# HAEMOSTASIS & THROMBOSIS
# LAB TESTS

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# ROUTINE HAEMOSTASIS LABORATORY:

:

- *PT/APTT*
- *TT/BT*
- *FIB-C*
- *D-Dimer*
- *Factors Assays: FVII, FVIII, FIX, FXI*
- *Inhibitor Screens*
- *Mixing Tests*

## *THE BETHESDA ASSAY FOR INHIBITORS*

- *Buffering the assay system with 0.1 M imidazole to pH 7.4 improves specificity and reliability.*
- *Glyoxaline buffer is used as the pre-incubation sample buffer in human Bethesda assays.*
- *These modifications allow better discrimination between positive & negative samples and improve reliability.*

# *THE MODIFIED BETHESDA ASSAY FOR NHIBITORS*

***FVIII inhibitors may exhibit 2 forms of kinetics***

***Type 1***

*-have a linear relationship with dilution*

*-follows the first order kinetics with FVIII progressively neutralized until either it or the inhibitor is used up.*

*-completely inactivate the FVIII & there is a linear relationship when the log of the residual FVIII activity is compared to the abs concentration*

*Type 2*

*-Inhibitors do not follow a linear relationship*

*-Exhibit a more complex order kinetics*

*-Give similar amounts of residual FVIII with different dilutions of patient's plasma*.

# PLATELET STUDIES LABORATORY:

***Tests done:***

- *Platelet aggregation tests*
- *Ri COF Assay*
- *PFA*
- *TEG test*
- *Platelet Nucleotides Bioluminescence Assay*

# PLATELET AGGREGATION TESTS

- *Five agonists routinely used are ADP, adrenaline, collagen and arachidonic acid. These are adequate to allow the major platelet function disorders to be discriminated.*
- *If there is biphasic wave with 2uM ADP & adrenaline, need not proceed with higher concentrations.*
- *If patient's platelets aggregate with 0.5mg/ml Ristocetin (indicating possible IIB VWD or Pseudo VWD), perform spontaneous aggregation for 15 minutes.*

# *PLATELET AGGREGATION TESTS*

- *-Most convenient and recommended to measure 3 mins after addition of agonist.*
- ***Interpretation of results:***
- *-ADP at 2 μM, clearly defined primary & secondary waves can usually be seen, above 3uM, masked secondary phase; shape changed from a disc to spiky sphere.*
- *-Adrenaline, patterns of response usually similar with ADP, except primary wave does not reverse nor is it so intense to mask secondary wave; no shape change.*

