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# Platelet Function Measurement

한진영

동아의대 진단검사의학교실



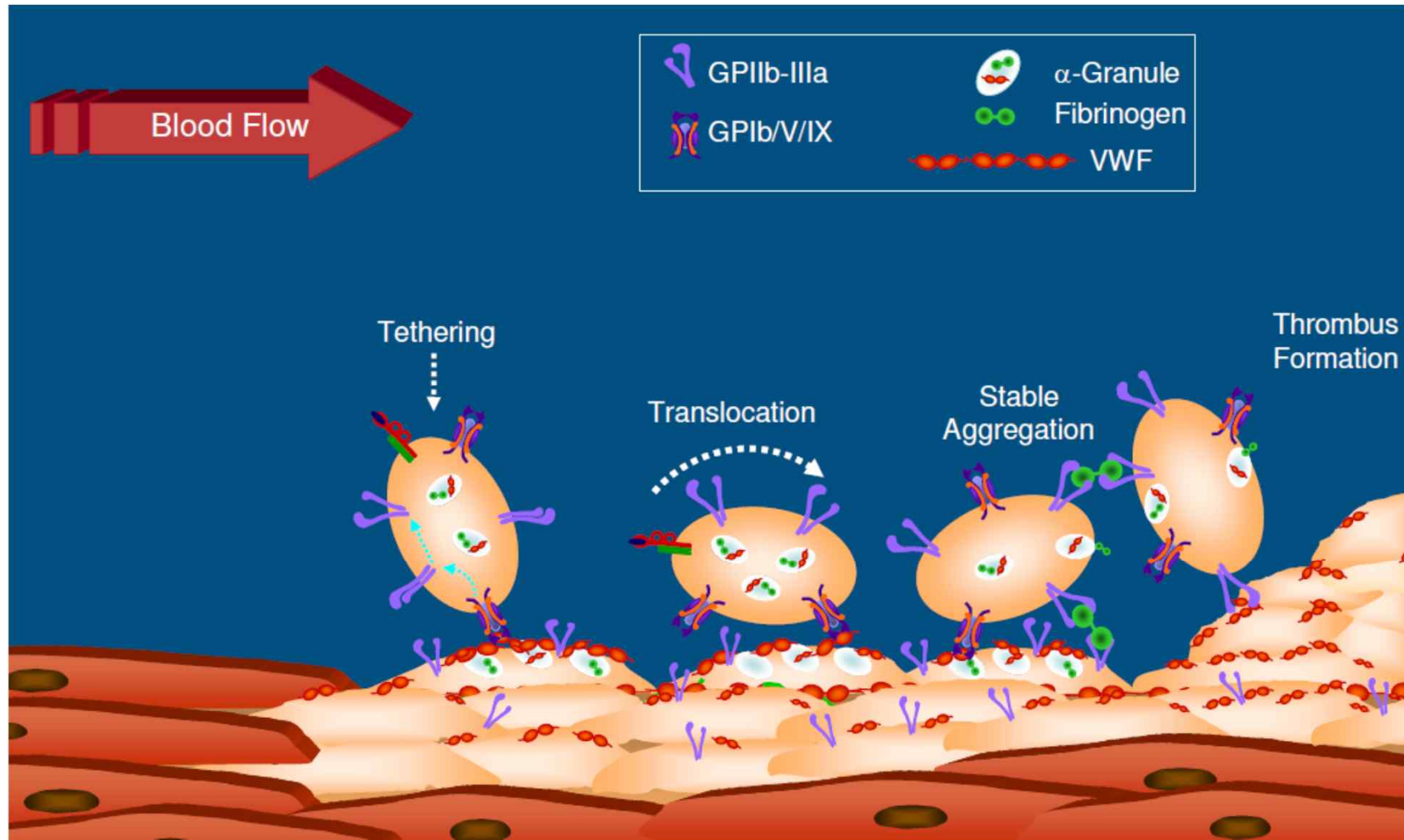
# Contents

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- ① Role of Platelets in Hemostasis
- ② Platelet Function Tests
- ③ Monitoring of Anti-platelet Drugs



# Platelet Adhesion and Aggregation



# Platelet Adhesion and Aggregation

## Platelet Adhesion



## Platelet Aggregation



VWF  
Collagen  
Fibrinogen





# Platelet Function Tests



Platelet aggregometry



Flow cytometry



Accumetrics®

Negative Sample no. 2715308720 Birth 1947/08/20 Ward CI Rack 245 Date 0007/04/02  
 Pat. ID 7844281 Sex Female Dr. Tube time 03:19  
 Name gangyansun Comment

