blood Sampling :-

- Blood samples for LTA should be drawn:
- with minimal or no venostasis
- using a needle of at least 21 gauge
- into plastic (polypropylene) or siliconized glass tubes
- into 109 or 129 mM sodium citrate, buffered anticoagulant
- The first 3-4 ml of blood drawn should be discarded or used for tests other than LTA
- When difficulties are encountered in obtaining sufficient blood for LTA, underfilled tubes may only be used to exclude severe platelet function disorders, such as Glanzmann Thrombasthenia or Bernard-Soulier Syndrome

ISTH SSC – LTA consensus document

Blood samples should be allowed to "rest" at room temperature for 15 min before centrifugation

Preparation of PRP for LTA:

- should be prepared by centrifuging blood samples at 200 x g for 10 min, at ambient temperature (approximately 21°C), without using a brake

- should be prepared by blood sedimentation for samples with very large platelets (it is uncertain whether it is advisable to keep the tubes at 45°)

Preparation of PPP for LTA: PPP should be prepared by centrifuging whole blood, or the tubes of blood from which PRP was removed, at ambient temperature, at 1500 x g for 15 min

Grossly hemolyzed samples should be discarded
If the sample tested is lipemic, the final report should indicate this

It is necessary to check the platelet count of the PRP sample tested

ISTH SSC – LTA consensus document

The results of LTA studies could be inaccurate when the platelet count in the PRP samples is lower than 150 x 10^9/L, therefore, caution should be taken when interpreting abnormal results in samples with low platelet counts

PRP with low platelet counts may be tested to exclude severe platelet function disorders (BSS, type 2B and platelet type von Willebrand disease)

Platelet count of PRP samples should NOT be adjusted to a standardized value with autologous PPP (uncertain for PRP samples with platelet counts > 600 x 10^9/L)

LTA studies must include a known normal subject, run in parallel with the subject(s) under study

ISTH SSC – LTA consensus document

After centrifugation, PRP samples should be allowed to sit at room temperature for 15 min before testing

PRP should be used to set 0% light transmission in the aggregometer

Autologous PPP should be used to set 100% light transmission in the aggregometer

LTA studies should be performed at 37°C

During LTA testing, PRP samples should be constantly stirred at 1,000 rpm using a disposable stirrer, unless otherwise specified by the manufacturer of the aggregometer

ISTH SSC – LTA consensus document

Before adding an agonist, baseline tracings for LTA should be observed for oscillations and stability for at least 1 minute

The volume of agonist added for LTA should be consistent, and never more than 10% of the total sample volume

Platelet aggregation should be monitored for:

- a minimum of 3 minutes after adding an agonist

- a minimum of 5 minutes after adding an agonist that does not cause maximal aggregation by 3 minutes with most control samples

- a minimum of 10 minutes after adding an agonist that does not cause maximal aggregation by 5 minutes with most control samples

LTA studies should be completed within a maximum of 4 hours after blood sampling

ISTH SSC – LTA consensus document

The following platelet agonists should be used for diagnostic LTA studies:

ADP: 2 µM

Epinephrine: 5 µM

Collagen: 2 µg/mL (Horm collagen)

Thrombin Receptor Activating Peptide (TRAP): 10 µM

The **thromboxane A2 mimetic U46619:** 1 µM

Arachidonic acid: 1 mM

} If any of these are abnormal then repeat with higher doses

Ristocetin: 1.2 mg/mL

In case platelet agglutination is normal, testing should be repeated using **Ristocetin 0.5-0.7mg/mL**

In case platelet agglutination is absent, testing should be repeated using **Ristocetin 2 mg/mL**.