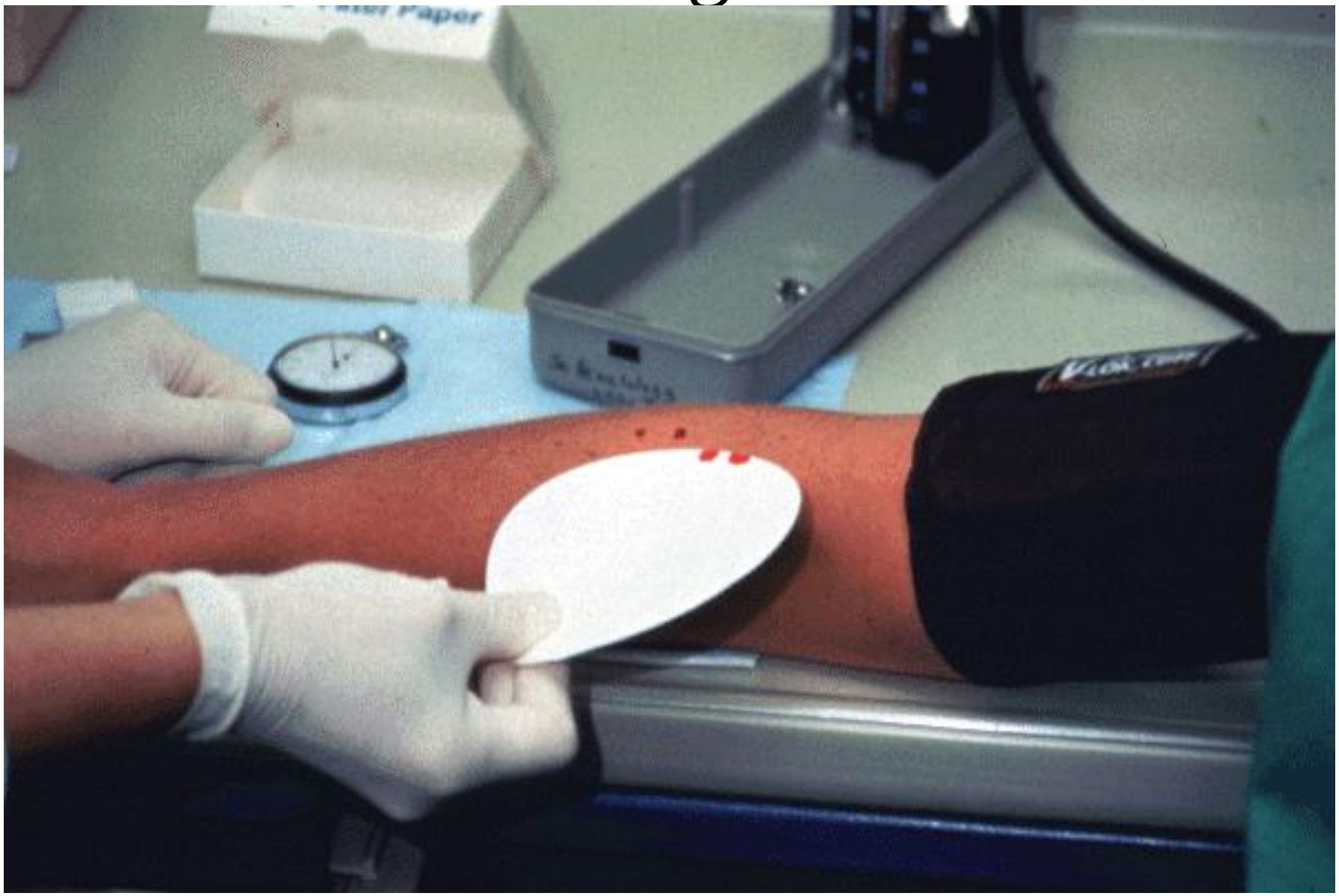
# *PLATELET AGGREGATION TESTS*

- *-With collagen, no primary wave occurs; response defined by duration of lag phase before onset of aggregation and its intensity.*
- *-With ristocetin, primary wave is a measure of the amount of VWF present in plasma; secondary wave is due to release of endogenous substances.*
- *-With arachidonic acid, aggregation is monophasic & preceded by a short lag phase.*