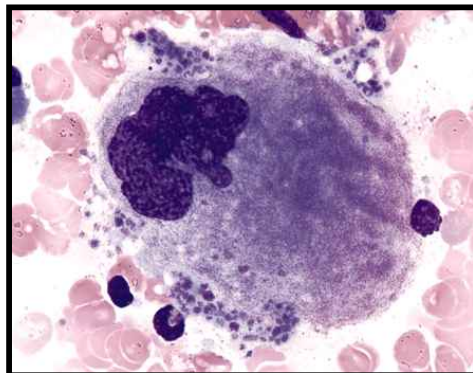
Item	Data	Unit	Item	Data	Unit
WBC	5.88	10 <sup>3</sup> /UL	NEUT#	2.93	10 <sup>3</sup> /UL
RBC	3.81	10 <sup>6</sup> /UL	LYMPH#	2.41	10 <sup>3</sup> /UL
HGB	12.2	g/dL	MONO#	0.39	10 <sup>3</sup> /UL
HCT	36.3	%	EO#	0.13	10 <sup>3</sup> /UL
MCV	95.3	fL	BASO#	0.02	10 <sup>3</sup> /UL
MCH	32.0	pg	NEUT%	49.9	%
MCHC	33.6	g/dL	LYMPH%	41.0	%
PLT	210	10 <sup>3</sup> /UL	MONO%	6.6	%
RDW-SO	45.8	FL	EO%	2.2	%
RDW-CV	13.2	FL	BASO%	0.3	%
PDW	13.1	FL			
MPV	11.1	fL			
P-LCR	33.1	%			
PCT	0.23	%			
RET%					
RET#		10 <sup>6</sup> /UL			
IRF		%			
LFR		%			
HFR		%			
HRBC#		10 <sup>3</sup> /UL			
HRBC%		/100wBC			



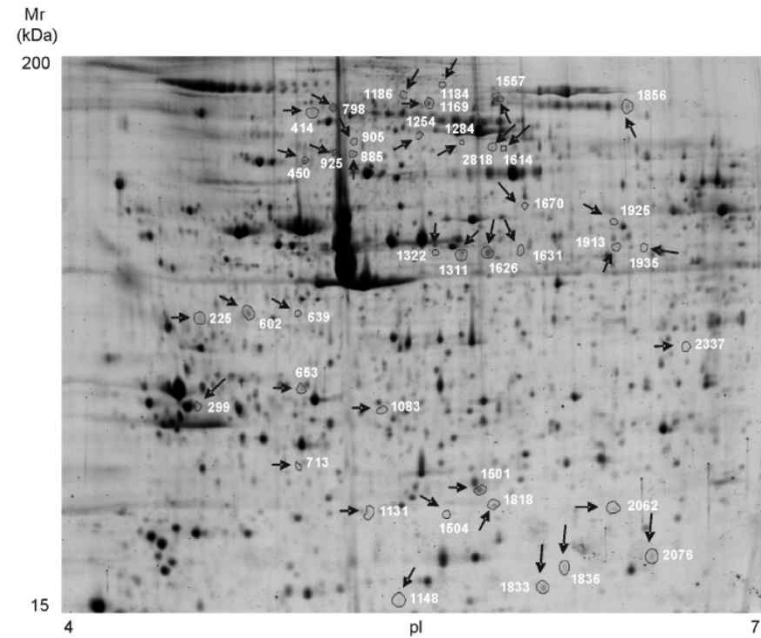
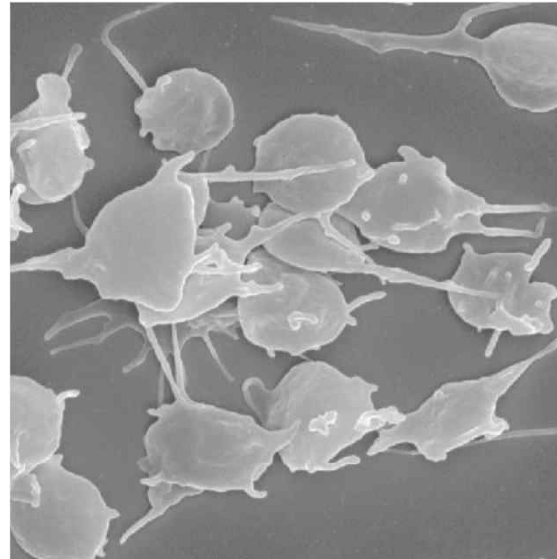
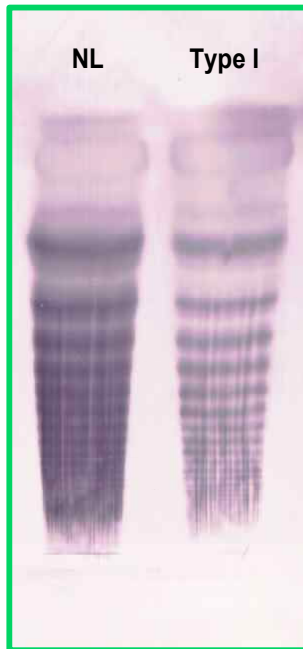
Multiplate® analyzer