ISTH SSC – LTA consensus document

The platelet aggregation tracing should be evaluated based on:

- presence of shape change
- length of the lag phase
- slope of aggregation
- maximal amplitude or % aggregation
- amplitude or % aggregation at the end of the observation
- disaggregation
- visual examination of the aggregation tracings

The presence of a "secondary wave" induced by epinephrine should be evaluated

Studies completed more than 4 hours after blood collection should be reported with a comment of this

Clinical laboratories must establish an appropriate reference interval and validate test performance with each lot of reagents

New ISTH SSC WP on ***Diagnosis of Congenital Platelet Function Disorders***

- What patients should be screened for platelet function disorders?
  - Type of bleeding manifestations
  - Usefulness of bleeding scores?
  - Criteria orienting towards an inherited defect
  - Presence of associated alterations in other cells/organs
  - Any role for global tests of primary hemostasis?
  - Do we need to rule out other bleeding disorders (e.g., VWD) before studying platelet function?
  - Drug history
- What first-line screening tests should we use?
  - Should platelet secretion be measured in parallel with platelet aggregation in all patients?
- What second-line, confirmatory tests should we use to test the diagnostic hypothesis that was raised based on the results of the first-line screening tests?
- Proposal of a diagnostic algorithm

Conclusions

- LTA remains the "gold standard" for diagnosing platelet disorders
- Since the late 1980's, many alternative tests have been developed
- Some are now widely used in clinical practice
  e.g. Flow cytometry, PFA-100 and Multiplate
- Quality assurance increasingly recognised
- New platelet function testing and LTA guidelines becoming available
- This should lead to improvements in inter-laboratory practice and the diagnosis of platelet disorders

Acknowledgements

CLSI

ISTH platelet physiology SSC (Chairman – Marco Cattaneo)

