



# Coagulopathy Case – 5

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# CLINICAL HISTORY

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- **PATIENT:** 45 year-old woman
- **CLINICAL HISTORY**
- This patient was admitted with massive hemoptysis. At the time of admission, she was receiving Coumadin (sodium warfarin) for superficial thrombophlebitis. The patient's prothrombin time was 21 seconds, although she had not taken Coumadin since the day preceding admission
- **MEDICAL HISTORY:** The patient had an episode of deep vein thrombosis after the birth of her second daughter when she was 30 years old. The patient was placed on heparin, which was then followed by oral anticoagulants for 6 months.

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- After a spontaneous abortion at the age of 32, the patient was placed on oral contraceptives. She subsequently experienced superficial left calf vein thrombophlebitis, and the oral contraceptives were discontinued. Two months prior to this admission, patient developed superficial thrombophlebitis of the legs that were not associated with trauma. Patient was started on Coumadin at that time.

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- **FAMILY HISTORY:** The paternal grandfather, who died at 32 years of age, had a history of “swollen legs”. The patient’s father had a pulmonary embolism at 27 years of age after appendectomy, and he died suddenly at 42 years of age after an episode of DVT of the left leg. Also, a cousin died at 39 years of age after several episodes of thrombophlebitis. Another cousin, 63 years of age, had a number of problems with thrombotic disease, including a history of arterial embolism that necessitated amputation of the left leg at the knee.
  - **DRUG HISTORY:** At the time of the initial evaluation, the patient was on no medication.

# PHYSICAL EXAMINATION

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- Superficial varices were noted over the legs.



# Subsequent workup

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- Positive for DVT/PE using imaging study

# SCREENING COAGULATION LABORATORY RESULTS

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- PT= 21 sec (Normal 8-14.6), INR=2.7
- aPTT= 38 sec (Normal 24-34.5)
- Plt= 315,000 / $\mu$ L (Normal 130,000-350,000)

# Differential Diagnosis

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Factor V Leiden

Prothrombin gene mutation

Protein C deficiency

Protein S deficiency

Antithrombin deficiency

Lupus anticoagulant (LA)

Anticardiolipin antibodies (ACA)

Hyperhomocysteinemia +/- secondary to  
Methylenetetrahydrofolate reductase  
mutation



## Further test results

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- F V Leiden mutation, heterozygous
- All other test results: negative

# Overview of Hypercoagulation (thrombophilia)

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- Hypercoagulation: poorly understood phenomena
- No definite cause is identified in > 40% of cases
- Three major factors in thrombus formation (Rudolf Virchow, 1845): decreased blood flow; changes in the circulating blood (coagulation factors & inhibitors); changes in the vessel wall

# Overview (cont'd)

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- Review is restricted to changes in circulating blood
- Identification of the etiology is critical for: specific treatment (LA in pregnancy); long-term treatment; counseling of family members (inherited disorders)
- Approach to diagnosis: clinical history, family history, laboratory tests

# Hypercoagulation Disorders

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- Well established:

Factor V Leiden (12-40% of hypercoagulation cases), V

Prothrombin gene mutation (6-18%), V

Protein C deficiency (6-10%), V

Protein S deficiency (5-10%), V

Antithrombin III (AT III) deficiency (5-10%), V

Lupus anticoagulant (LA) (10-20%), A+V

Anticardiolipin antibodies (ACA) (5-10%), A+V

Heparin-induced thrombocytopenia, A+V

Hyperhomocysteinemia (10-20%) +/- secondary to

Methylenetetrahydrofolate reductase mutation, A+V

- Legends: A (arterial thrombosis), V(venous thrombosis)

## F V LEIDEN: INTRODUCTION

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- Dahlback described an inherited (autosomal dominant) disorder associated with venous hypercoagulation (1993).
- This disorder is due to a mutation in Factor V gene on chromosome 1 (the mutated gene is called Factor V Leiden). Mutation at nucleotide 1691: Guanine-> Adenine, causing substitution at position 506: Arginine-> Glutamine