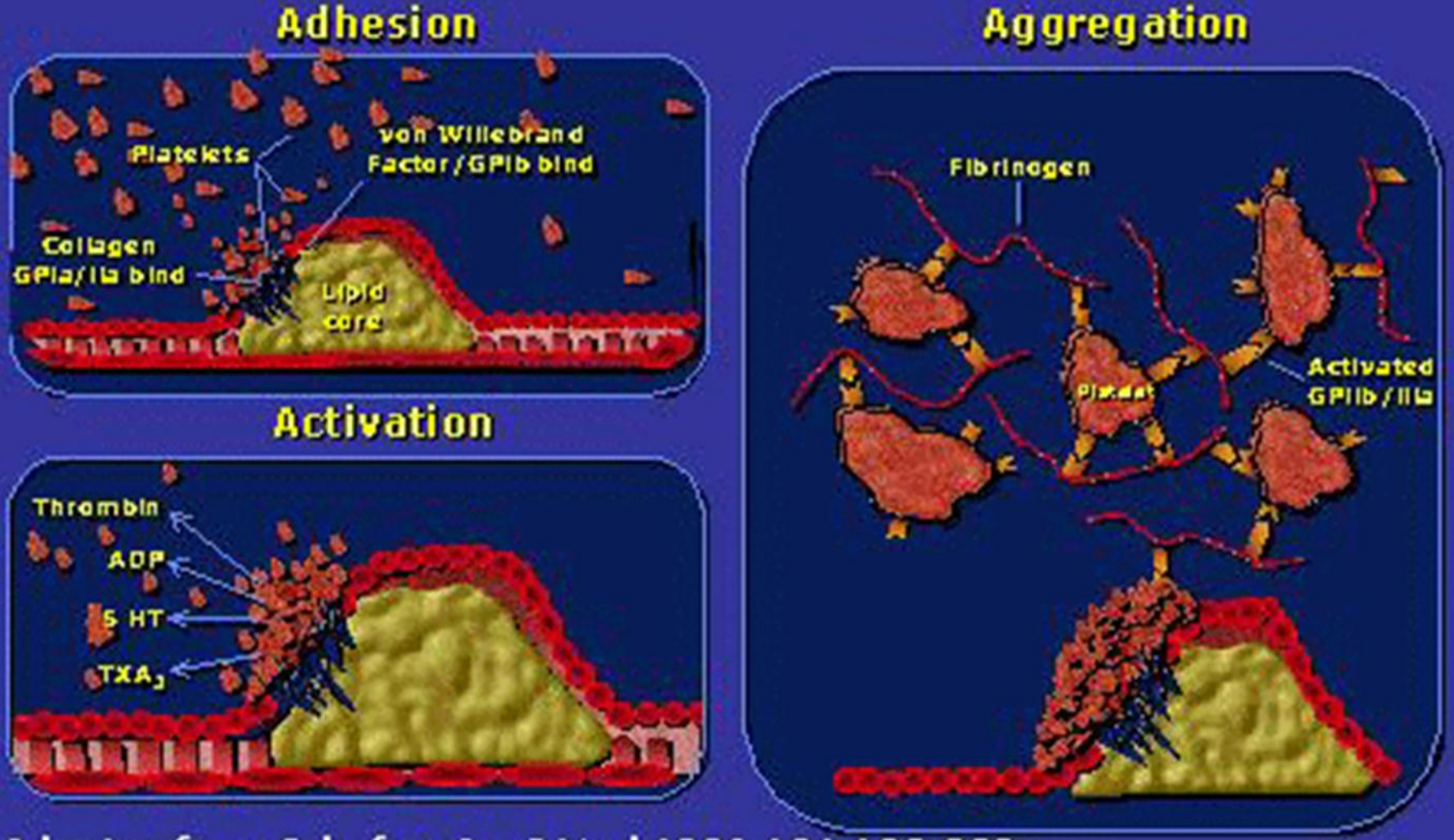
# *PLATELET AGGREGATION TESTS*

- ***Further investigation of platelet function:***
- *Always repeat at least one occasion, if an abnormal aggregation pattern is observed.*
- *In the presence of abnormal aggregation, further investigations include platelet nucleotides and beta-thromboglobulin;*
- *Quantitation of membrane glycoproteins for diagnosis of Bernard-Soulier syndrome & Thrombasthenia*