BCSH

David Keeling
Kampta Sukhu
James Beavis

Oxford Haemophilia & Thrombosis Centre

Multiplate analyzer

multiple
platelet
function
Analyzer

Or MEA

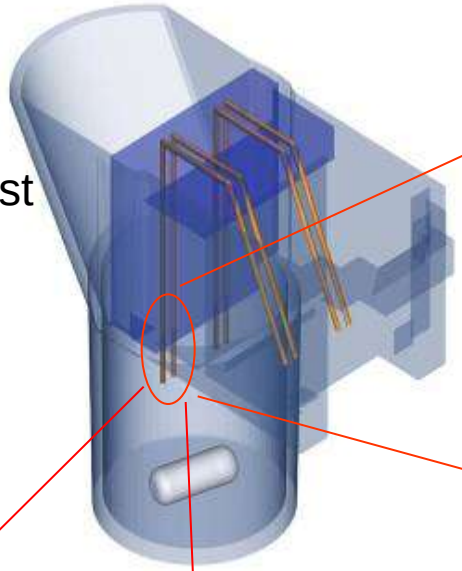
Multiple
Electrode
Aggregometry

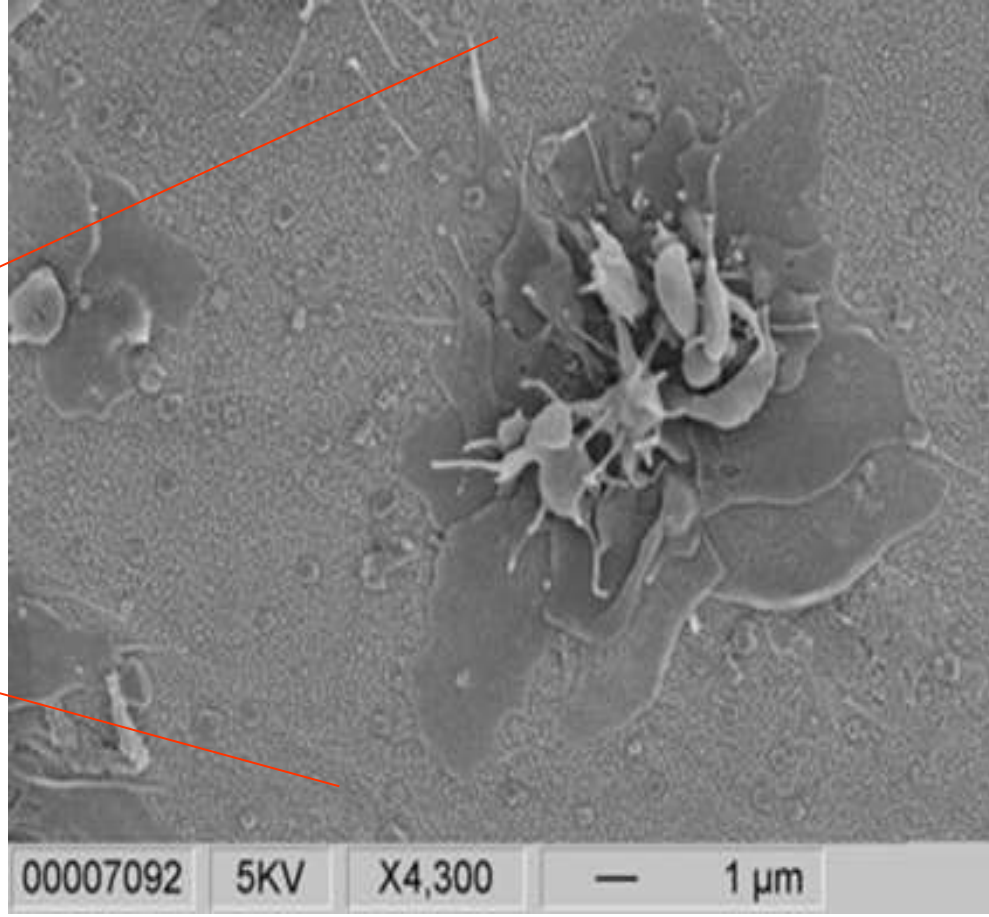
- Compact (10“ x 15“ x 4“)
- 5 independent channels
- Integrated computer
- Windows XP based software
- Automatic analysis and documentation
- Electronic pipetting available