Note: FV HR2 haplotype (A4070G, His199Arg) has unknown risk

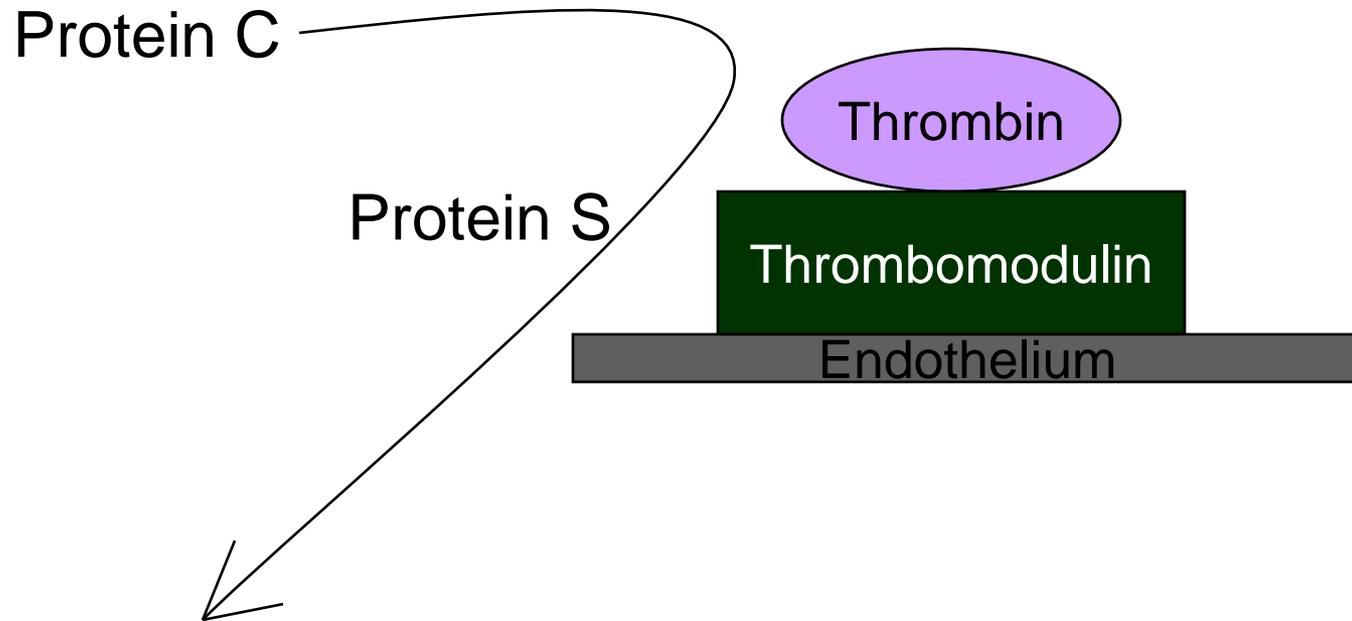
## INTRODUCTION (cont'd)

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- Review of Protein C pathway: down-regulation of coagulation with activated protein C complex (APC)

# Protein C Pathway

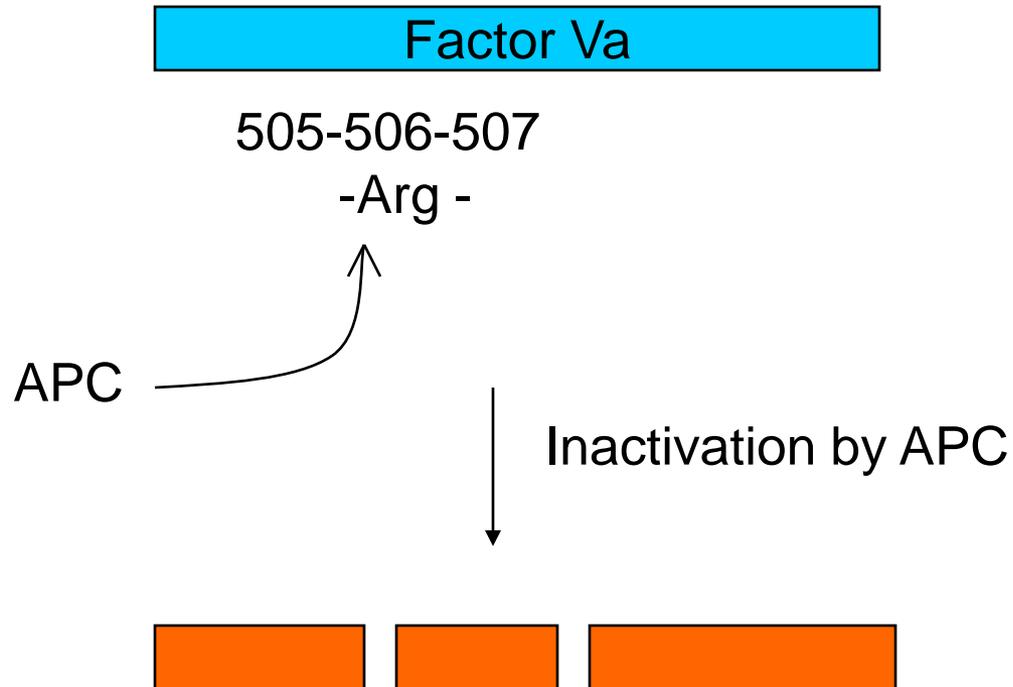
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Activated Protein C → Inactivates F Va , F VIIIa

