



Manufactured By: HYPHEN BioMed

ASSAY METHODS FOR THE EXPLORATION OF FIBRINOLYSIS

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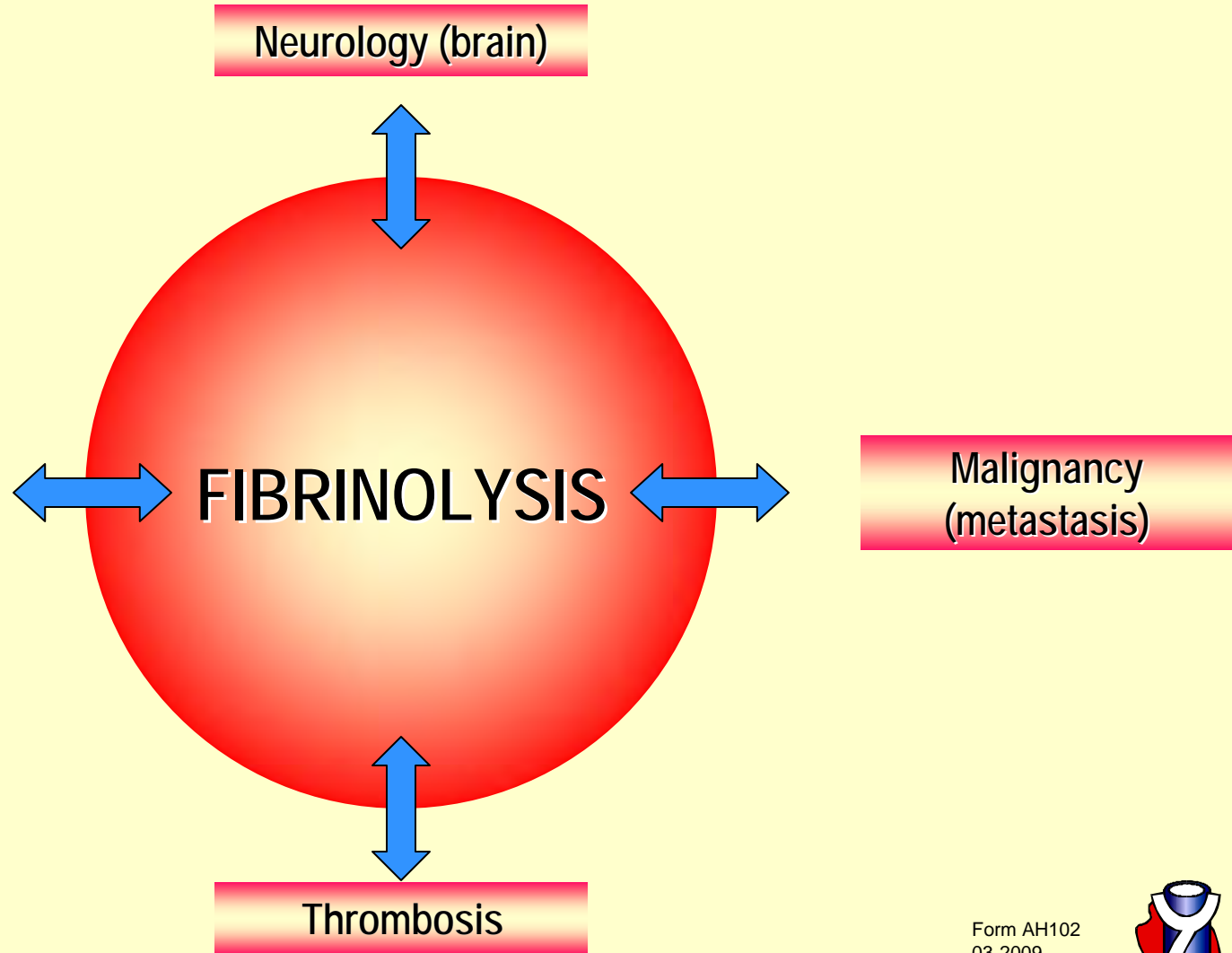
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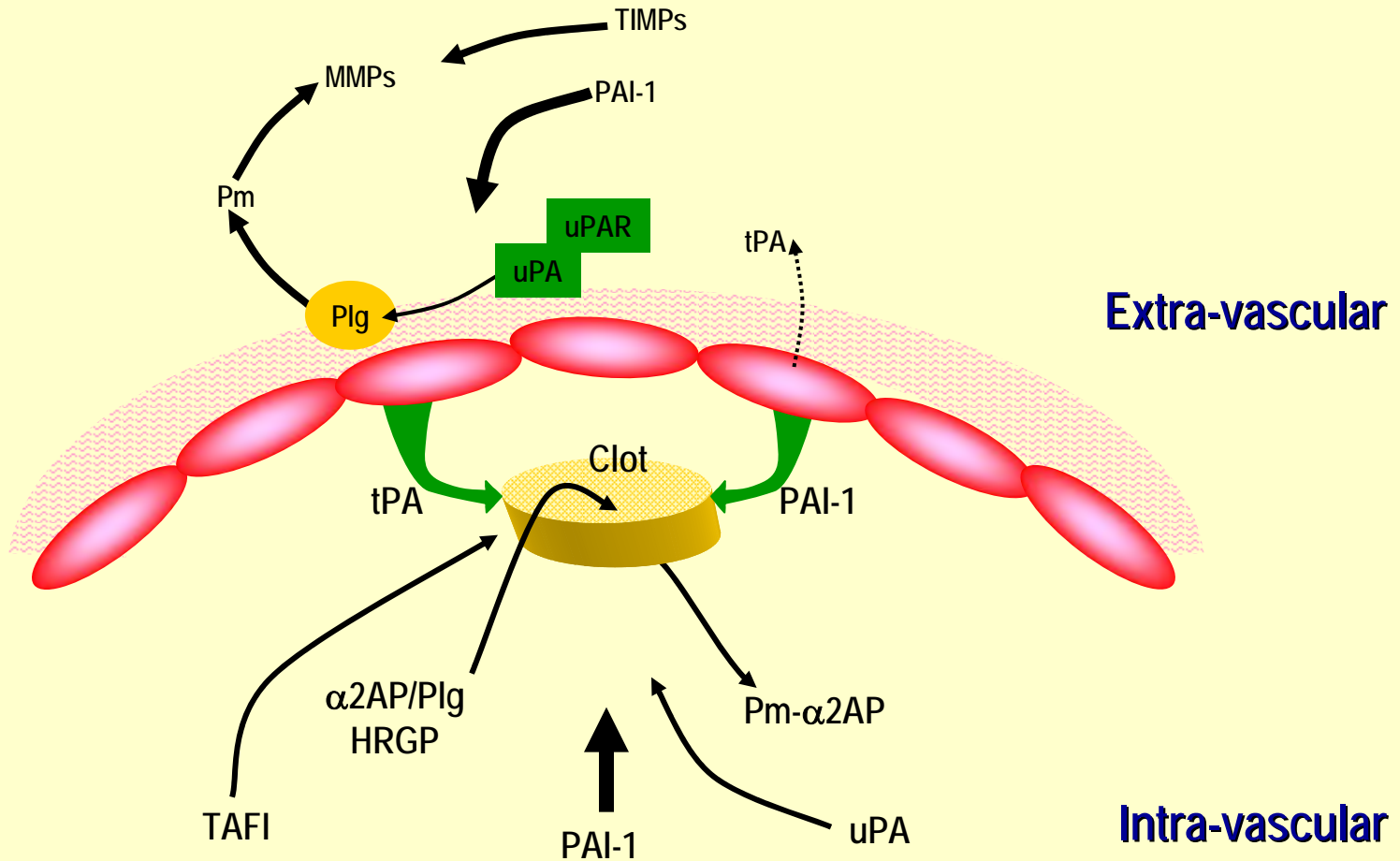
Jean AMIRAL - June 2005