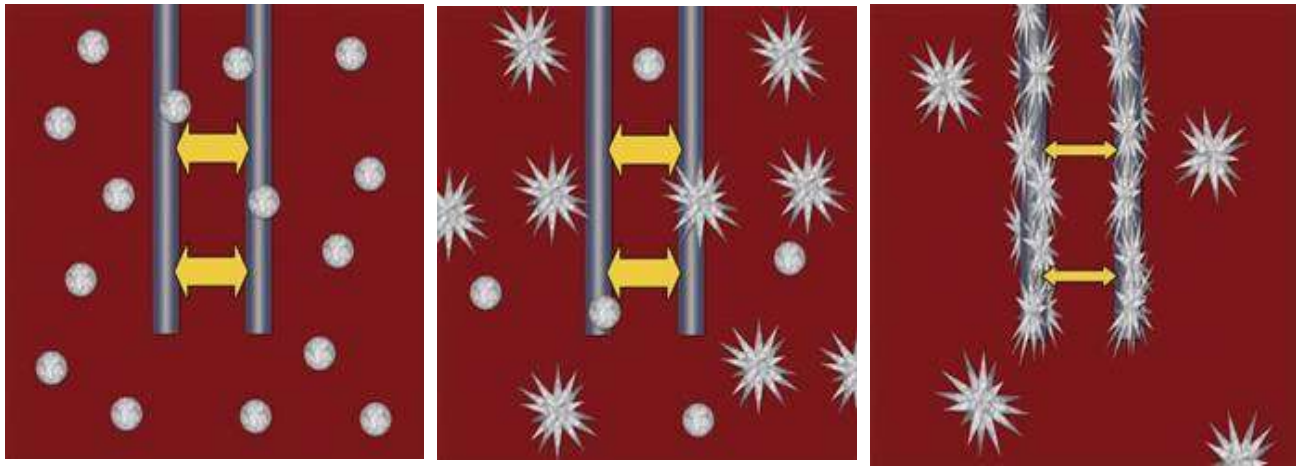
principle of Multiplate analysis

single use test cell with twin impedance sensor

sample volume 0.3 ml/ test

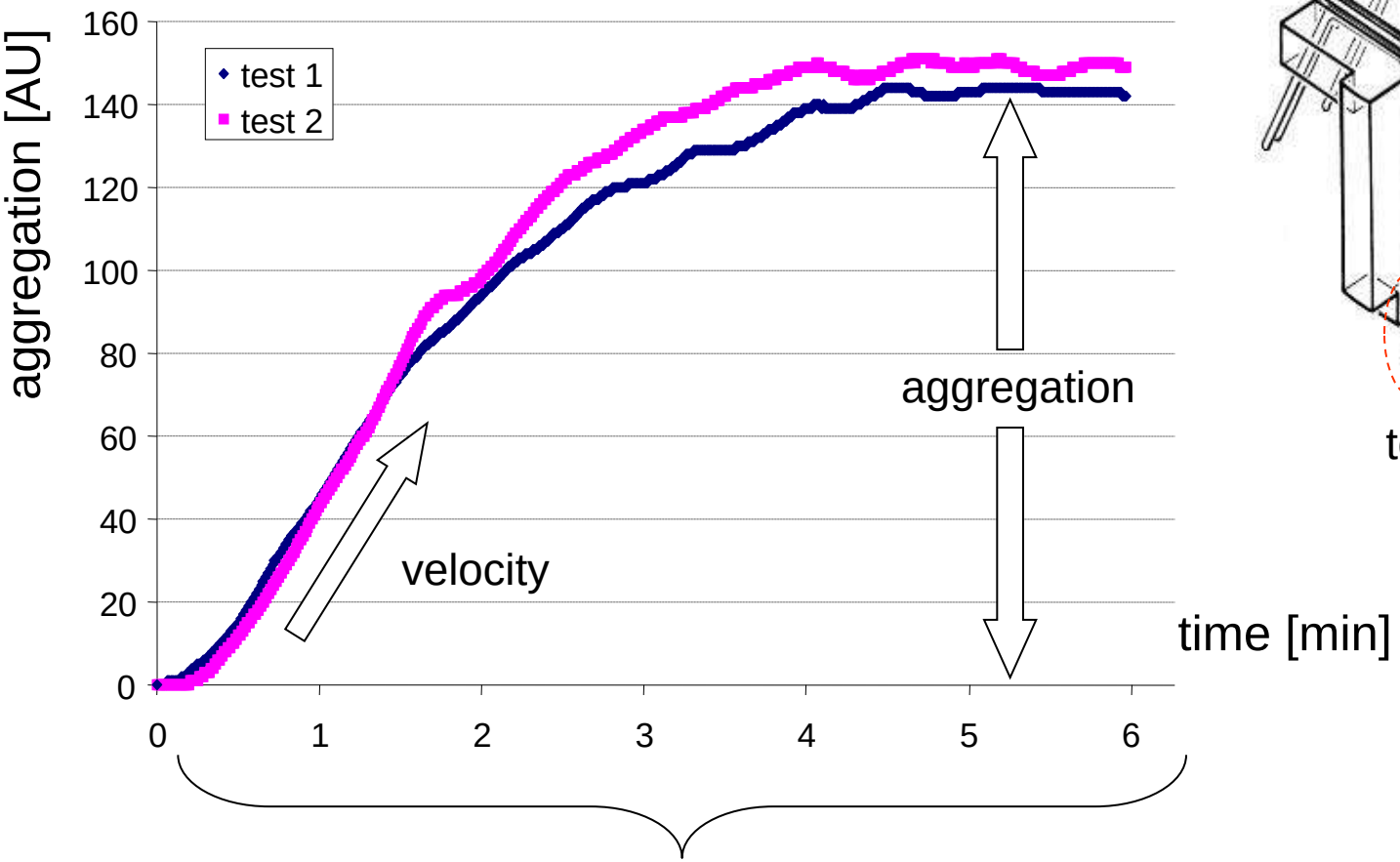


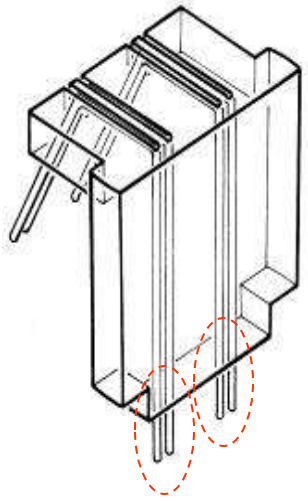




firm adhesion and aggregation of platelets on the sensor surface enhances the electrical resistance between the 2 sensor wires