PFA-100®

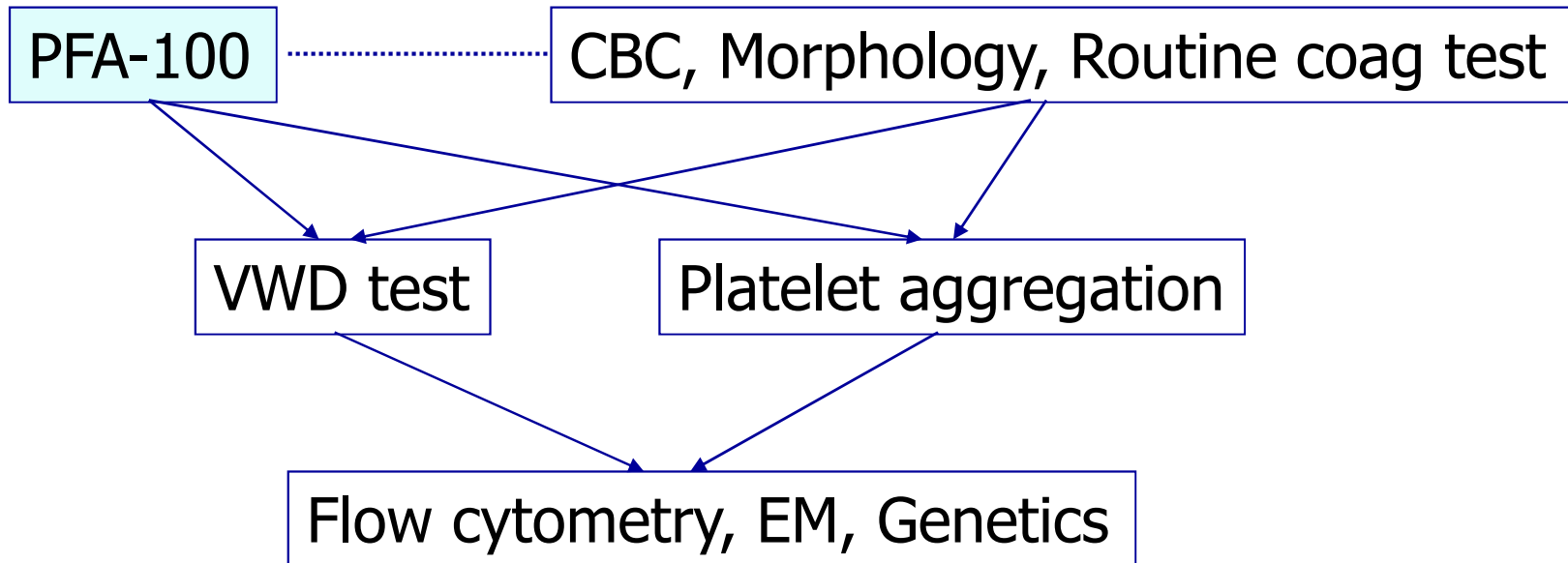


# Platelet Function Tests



# Platelet Function Tests

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# Platelet Function Tests

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## Bleeding Time (BT)

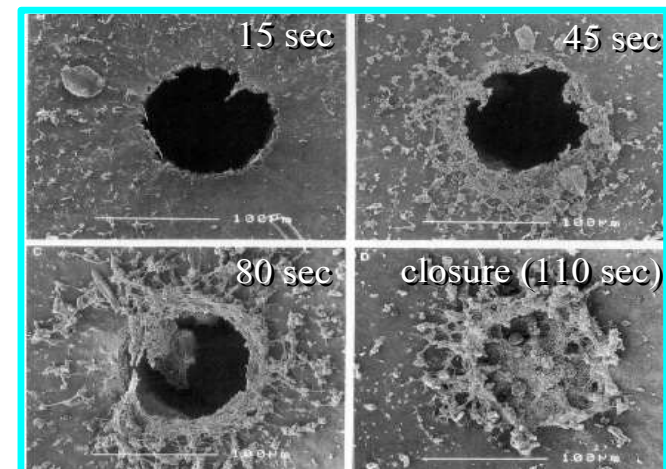
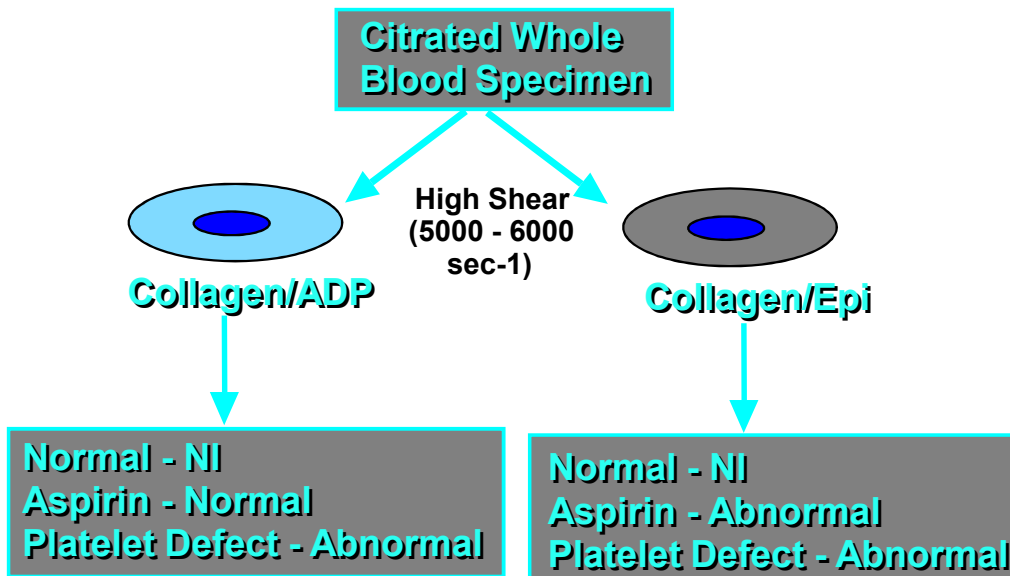