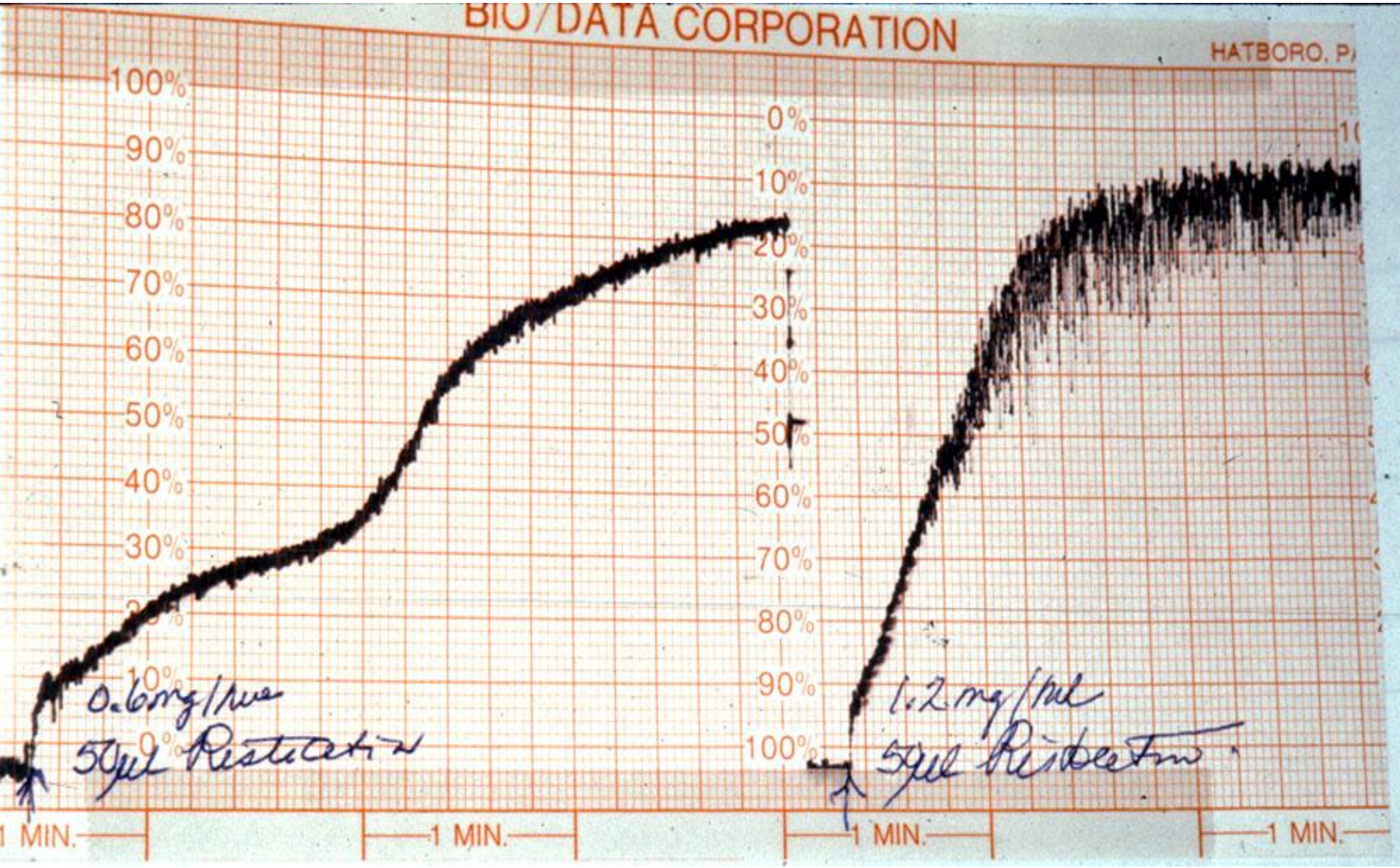
# Bleeding Time



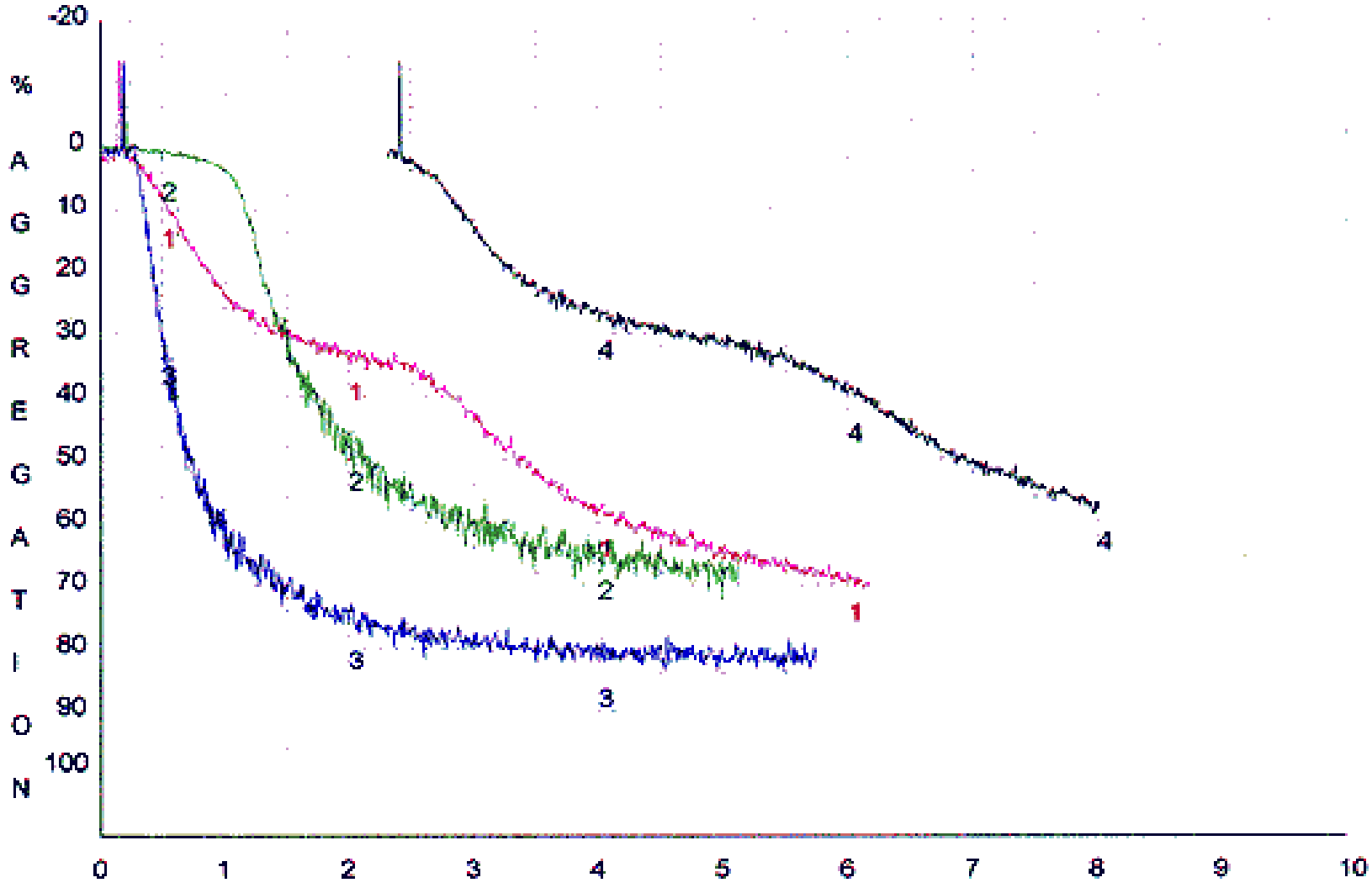
# The 3 Steps of Platelet Aggregation




Adaptec from Schafer. Am J Med 1996;101:199-209.




# Platelet Aggregation




Platelet Aggregation: 1) Epinephrine,
2) Arachadonic acid, 3) ADP 4) Epinephrine

# *RISTOCETIN CO-FACTOR ASSAY*

- *Fresh washed platelets:*
- *-Prepared from  PRP .*
- *Advantages over lyophilized platelets cheap*
- *-Wider aggregation range than lyophilised platelets*
- *-Disadvantage: time consuming*

# *ELISA LABORATORY TESTS:*

- *Monoclonal Free Protein S Antigen*
- *Total Protein S*
- *Protein C Ag*
- *VWF Ag*
- *Factor VIII:C Levels*

# *FACTOR VIII:C ASSAY*

- *-Always include a test, an abnormal & a normal plasma control*