Multiplate parameters



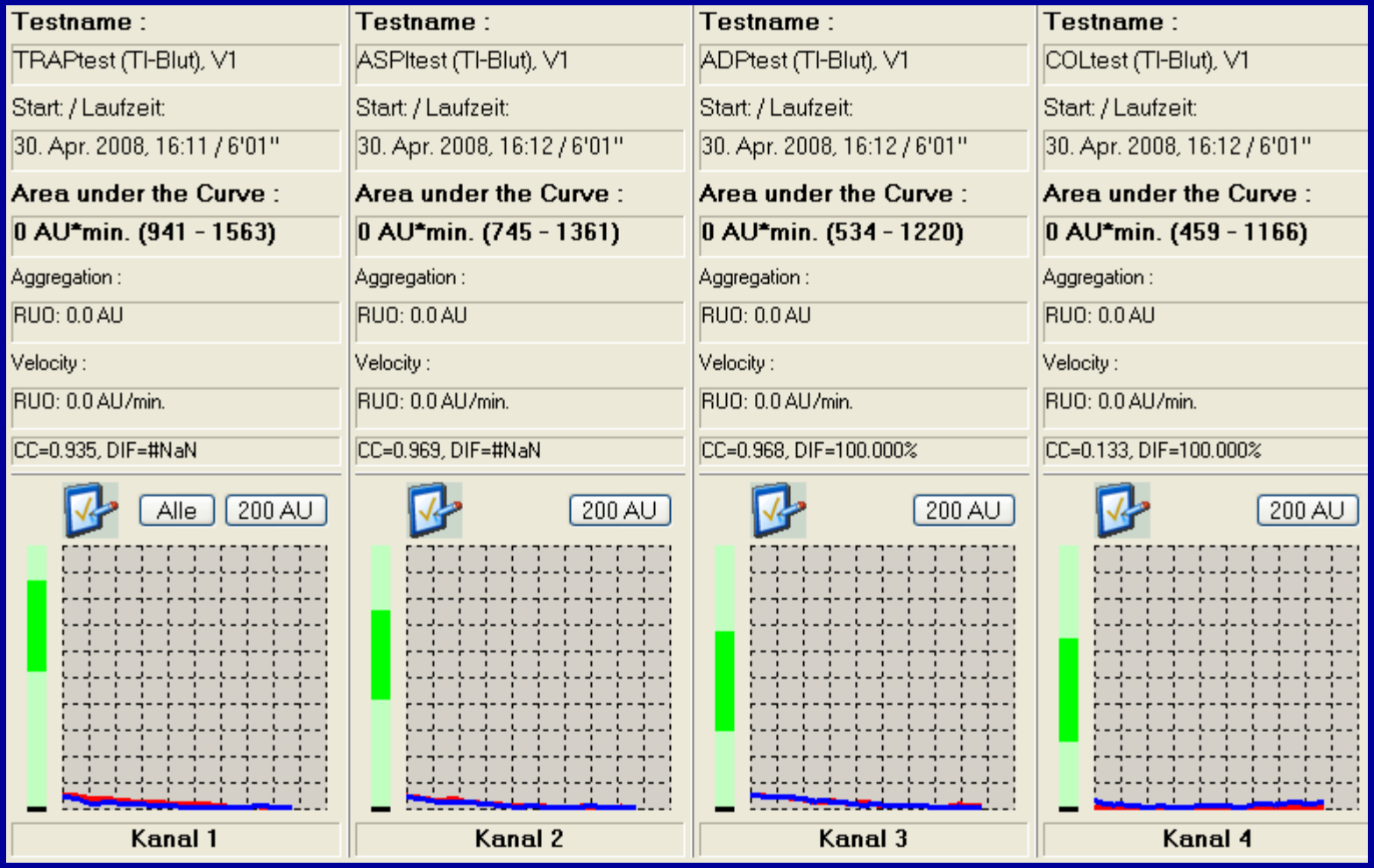


test 1+2

Area under the curve = AUC

- *most important parameter*
- *expressed in AU*min or U (10 AU*min = 1 U)*

C.H. *1965

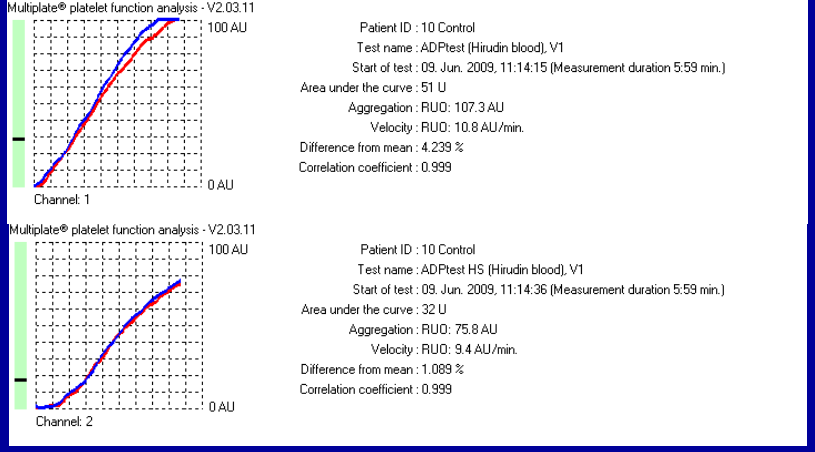


→ absent aggregation in all tests due to Glanzmann Thrombasthenia