- By Duke (1910) & Ivy (1941)
- In vivo test
- Measure of primary hemostasis
- **Rarely used in developed nations**
- Highly dependent on operator
- Influenced by a variety of variables
- Insensitive to mild disorders
- Now replaced by many alternatives



# Platelet Function Tests

## Platelet Function Analyzer (PFA)-100



- Microprocessor-controlled cartridge system
- Using citrated whole blood
- Designed to measure platelet function

# Platelet Function Tests

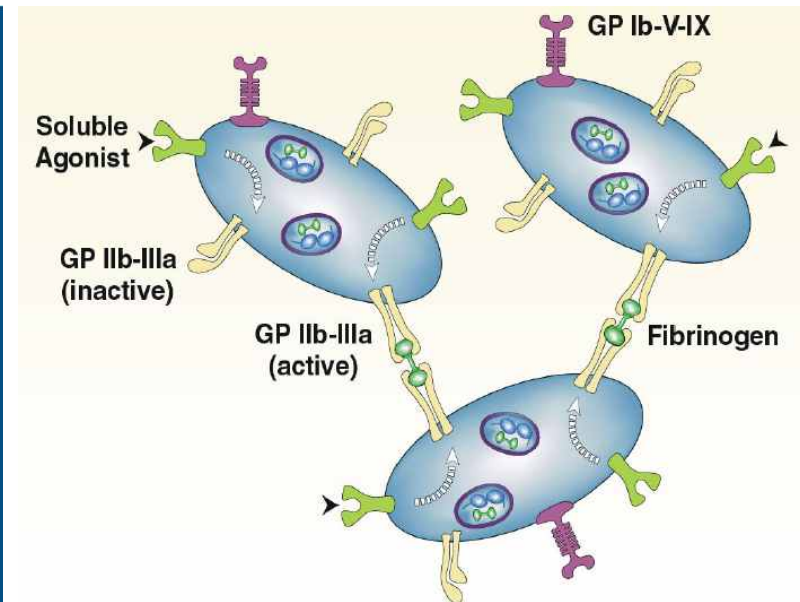
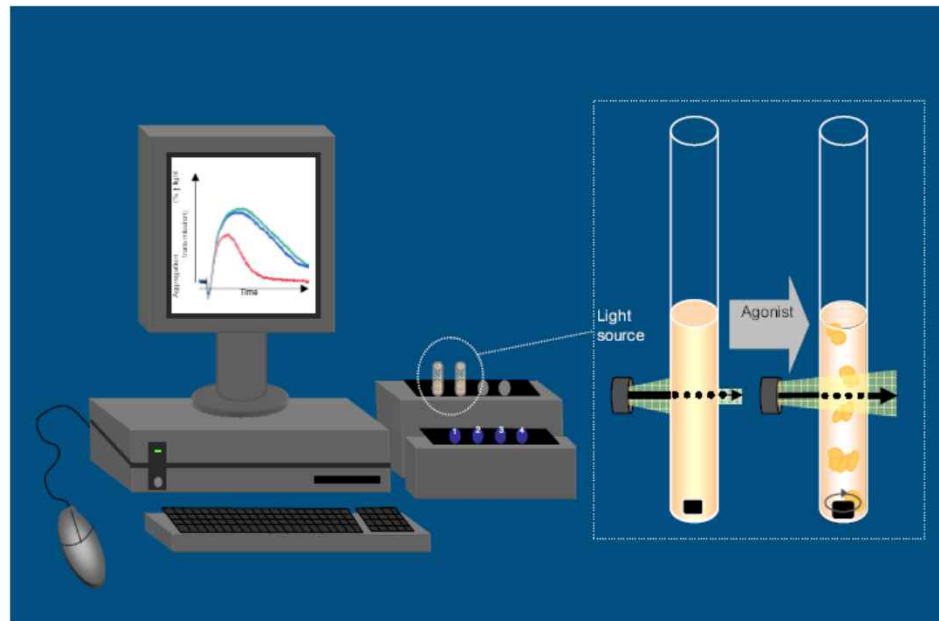
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## Platelet Function Analyzer (PFA)-100

- **Most widely used for global primary hemostasis**
- Very quick and easy to perform, well standardized
- Small volume of venous blood (0.8 mL)
- Low platelet count ( $<100,000/\mu\text{L}$ ) or low Hct ( $<30\%$ ) may cause long closure time (CT)
- Very sensitive, but non-specific screening tool
- Normal PFA-100 results can help exclude some severe platelet dysfunctions (Glanzmann's, Bernard-Soulier)
- Role in therapeutic monitoring remains to be established