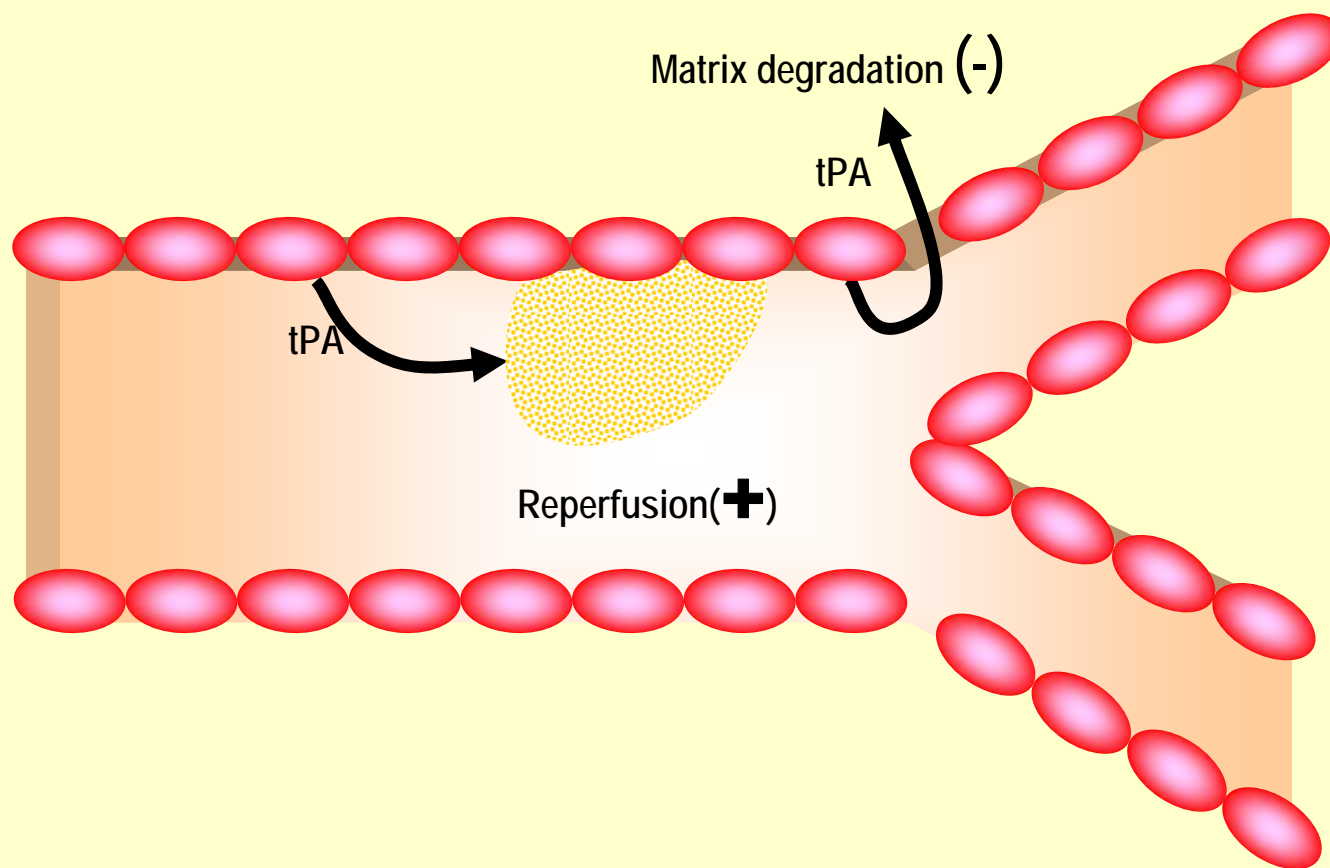
Fibrinolysis Functions



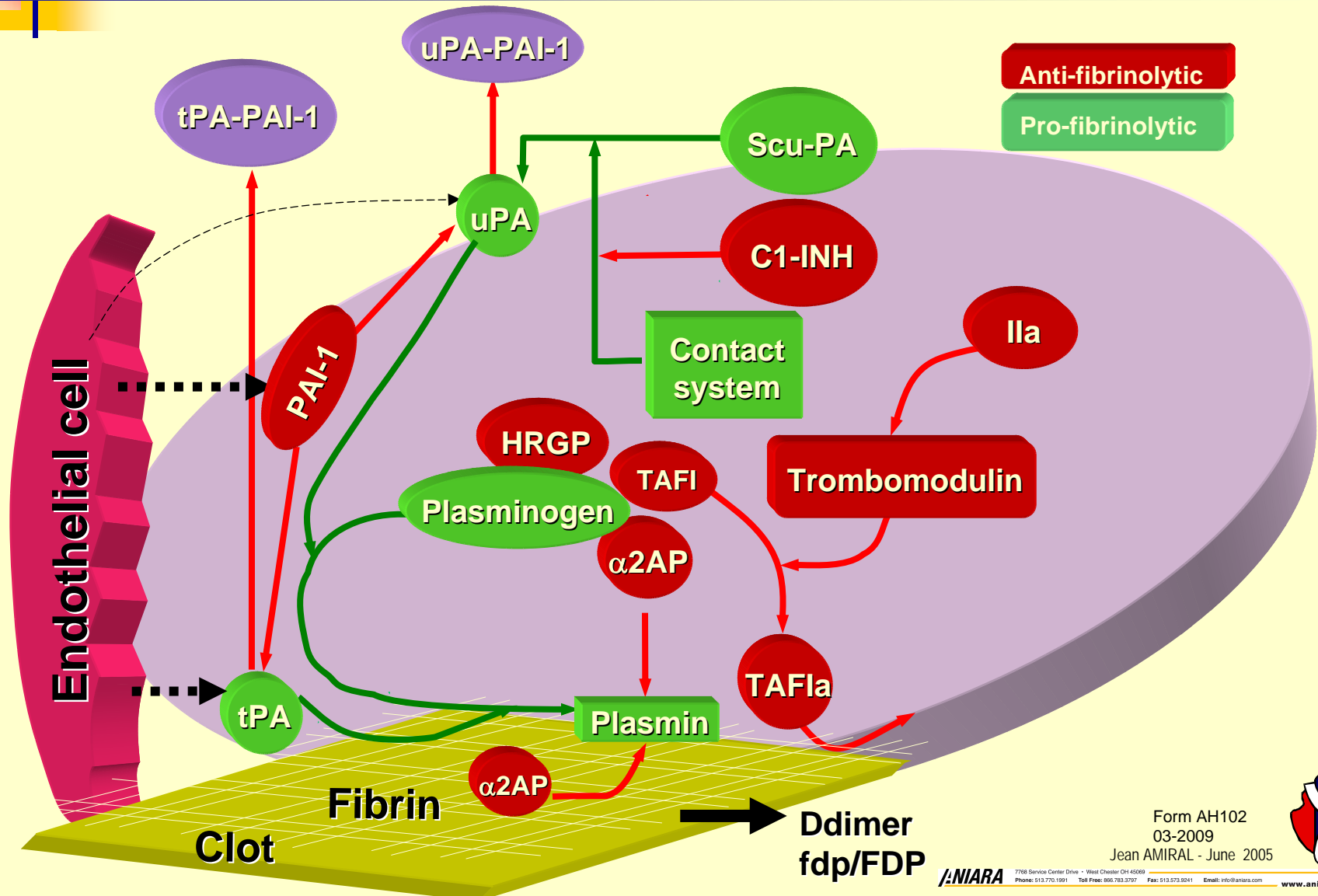
Fibrinolysis Actions



Yin and Yan effect of tPA in brain



FIBRINOLYSIS



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Analytes involved in Fibrinolysis

- **Triggers:** tPA, uPA
- **Proteases:** Plasminogen → Plasmin, MMPs
- **Regulators of clot degradation:** TAFI, α_2 AP
- **Reg. of Plg binding to clot:** α_2 AP, HRGP
- **Inhibitors:** PAI-1, α_2 AP, (PAI-2), TIMPs, ...





Major diagnostic analytes for fibrinolysis

■ Intra-vascular (plasma)

- tPA
- PAI-1
- uPA (?)

■ Extra- vascular

- uPA
- uPA-R
- PAI-1
- MMPs/TIMPs (?)





Assay Methods for Fibrinolysis

- **Functional**: Plg, TAFI, Anti-Plasmin, tPA, PAI-1, etc...
- **Immunoassays**: Plg, TAFI, Anti-Plasmin, tPA, PAI-1, tPA- or uPA- PAI-1 complexes, PAI-2, PAI-3, MMPs, TIMPs, etc...
- **Global Assays** for Fibrinolytic Potential





Functional Assays for tPA or PAI-1

■ tPA:

- Specimen + Fibrin Monomers (Eq.) + Plasminogen
- Generation of Plasmin
- Chromogenic substrate for Plasmin (OD 405)

■ PAI-1:

- Specimen + tPA (or uPA)
- Activator + Plasminogen + Substrate
- OD 405 (Measurement of tPA or uPA in excess)