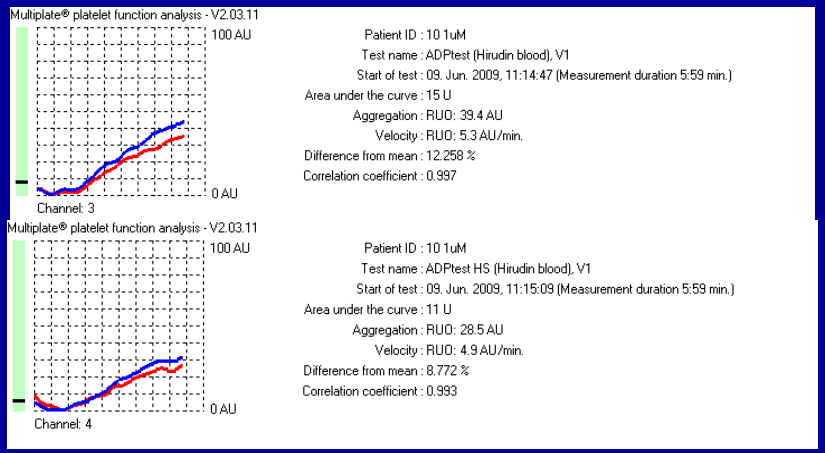
Detection of

$P2Y_{12}$ inhibition

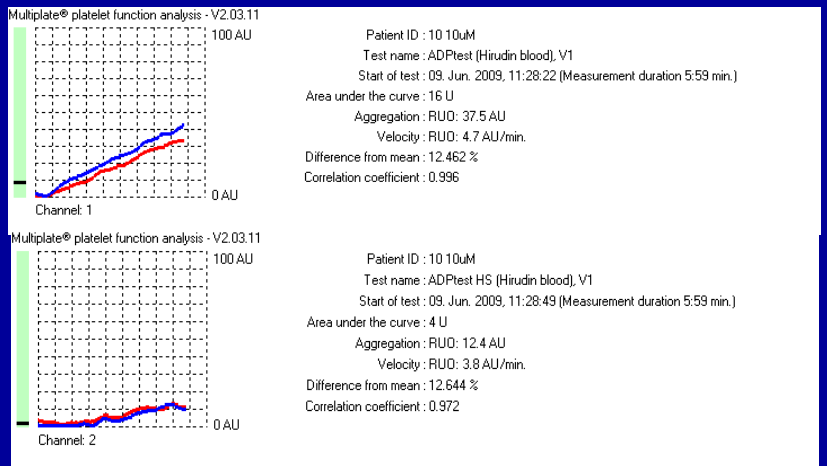
(Edwards et al, unpublished data)



Control (DMSO)



1 μM R138727



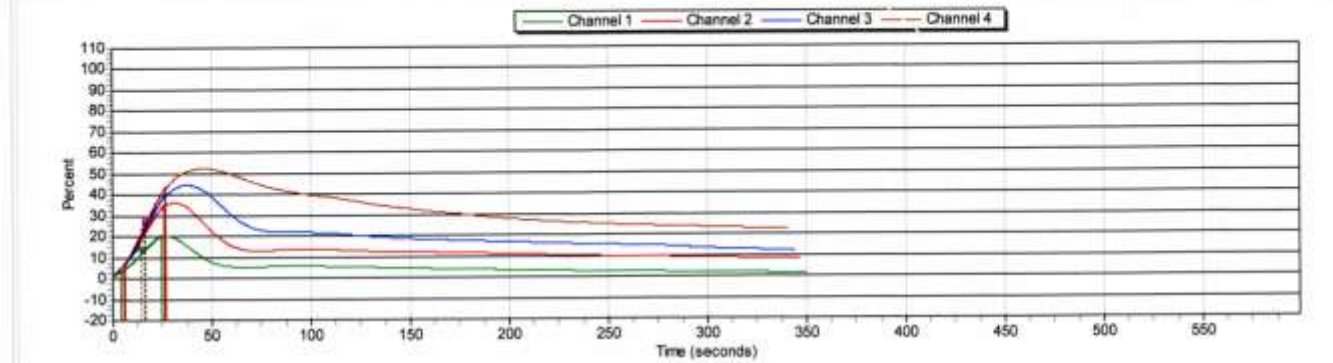
10 μM R138727

Detection of $P2Y_{12}$ Defect in a patient with a lifelong bleeding history?