# Cleavage Site on Factor V by APC: Inactivation of Factor V in normal patient

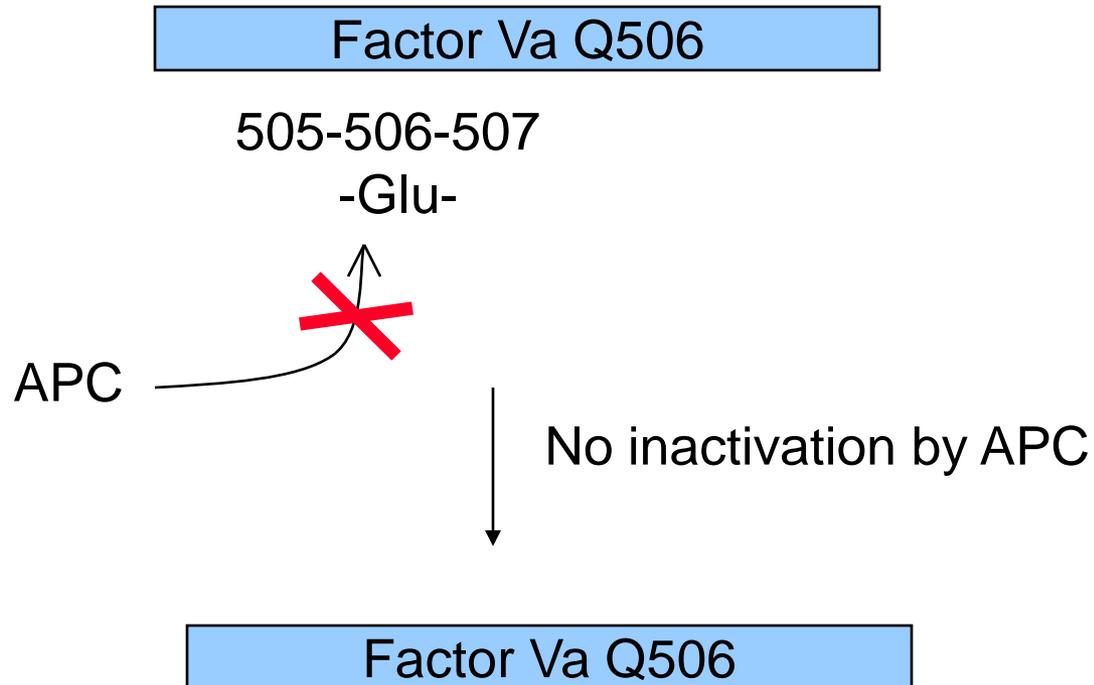
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## Cleavage Site on Factor V by APC:

No inactivation of Factor V in patient with  
Factor V Leiden (95% of APC resistance cases)

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## Two Forms of Factor V Leiden

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- Heterozygous: 3-7% of general population, 3-5 fold increase in risk of deep vein thrombosis, 20% have thrombosis by 33 y/o (mean age of first thrombotic episode)
- Homozygous: 0.06-0.25% of general population, 50-100 fold increase in risk of deep vein thrombosis, 40% have thrombosis by 33 y/o

## Other Relevant Information on Factor V Leiden

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- Some patients do not have thrombosis unless exposed to hemostatic challenge
- Increased risk for hypercoagulation in combination with other risk factors (such as Protein C or S deficiency)
- Factor V procoagulant activity is normal
- Treatment: heparin, coumadin

# Testing for Factor V Leiden

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- Clot-based testing (blue top tube)
- Polymerase chain reaction (PCR) testing (purple top tube)

## Clot-based Testing

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- Determines the resistance to APC, using platelet-poor plasma
- Principle of test: in patient with APC resistance, Factor V is not inactivated by APC, hence (PTT with APC) is not prolonged. This will shorten the APC Ratio (APCR)

## Clot-based Testing (cont'd)

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- $APCR = (PTT \text{ with APC}) / (PTT \text{ without APC})$

$APCR > 2$  -> negative for APC resistance

$APCR < 2$  -> positive for APC resistance

- Considerable overlap between FV Leiden heterozygous and normal

Note: cut-off value is dependent on particular test kits

## Clot-based Testing (cont'd)

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- Inaccurate result with: intrinsic factor deficiency, lupus anticoagulant, anticoagulant (need to get pre-treatment sample)
- New generation test (COATEST by Chromogenix)
  1. Predilution of patient sample with FV deficient plasma before testing: alleviates coumadin interference
  2. Polybrene: alleviates heparin interference

## PCR Testing

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- Amplifies the mutated gene fragment. Results: negative, heterozygous, homozygous.
- Results not effected by factor deficiency, lupus anticoagulant, anticoagulant
- PCR testing cannot detect APC resistance that is not due to FV Leiden