- *-Test plasma in 3 dilutions, 1:5; 1:10 &1:20.*
- *If values obtained >10% of each other, repeat test.*

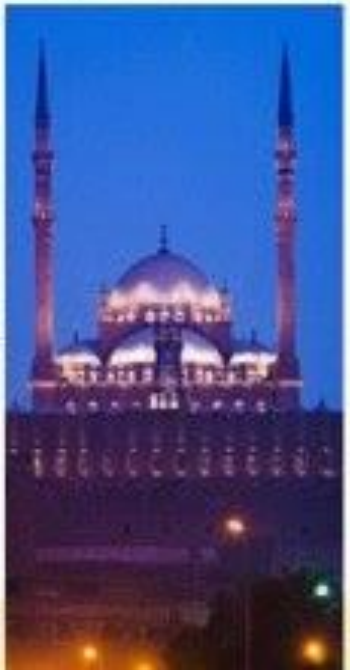
# THROMBOPHILIA STUDIES:

## Tests done:

- Lupus Anticoagulant
- KCT Test
- Protein S activity
- Protein C activity
- APC R Assay
- Factor V Leiden/ PCR

# *LUPUS ANTICOAGULANT (LA)*

- *An auto-antibody directed against , or cross-reacting with, specific chemical groups that are found in negatively charged phospholipids such as phosphatidyl serine and cardiolipin.*
- *They are usually immediate reacting, are detected by their effect on phospholipid-dependent coagulation tests.*
- *No one method has 100% reliability, usually > than one test is carried out.*
- *LA is transient in nature.*