# Platelet Function Tests

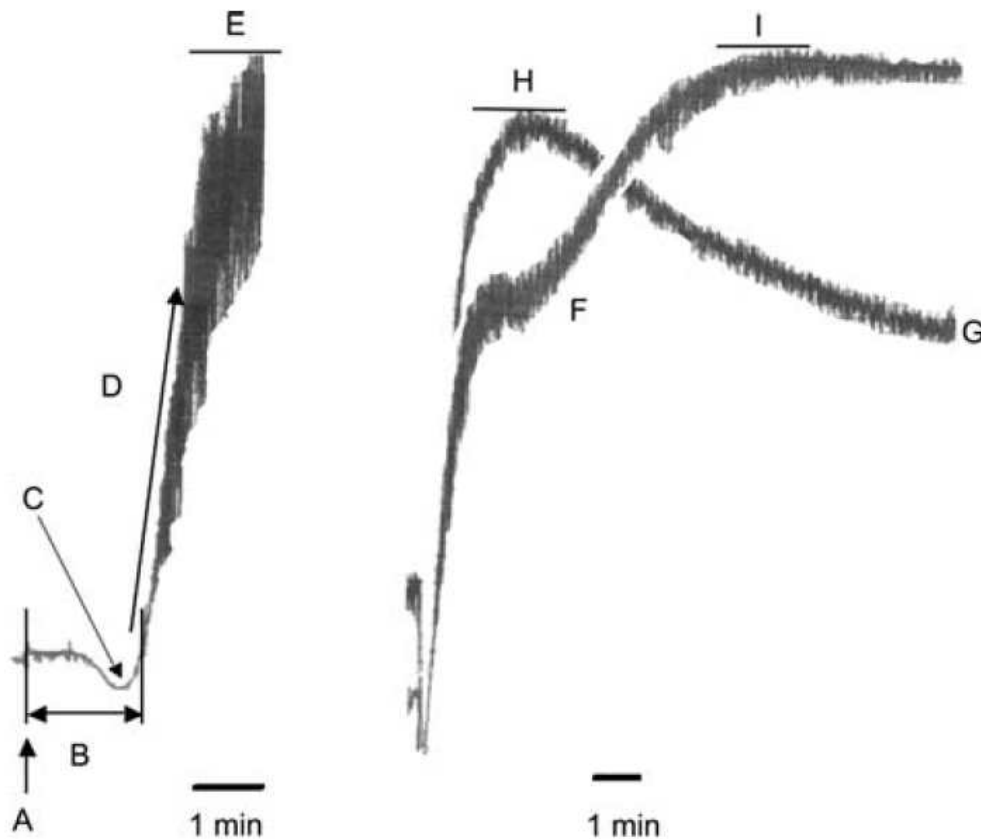
## Platelet Aggregometry



Measure light transmission through a test sample containing platelets in suspension that increases when platelets are aggregated by agonists

# Platelet Function Tests

## Platelet Aggregometry



### Left: Collagen 2 ug/mL

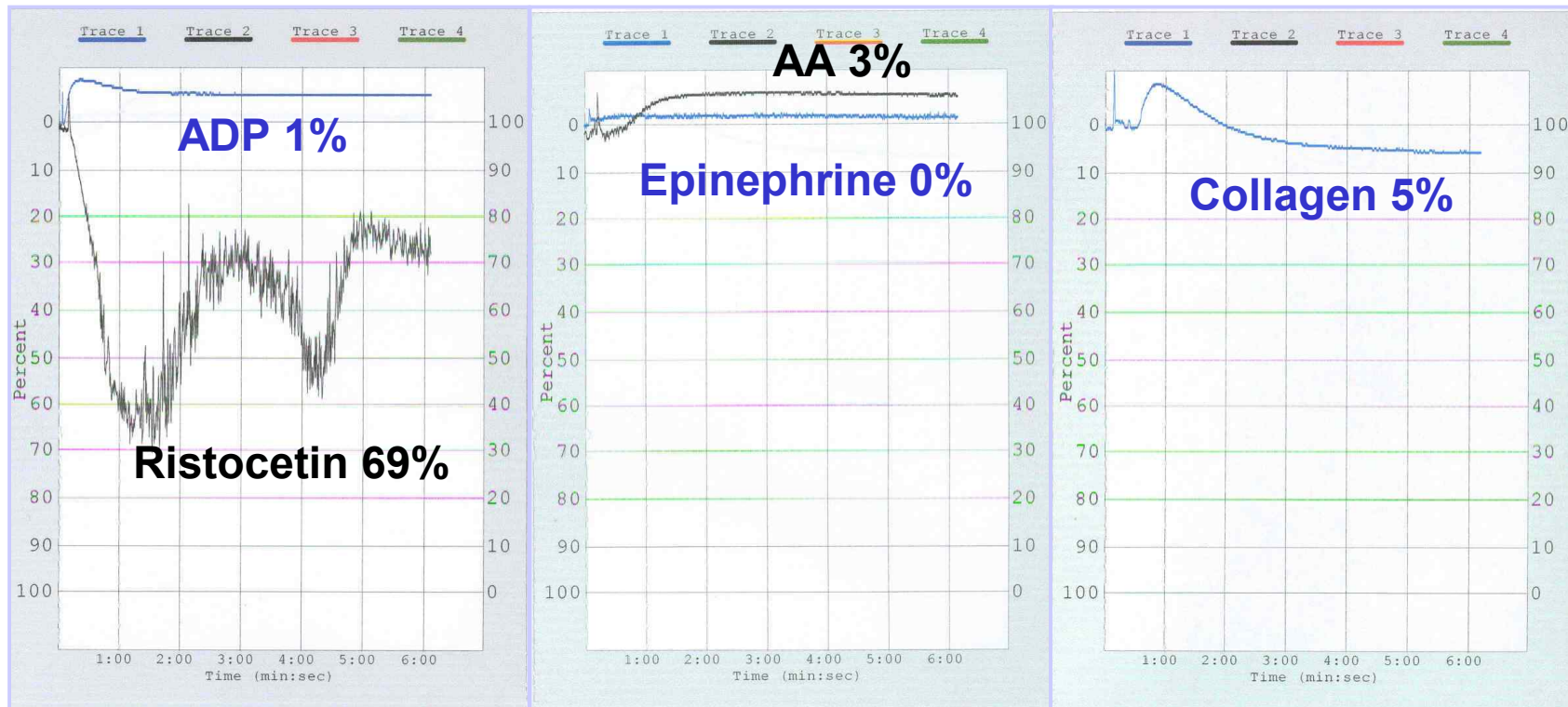
- A: Addition of agonist
- B: Lag phase
- C: 'Shape change'
- D: Slope of aggregation