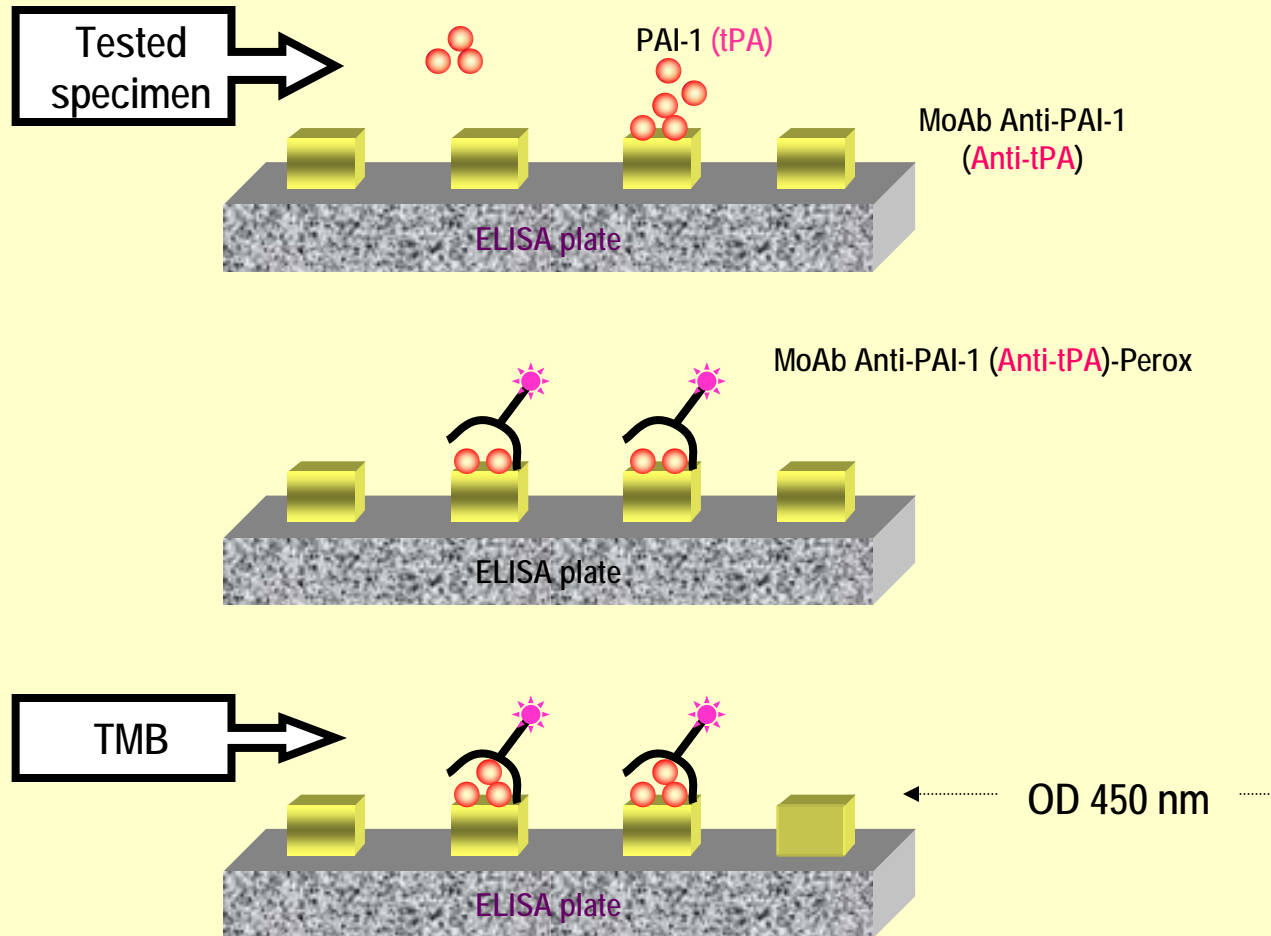


Reactivity of Antigen Assays for PAI-1, tPA, uPA

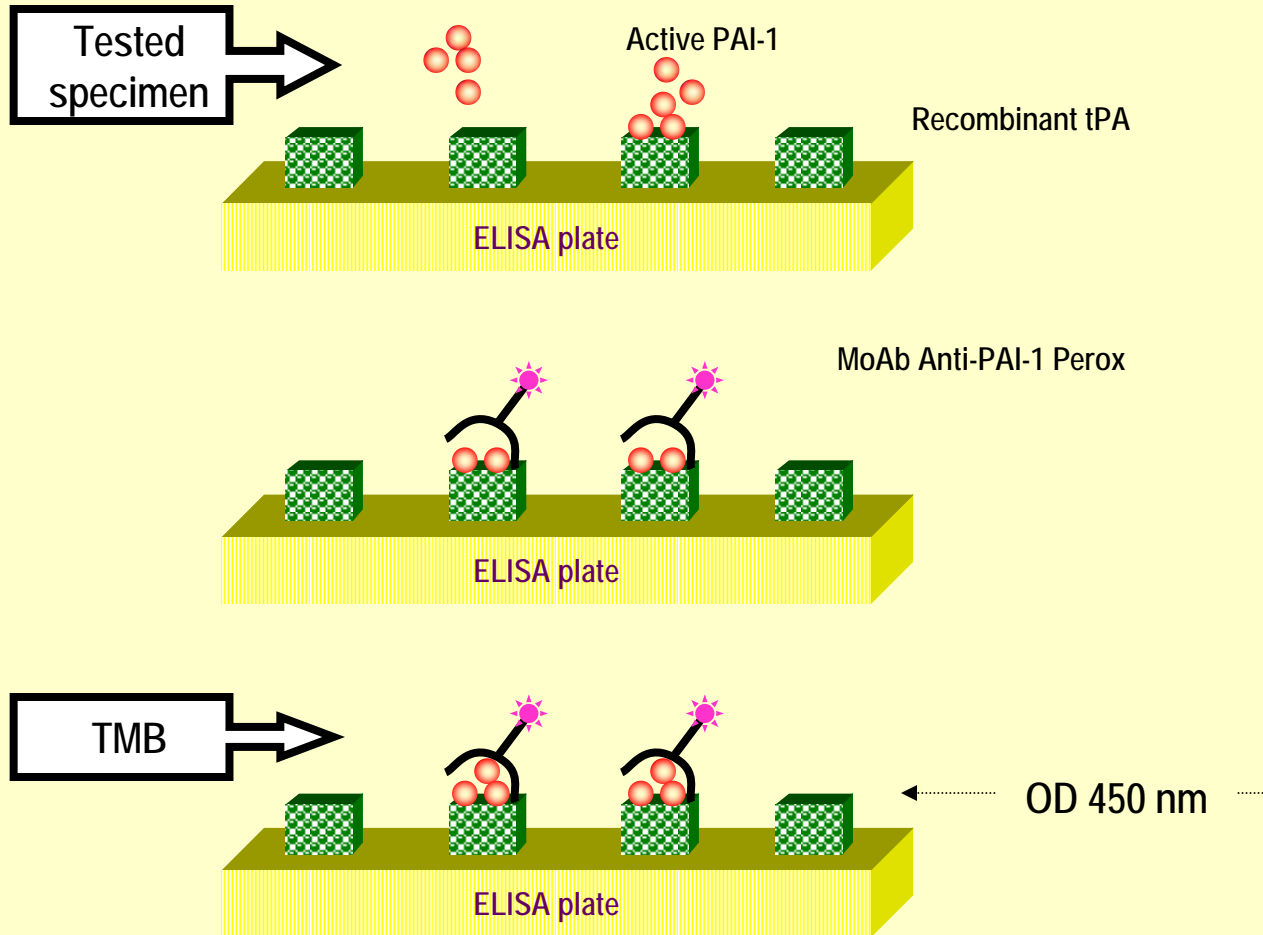
- Should measure homogeneously all the protein whether the presentation is:
 - PAI-1 (Active, Bound to Vitronectin, Latent, Complexed to tPA or uPA, etc...)
 - tPA (Free or Complexed with PAI-1, etc ...)
 - uPA (Free or Complexed with PAI-1, etc ...)
- Importance of the selection of the MoAb pair used for designing the assays.