## *SAMPLE PREPARATION (LA)*

- *Double centrifugation*
- *Aliquot and store at –45 °C*

- *The following tests are run prior to DRVVT/DRVVC:*

*PT; APTT; APTT 50:50; TT; Fib-C*

# *LUPUS ANTICOAGULANT-CALCULATION OF RESULTS*

- ***Pathromtin Reagents:***
- ***LA screen***
- *-The 20NP should give a value of 45-65 s (mean 56.2)*
- *-A test/normal ratio of 1.16 or > indicates a positive result.*
- ***LA confirm***
- *-The 20NP should give a value of 47-56 s (mean 51.5)*
- *-If correction is > 65%, LA positive.*

# *LUPUS ANTICOAGULANT-CALCULATION OF RESULTS*

- ***Stago Diagnostic:***
- ***LA screen***
- *-The 20NP should give a value of 35-45 s*
- *-Reference range for DRVVT is 27-46 s*
- ***LA confirm***
- *-The 20 NP should give a value of 29-37s*
- *-Reference Ratio of DRVV Test/ DRVV Confirm is 0.9-1.2*
- *-A ratio of > 1.2 is considered LA positive.*

# *LA TEST: IMPORTANT POINTS TO NOTE*

- *20 Normal Pool ( NP)used must be similarly treated as the test plasma*
- *Warfarin samples must be mixed with equal parts of normal pooled before tested for LA.(to compensate for the effect of warfarin on FX)*
- *If LA is negative but the prolonged APTT didn't correct well, perform KCT*
- *Heparinized samples cannot be tested for LA.*
- *Patients on warfarin cannot be tested with an EXNER test.*

# *THROMBOPHILIA STUDIES*:

## ❑ *Free Protein S*

*-Recommendations of WHO/ISTH*

*-Free Protein S assay have higher specificity & sensitivity for genetic defects causing Protein S deficiencies than total Protein S assay.*

*-Only free PS is functionally active & able to bind with aPC,*

*-There is a great overlap in the total Protein S levels between normal and those with genetic defect.*

## *THROMBOPHILIA STUDIES:*

*Note:*

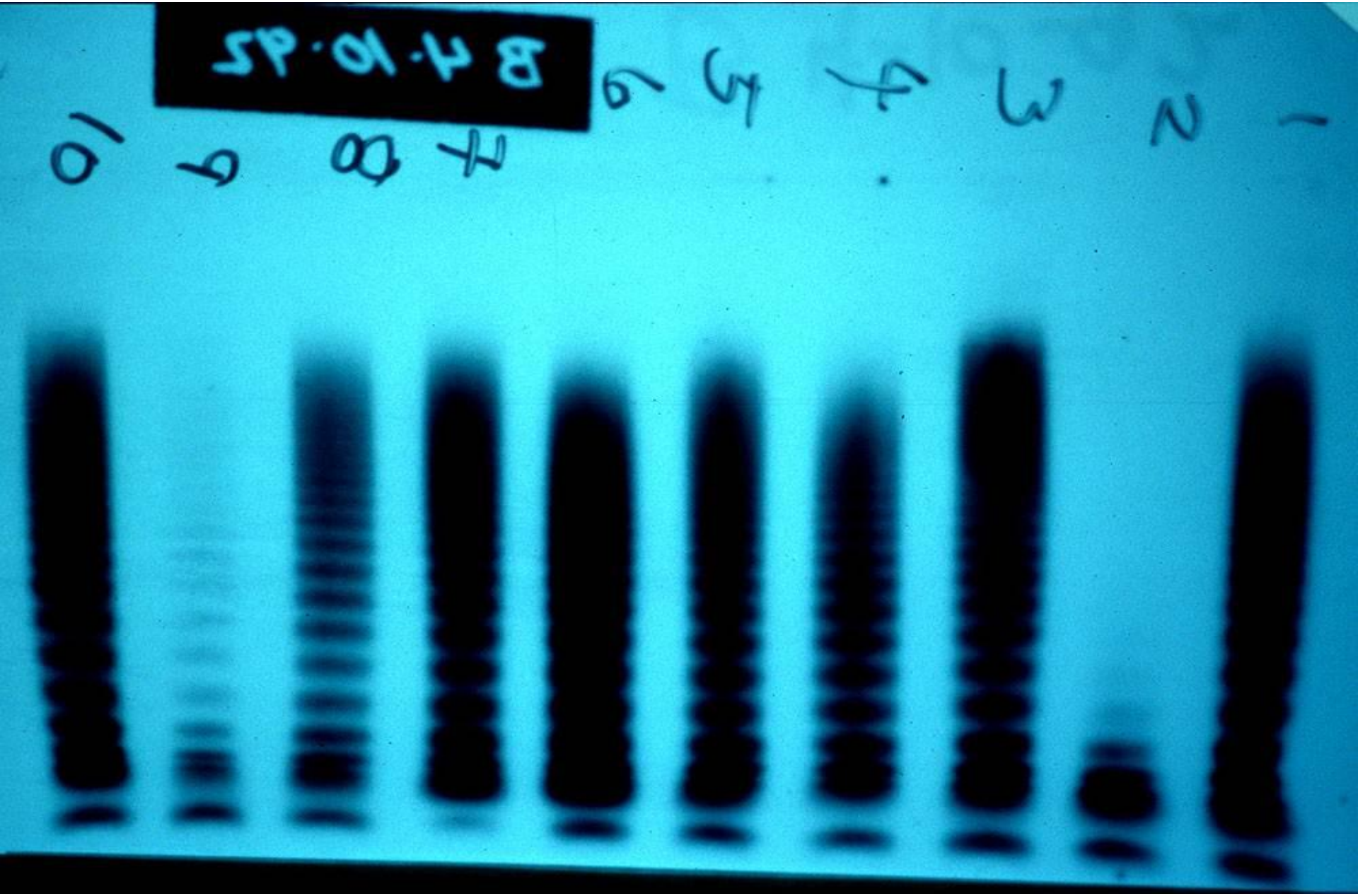
- *If Protein C activity is normal, need not do PC Ag.*
- *If free Protein S is normal, need not do total PS. (Free PS latex particle enhanced immunoassay, IL).*
- *If AT activity is normal, need not do AT Ag.*