### Right: ADP 2.5 uM

- F: Secondary response
- I: Maximal aggregation
- H: Maximal aggregation
- G: Only primary aggregation

# Platelet Function Tests

## Platelet Aggregometry



Glanzmann thrombasthenia



# Platelet Function Tests

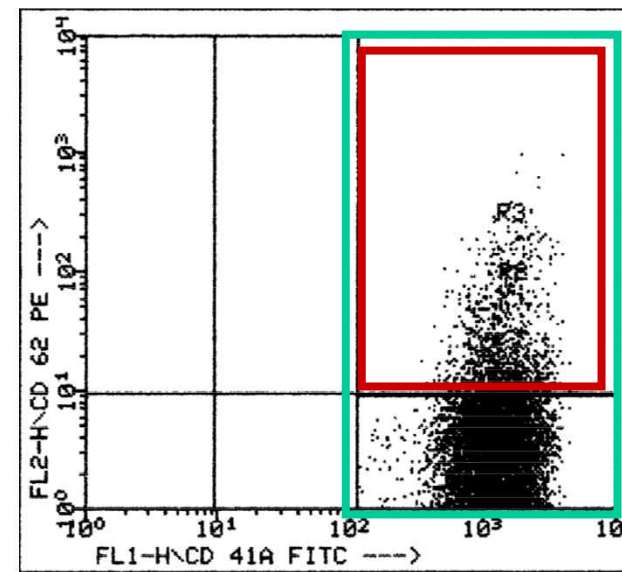
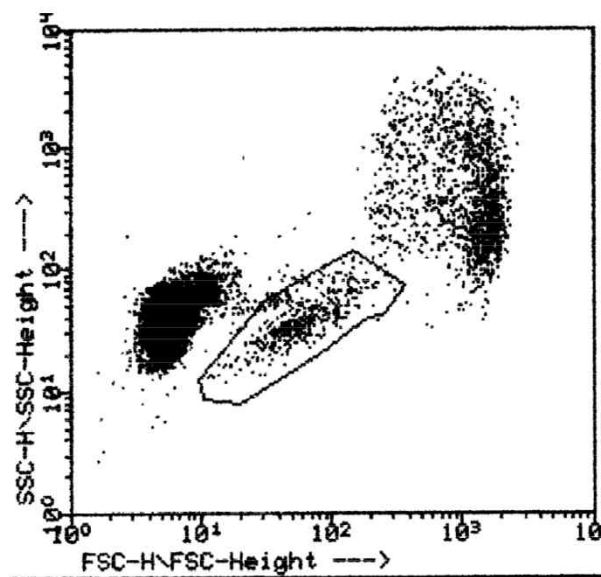
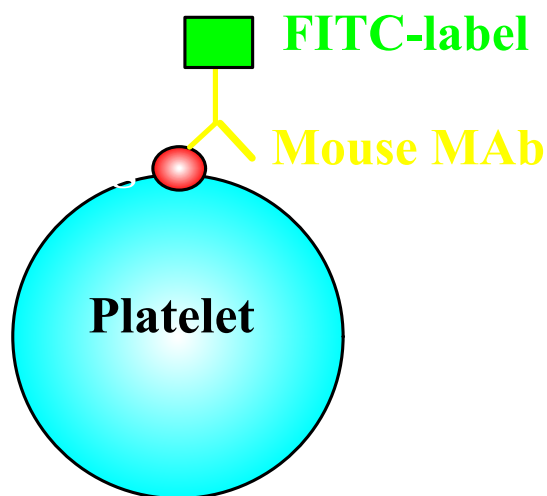
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## Platelet Aggregometry

- **Most common test used to assess platelet function**
- Time consuming, technically challenging
- Affected by many pre-analytical and analytical variables
- Proper use of agonists and concentration
- Diagnosis of several bleeding disorders associated with inherited or acquired platelet dysfunction
- Monitoring of anti-platelet agents: Not encouraged by ISTH
- Standardization: CLSI or ISTH guidelines
- Quality assurance issues: CAP, NASCOLA, UK NEQUAS