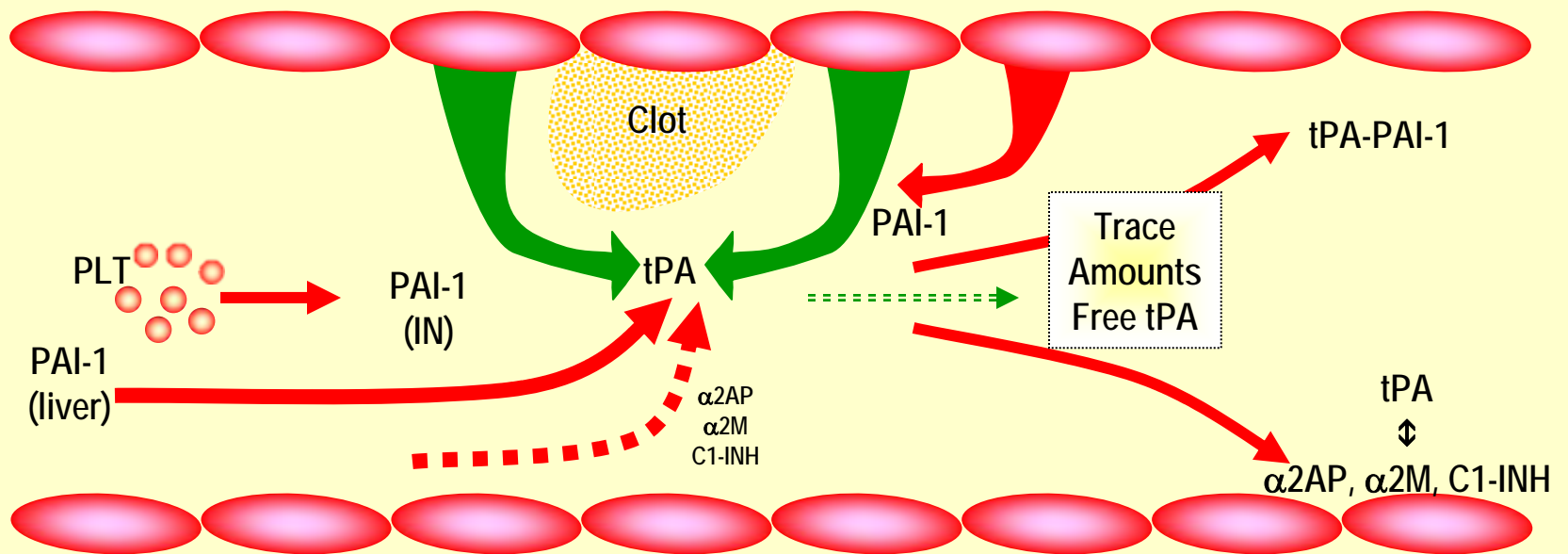
Two Site ELISA for PAI-1 (tPA):Antigen



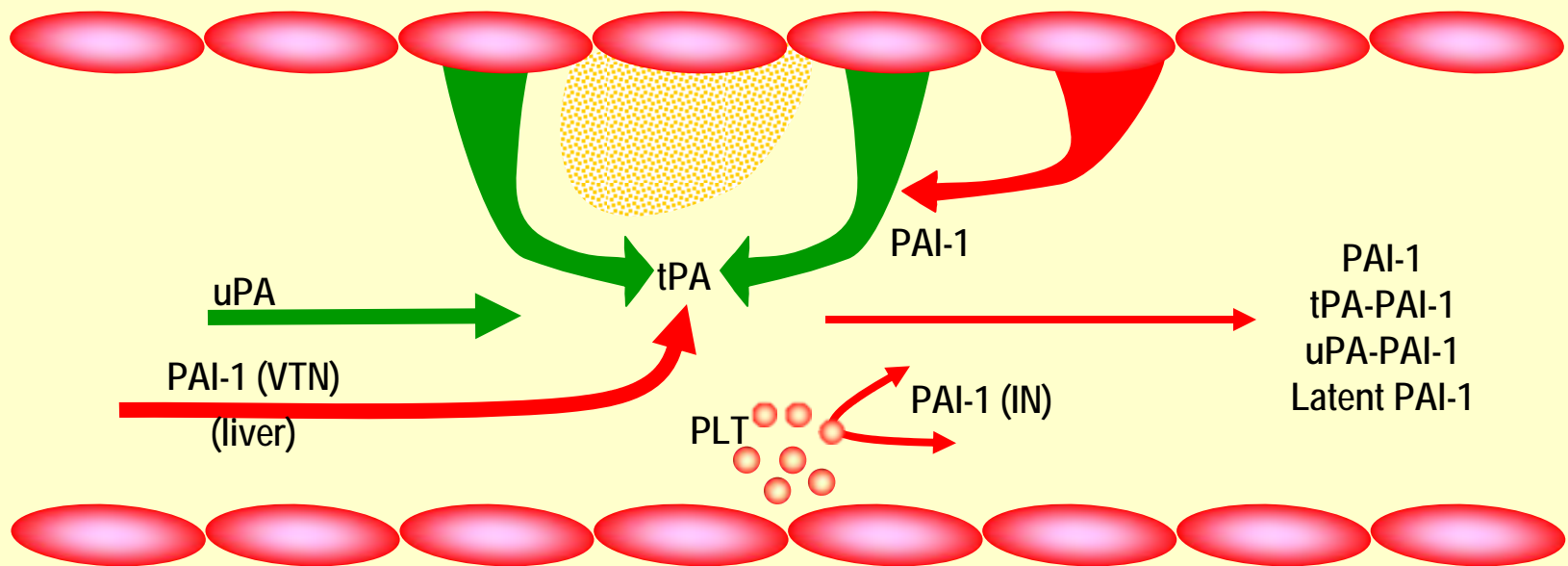
Bio-Immuno-Assay for PAI-1: Activity

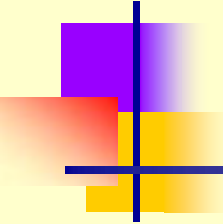


tPA concentration in the micro-environment and in blood circulation



PAI-1 in blood vessels





Specimen collection

- Citrate, CTAD or EDTA anticoagulated plasma: avoid blood activation ex-vivo (PAI-1 release from platelets).
- Clean venipuncture.
- Avoid tourniquet (tPA-release).