# *VON WILLEBRAND DISEASE (VWD)*

- *The most common inherited bleeding disorder caused by quantitative or qualitative defects of VWF.*
- *Prevalence of 1-2 % in the general population.*
- *Different management strategies in the various types of vWD underlie the importance of classification.*

# *CLASSIFICATION OF VWD*

- *Type 1 (partial quantitative deficiency, most common type)*
- *Type 2 (qualitative defect)*
- *Type 3 (total deficiency)*
- *Based on specific structural abnormalities, type 2 vWD is further classified into 4 subtypes (2A, 2B, 2N, 2M)*



Von Willebrand Multimers

# PATTERNS OF FVIII RESULTS IN VWD

| | FVIII:c | VWF:Ag | VWF:Ac | CBA | Ag:CBA |
|---|---|---|---|---|---|
| Normal | N | N | N | N | N |
| Type 1 vWD | N-↓ | ↓ | ↓ | ↓ | N |
| Type 3 vWD | Absent | Absent | Absent | Absent | - |
| Type 2A vWD | N-↓ | N-↓ | N-↓ | ↓↓↓ | ↑↑↑ |
| Type 2B vWD | N-↓ | N-↓ | N-↓ | ↓ | ↑ |
| Type 2M vWD | N-↓ | N-↓ | ↓↓ | N-↓ | N |
| Type 2N vWD | ↓↓↓ | N | N | N | N |

## *INITIAL LABORATORY EVALUATION OF VWD*:

- ❑ *Bleeding time*
- ❑ *Platelet count*
- ❑ *APTT*
- ❑ *FVIII:c*
- ❑ *vWF Activity*
- ❑ *CBA*
- ❑ *Blood Group*
- ❑ *RIPA*

## *MULTIMER ANALYSIS*

- *Performed when Type 2vWD is suspected.*
- *A plasma sample is electrophoresed on a gel to separate the multimers by size.*
- *Type 2A:distribution of HMW vWF multimers lacking.*
- *Type 2M: normal distribution.*

# TESTS ASSAYS IN vWD

| Type | RIPA | Multimers | Plat. GP1b | Collagen | FVIII |
|---|---|---|---|---|---|
| 1 | N-↓ | N | - | N-↓ | - |
| 2A | ↓ | Lack MMW& HMW | ↓ | ↓ | - |
| 2B | ↑↑ | Lack HMW | ↑↑ | ↓ | - |
| 2M | ↓ | N | ↓ | - | - |
| 2N | N | N | - | - | Low |
| 3 | absent | undetectable | - | undetectable | - |

كن خيرًا و سيعو د الخير لك.

Do good. and good will come to you.