# Platelet Function Tests

## Flow Cytometry

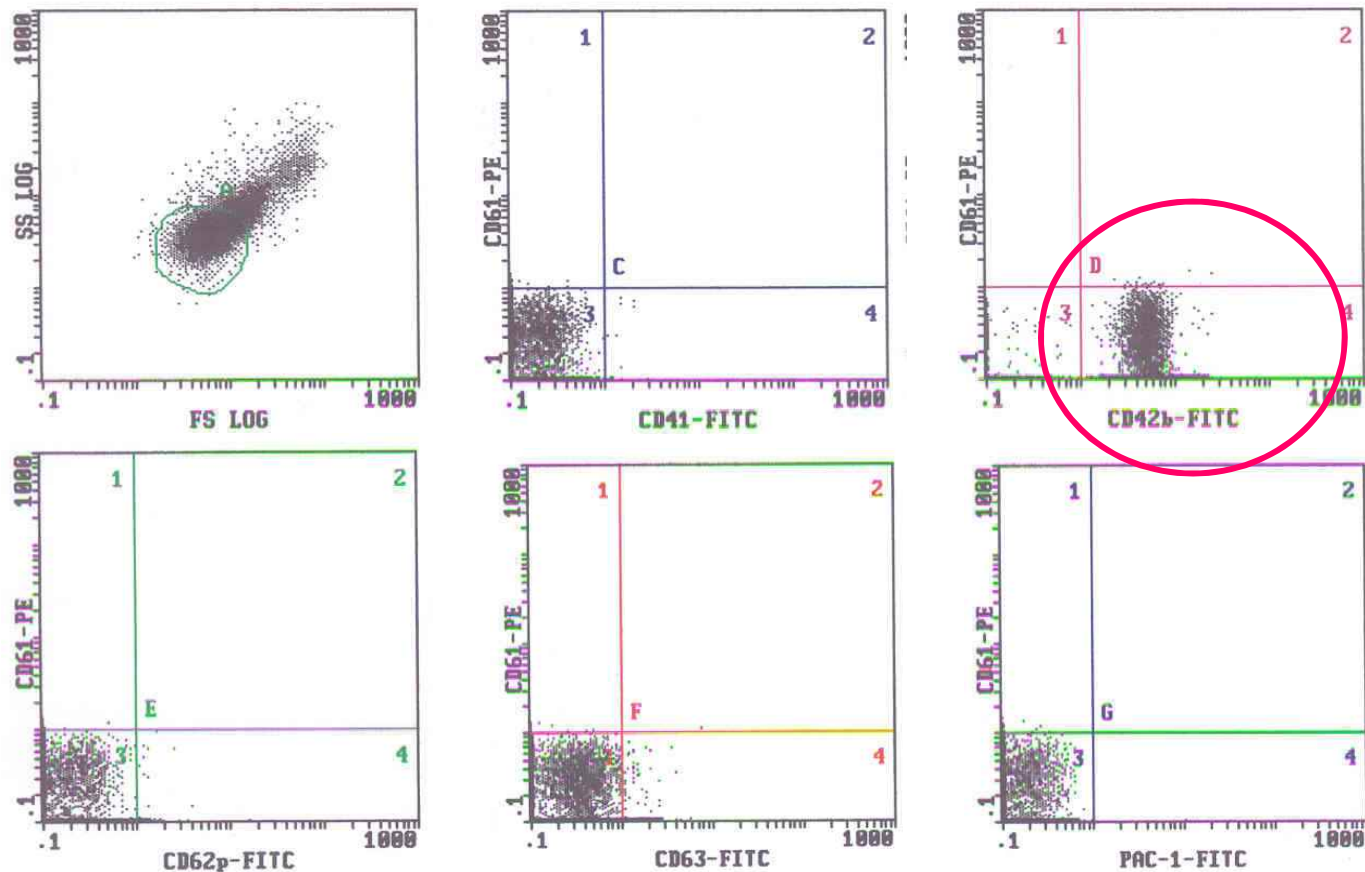


- Platelet glycoprotein analysis
- Platelet secretion studies
- Examination of microparticles