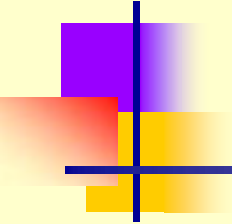




Standards for tPA, uPA or PAI-1

- **NIBSC International Standards Available:**
 - Defined by Activity
 - Antigen Amount \pm well defined (acceptable for tPA, discussable for PAI-1)
 - Are dependent on Assays used for their evaluation
- **Practically:**
 - Established International Standards for Activity (UI)
 - High difficulties to standardize and harmonize antigen concentrations





Available NIBSC Int. Stds. For Fibrinolysis

- **tPA: Human, Recombinant 3rd IS (98/714), 1999,** 10,000 IU per ampoule.
- **Urokinase HMW: 1st IS (87/594), 1989, 4,300 IU** per ampoule.
- **PAI-1, Plasma Human: 1st IS, 1995 (92/654), 27.5** IU (tPA neutralization) or 7.0 IU (uPA neutralization) per ampoule.
- **Others: Plasmin, SK (All activities), NIBSC Res. Reagent (tPA:Ag, Plasma, 25 ng/ml).**





Other ways to Establish Standards

- **Highly purified protein preparations:**
 - High purity grade (> 99%)
 - Exact protein level (Lowry, BCA/Bradford, AA sequence, etc...)
 - Native (difficult) or Recombinant (Wild Type)
- **Remaining Issues:**
 - Matrix effect (?), milieu incidence
 - Assay reactivity with the various presentations



PAI-1 Normal ranges

Stago	MoAb/MoAb	< 50 ng/ml
Coaliza	MoAb/MoAb	40 ± 29 ng/ml
Imubind	MoAb/MoAb	4 – 43 ng/ml
Imulyse	MoAb/MoAb	4 – 43 ng/ml
Tintelize	MoAb/MoAb	4 – 43 ng/ml
Zymutest	MoAb/PoAb	0 – 25 ng/ml (4 – 43 ng/ml)

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PAI-1 Ag with various assays (ng/ml)

(Declerck et al. Thromb Haem 70 (5), 1993)

Sample	Stago	Coaliza	Imubind	Imulyse	Tintelize
1	16	28	8.6	10	12
2	117	110	53	68	83
3	45	47	20	21	28
4	3.8	8.5	4.1	3.1	1.6
5	1.5	2.2	0.2	0.6	0.7
6	13	31	8.4	9.2	10
7	6.2	17	4.5	60	4.0
8	32	62	17	20	20





Normal ranges for Fibrinolysis proteins

PAI-1

0 – 25 ng/ml
(4- 43 ng/ml)

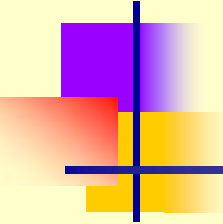
tPA

0 – 10 ng/ml

uPA

0 – 5 ng/ml



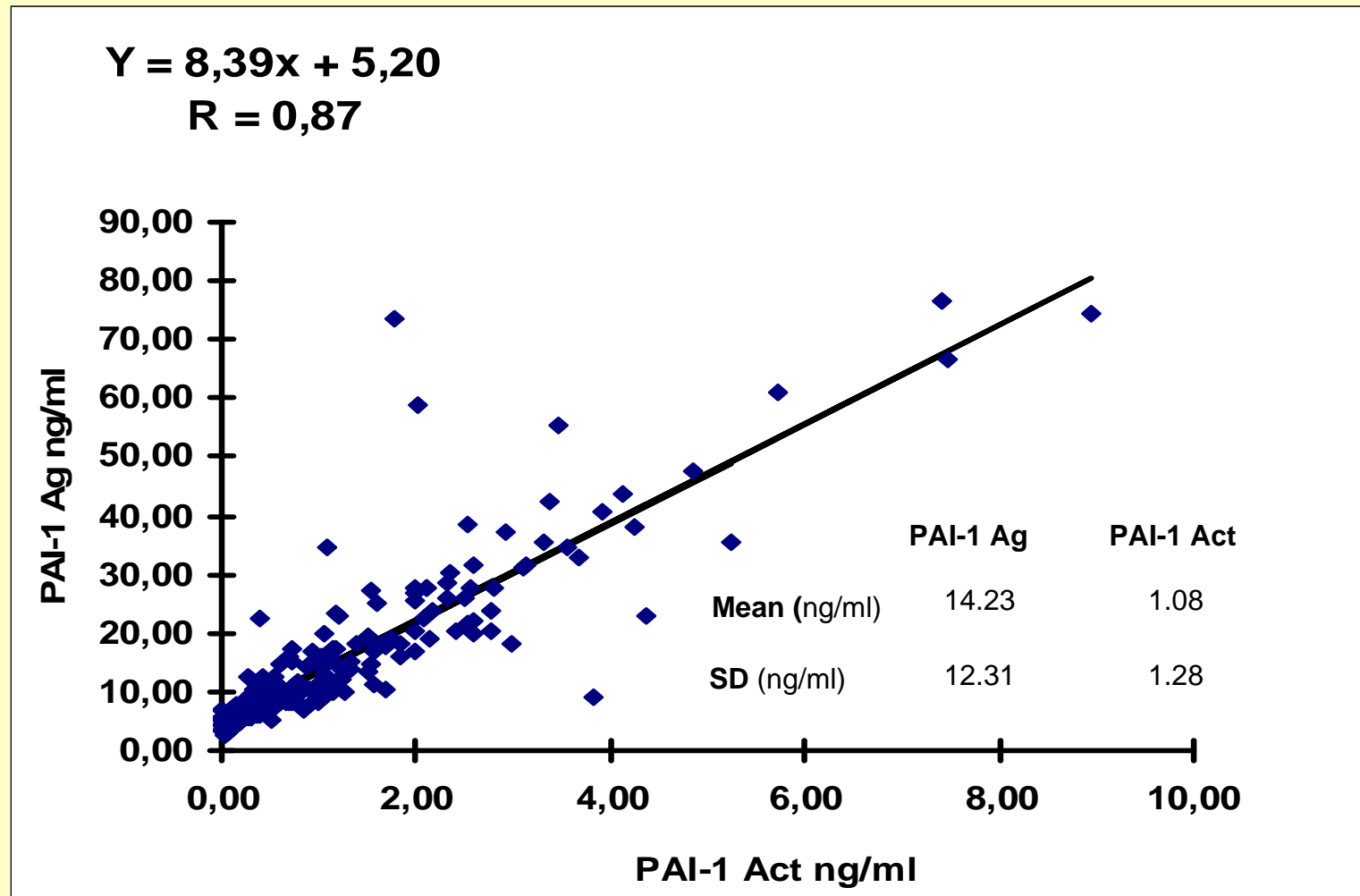


ZYMUTEST PAI-1 :Ag normal range

- N= 57
- Mean: 6.58 ng/ml
- S.D. : 5.21 ng/ml
- Min.: 1.19 ng/ml
- Max: 25.28 ng/ml