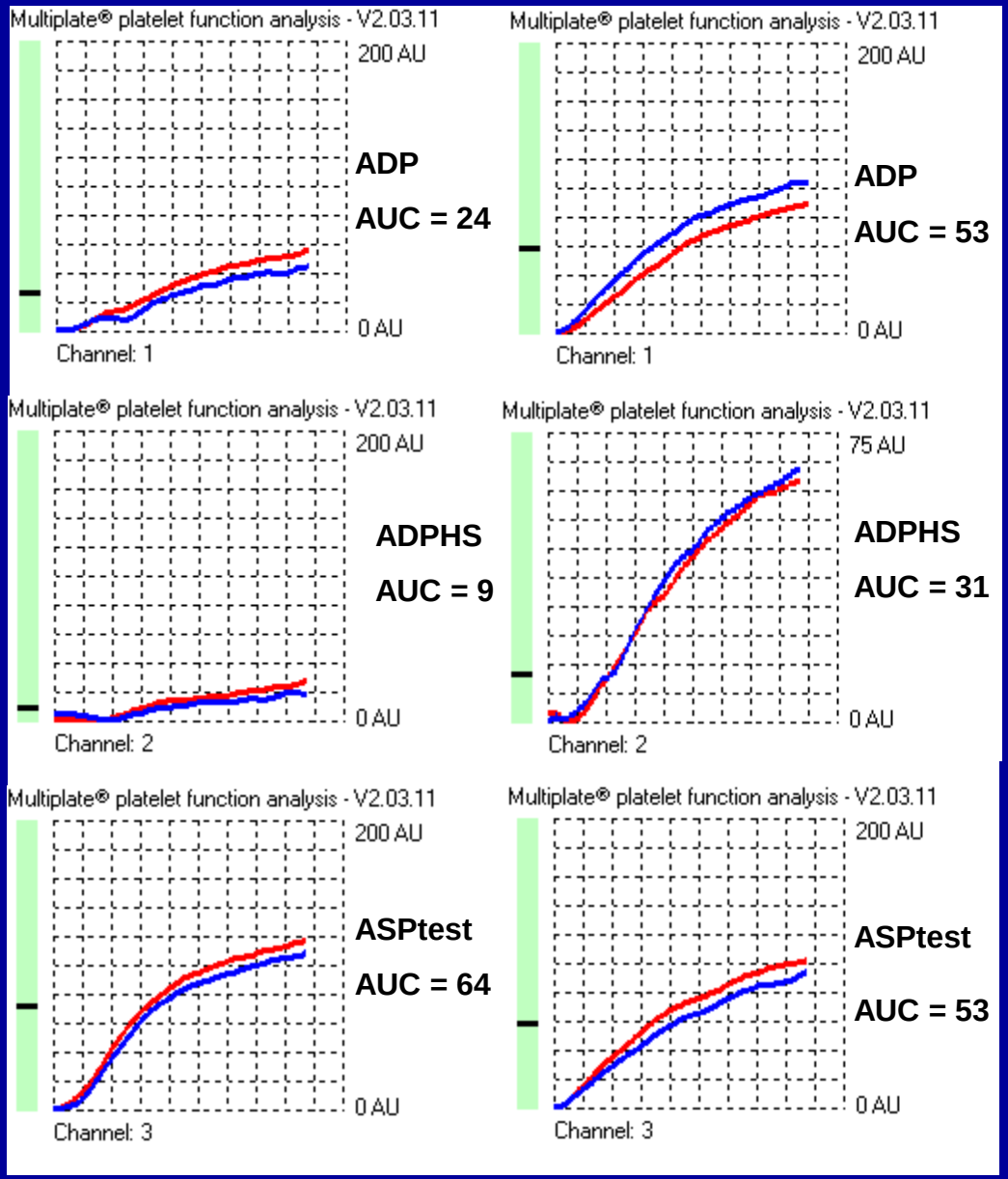
Absence of Secondary Aggregation to all doses of ADP by LTA

Chnl	Reag	Lot No.	Reag. Conc.	Units	PPP	PRP	Date and Time
1	Adenosine Diphosphate		2.5	uM	0.076	1.075	11/03/2010 12:33:51
2	Adenosine Diphosphate		5	uM	0.05	1.014	11/03/2010 12:33:57
3	Adenosine Diphosphate		10	uM	0.035	1.051	11/03/2010 12:34:04
4	Adenosine Diphosphate		20	uM	0.07	1.069	11/03/2010 12:34:11

Chnl	Max %	TMax	Slope	Slope2	Lag	% Area Under Curve	Comment
1	20.2	27	55.1		0		
2	36.1	31	90.5		0		
3	44.7	38	104.4		0		
4	52.5	47	113.4		0		



Detection of $P2Y_{12}$ Defect in a patient with a lifelong bleeding history?



Patient Control

Conclusion

- Multiplate is a platelet function analyzer using whole blood
- platelet function is recorded with a single-use test cell with a double sensor unit
- the device has 5 channels for parallel determinations
- Standardized reagents
- good sensitivity for anti-platelet drugs Aspirin®, Plavix® and ReoPro®
- Does not measure released nucleotides

other applications include:

- the peri-operative assessment of platelet function
- evaluation of platelet disorders
- Clinical experience limited but promising
- Experience growing
- More studies on platelet defects required