- All Platelets
- Activated Platelets

# Platelet Function Tests

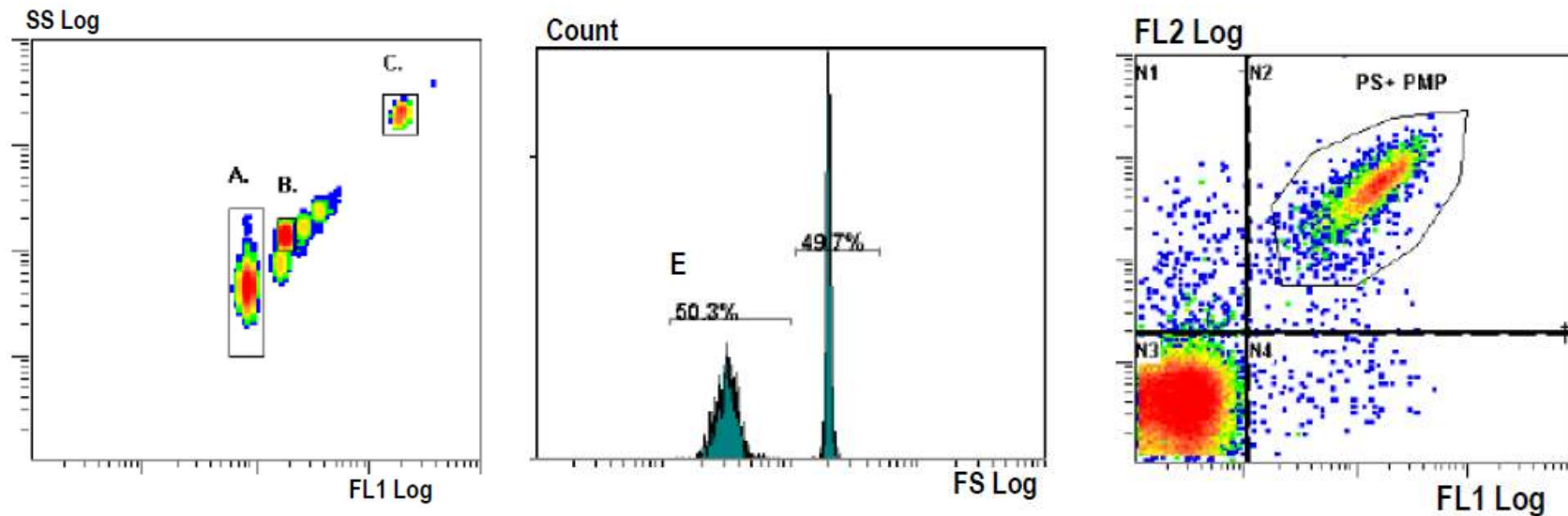
## Flow Cytometry



Glanzmann thrombasthenia

# Platelet Function Tests

## Flow Cytometry



- Megamix (Biocytex, Marseille, France)
- Standardize set-up of microparticle analysis region (0.5 ~ 1.0  $\mu$ M)
- Optimal compromise between microparticle and background exclusion

# Monitoring Anti-Platelet Drugs

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- Many patients experience recurrent ischemic events despite optimal antiplatelet therapy.
- 'Resistant' 'Non-responders' 'Variable response' 'Treatment failure' 'Clinical resistance' 'Laboratory resistance'
- Laboratory tests revealed:
  - 5-60% are resistant to aspirin
  - 4-30% are resistant to clopidogrel
  - ? to combined application
- There is currently no consensus regarding the most appropriate method to quantify the magnitude of on-treatment platelet reactivity.





# Monitoring Anti-Platelet Drugs

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## Mechanism of Aspirin Resistance

- **Clinical Factors**
  - Non-compliance
  - Drugs interactions with NSAID
  - Aspirin formulation
  - Duration of therapy
- **Biological Factors**
  - Alternate pathways for platelet activation
  - Generation of 8-iso-PGF2 alpha
  - Vascular inflammation
  - Reticulated platelet and platelet size
- **Genetic Factors**
  - Polymorphisms and mutation of COX-1, GP IIIa receptors, collagen receptors, vWF receptors

# Monitoring Anti-Platelet Drugs

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## Mechanism of Clopidogrel Resistance

- **Clinical Factors**
  - Non-compliance
  - Under dosing
  - Drug-drug interactions
  - DM, acute coronary syndrome, elevated BMI
- **Biological Factors**
  - Accelerated platelet turnover
  - Increased ADP exposure
  - Reduced CYP activity
  - Up-regulations of P2Y12, P2Y1, P2Y-independent pathways
- **Genetic Factors**
  - Polymorphisms and mutation of MDR1, CYP isoforms, P2Y12, GP IIb/IIIa

# Monitoring Anti-Platelet Drugs

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## Available Methods

### Aspirin

- Aggregation – AA
- PFA-100 Coll/Epi
- VerifyNow ASA
- TxB2 release
- Urinary TxB2
- Multiplate – ASPItest

### Clopidogrel

- Aggregation – ADP
- *PFA-100 P2Y*
- VerifyNow P2Y12
- VASP-P
- Multiplate - ADPtest

# Monitoring Anti-Platelet Drugs

## Comparison of 4 Methods

66 Coronary Artery Disease Patients with Triple Anti-platelet Therapy

Variables	Prevalence (%)	
	Aspirin Resistance	Clopidogrel Resistance
Aggregometer	0 (0.0)	6 (9.1)
VerifyNow	2 (3.0)	28 (42.4)
VASP	-	32 (48.5)
Multiplate analyzer	15 (22.7)	14 (21.2)

# Monitoring Anti-Platelet Drugs

## Degree of Agreement

Variables	$\kappa$ Statistics
VerifyNow	
ARU value	0
PRY 12% inhibition values	0.25
Multiplate analyzer	
ASPI test	0
ADP test	0.21
VASP/P2Y12 assay	
PRI	0.14

- 0~0.2: Slight agreement
- 0.2~0.4: Fair agreement
- 0.4~0.6: Moderate agreement
- 0.6~0.8: Substantial agreement
- 0.8~1.0: Almost perfect agreement



# Conclusions & Summary

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- The investigation of platelet function disorders requires a step wise process and should involve collaborative interaction between both clinical and laboratory personnel in order to achieve the best outcome.
- A consensus on a definition of anti-platelet drug resistance is still lacking. The platelet function assay that closely correlates platelet responsiveness to the adverse clinical outcome has yet to be determined. The beneficence of adapting anti-platelet therapy on the basis of laboratory findings is not clearly demonstrated.



# Welcome to Busan

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