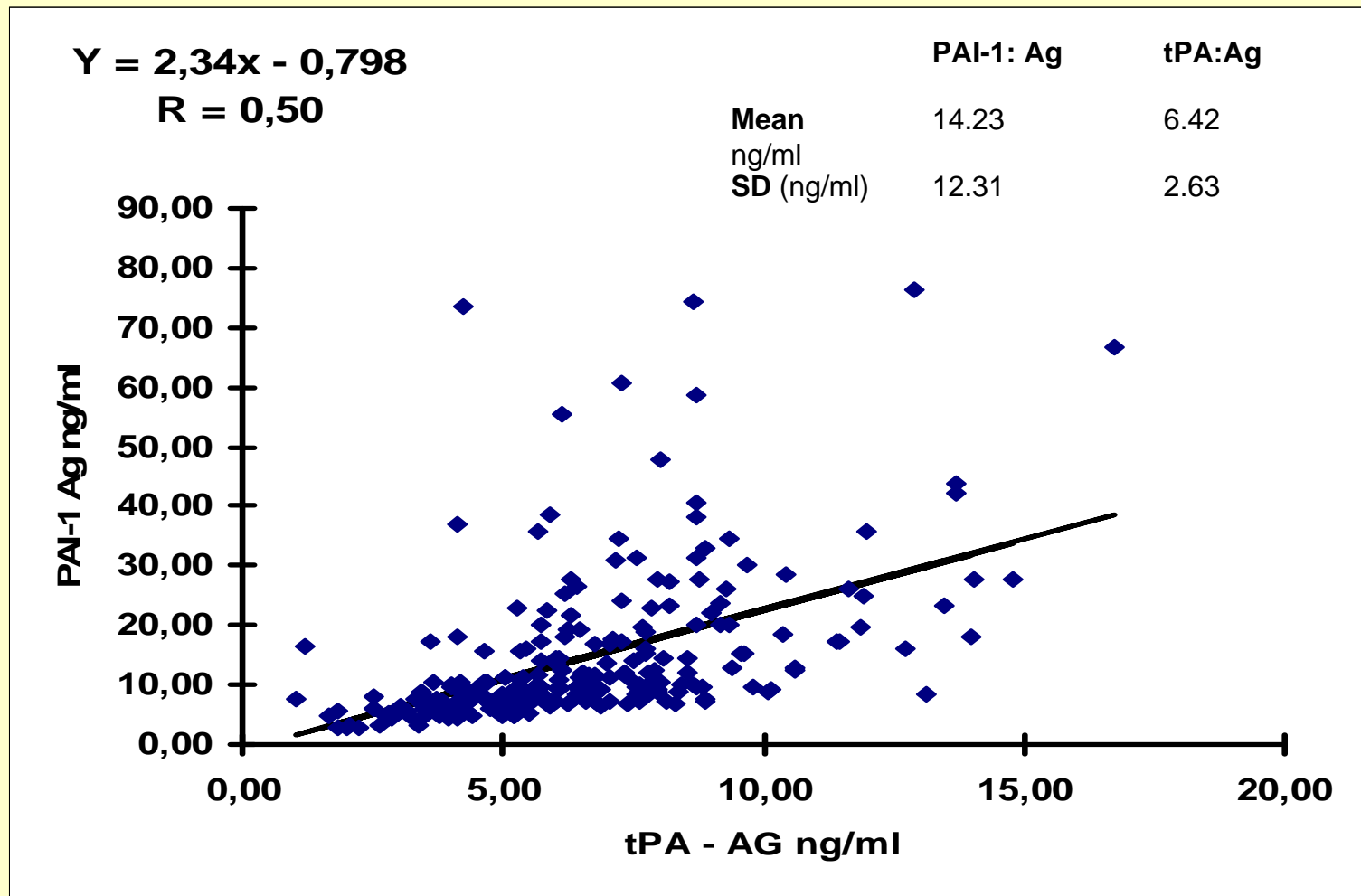
Correlation between PAI-1 Ag and Activity (N=253)



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Correlation between tPA and PAI-1 Ag (N=253)



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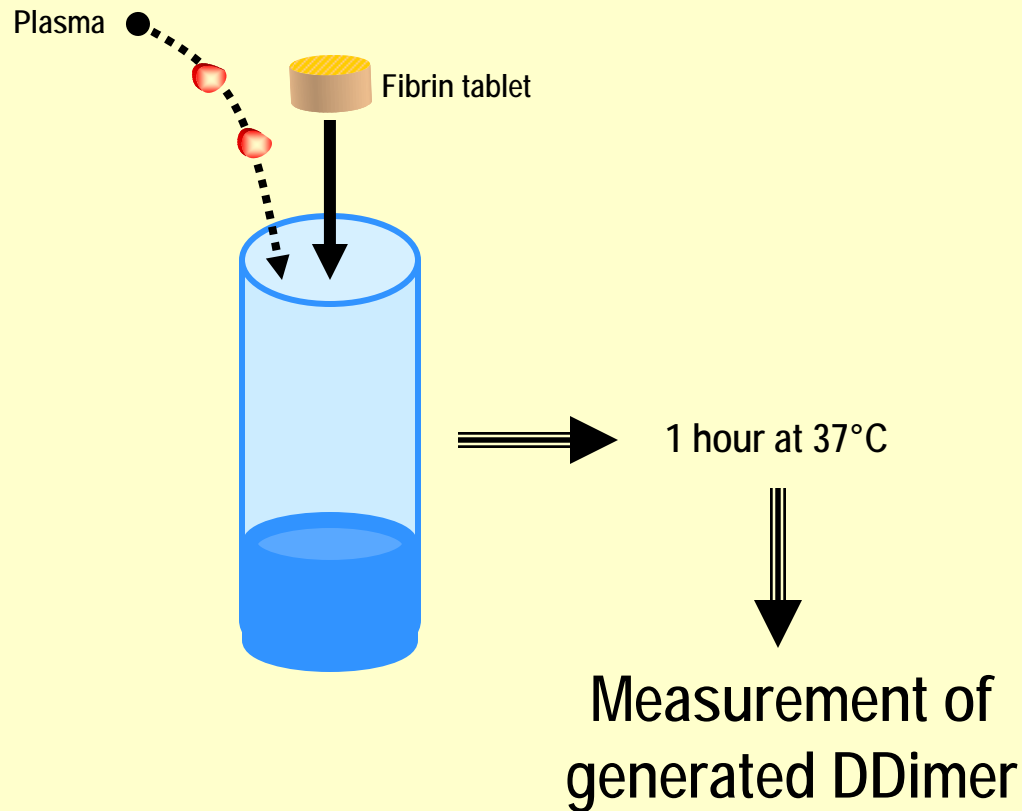


Evaluating Body's Fibrinolytic Potential

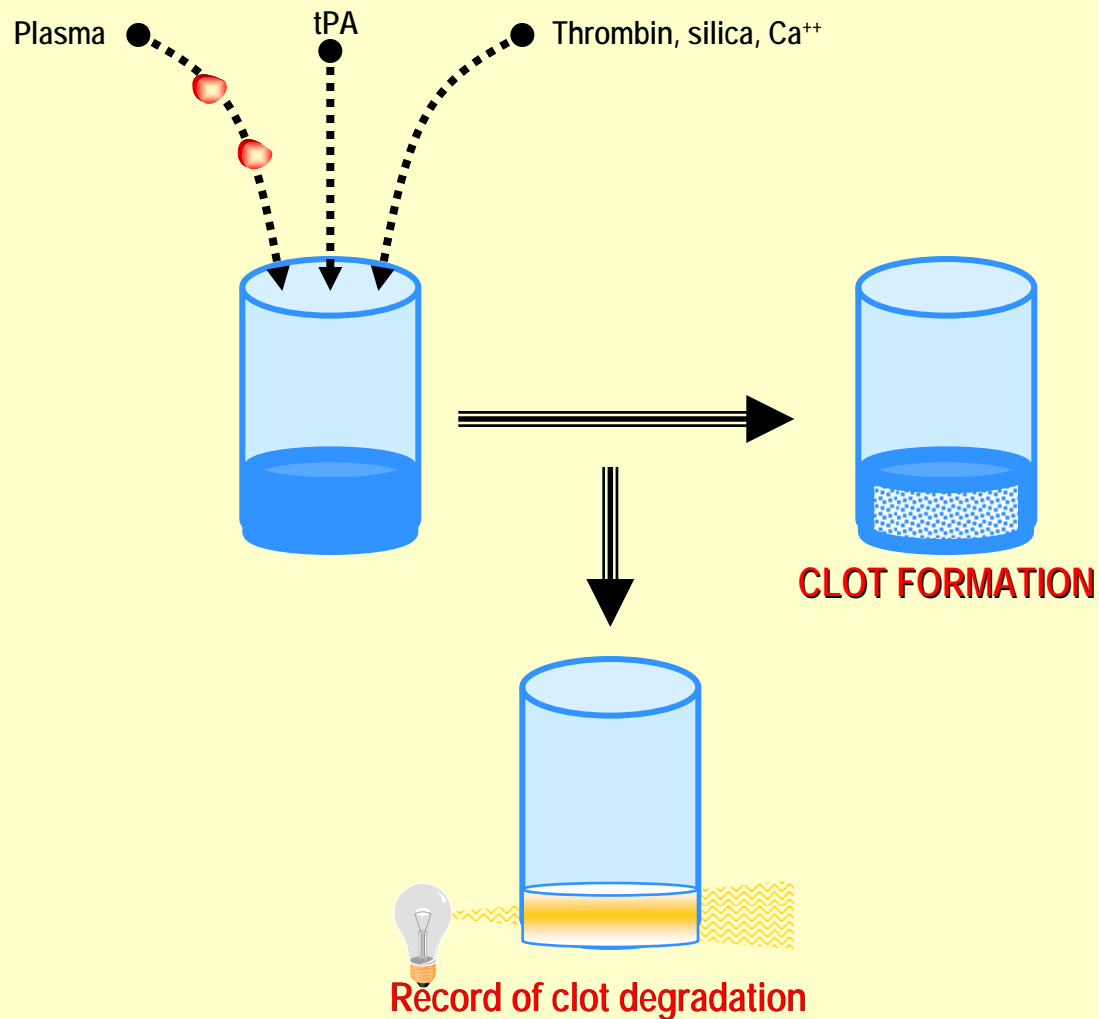
- **Can we Evaluate the Global Fibrinolysis Potential in Body?**
- Fibrinolysis is Initiated, Regulated and Inhibited in the Micro-Environment.
- Its activity is delayed, then stimulated and finally stopped.
- **Is there any Plasma Assay linked to body's capacity?**



Global Fibrinolytic Capacity (GFC)



Global Fibrinolytic Capacity assay





Assays for global fibrinolytic capacity

- Strong correlation with cardiovascular risk factors (obesity, triglycerides, blood pressure, LDL- Cholesterol, glucose,...), and type II diabetes or X-syndrome.
- Strong contribution of PAI-1.
- Inverse relationship with tPA concentration.





Clinical applications of Fibrinolysis

- Metabolic Syndrome (X-Syndrome)
- Diabetes, Type II (not affected by Type I)
- Cardiovascular diseases (predictivity of tPA?, PAI-1?, ...)
- Malignancy (Breast Cancer, ...), etc ...





Conclusions

- Fibrinolysis is a key system in life, probably still under-evaluated.
- Important (but occult?) function in regulating many biological functions.
- Diagnostic and Prognostic Value for the key parameters involved in Fibrinolysis (tPA, PAI-1, uPA, ...).
- Diagnostic Potential of Other Factors (TAFI, PAI-2, MMPs, TIMPs, ...)?