## PCR Testing (cont'd)

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- Genomic DNA from lymphocytes
- DNA sequence flanking the mutation site is amplified by PCR, resultant product is analyzed by restriction enzyme digestion
- Normal (wild type): two normal FV alleles  
Heterozygous: one abnormal allele  
Homozygous: two abnormal alleles

## New PCR Testing: LightCycler (Roche)

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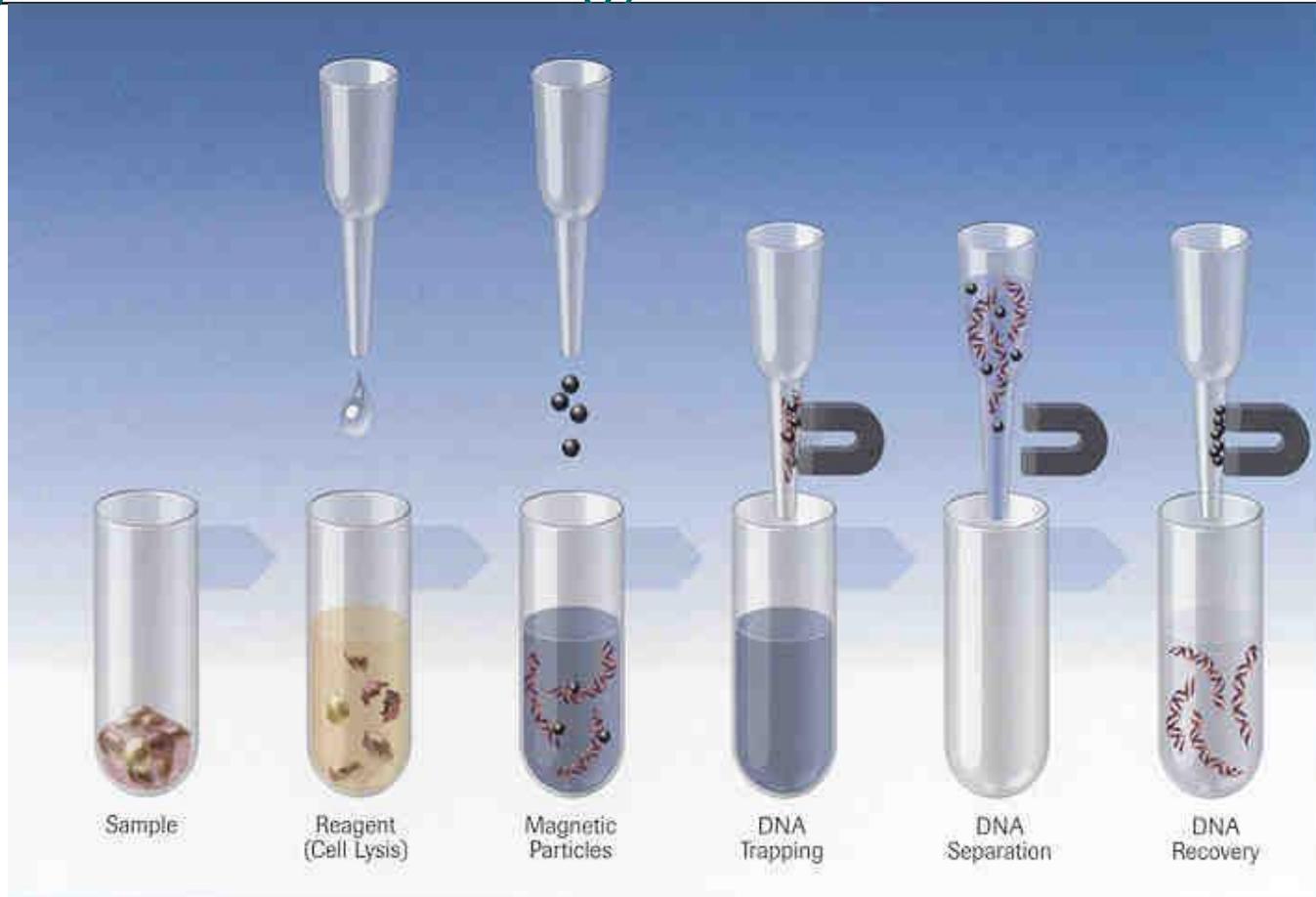
- Melting curve analysis method
- “Real time” analysis
- 35 thermal cycles in 25 min, followed by melting curve analysis in 5 min -> results in 30 min
- Batch of 32 samples
- Designed for clinical lab setting
- Optional module for automated DNA extraction (60 min for 32 sample extraction)

# MagNA Pure LC and LightCycler

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# Magnetic Bead Technology for DNA Extraction



# LightCycler Schematics

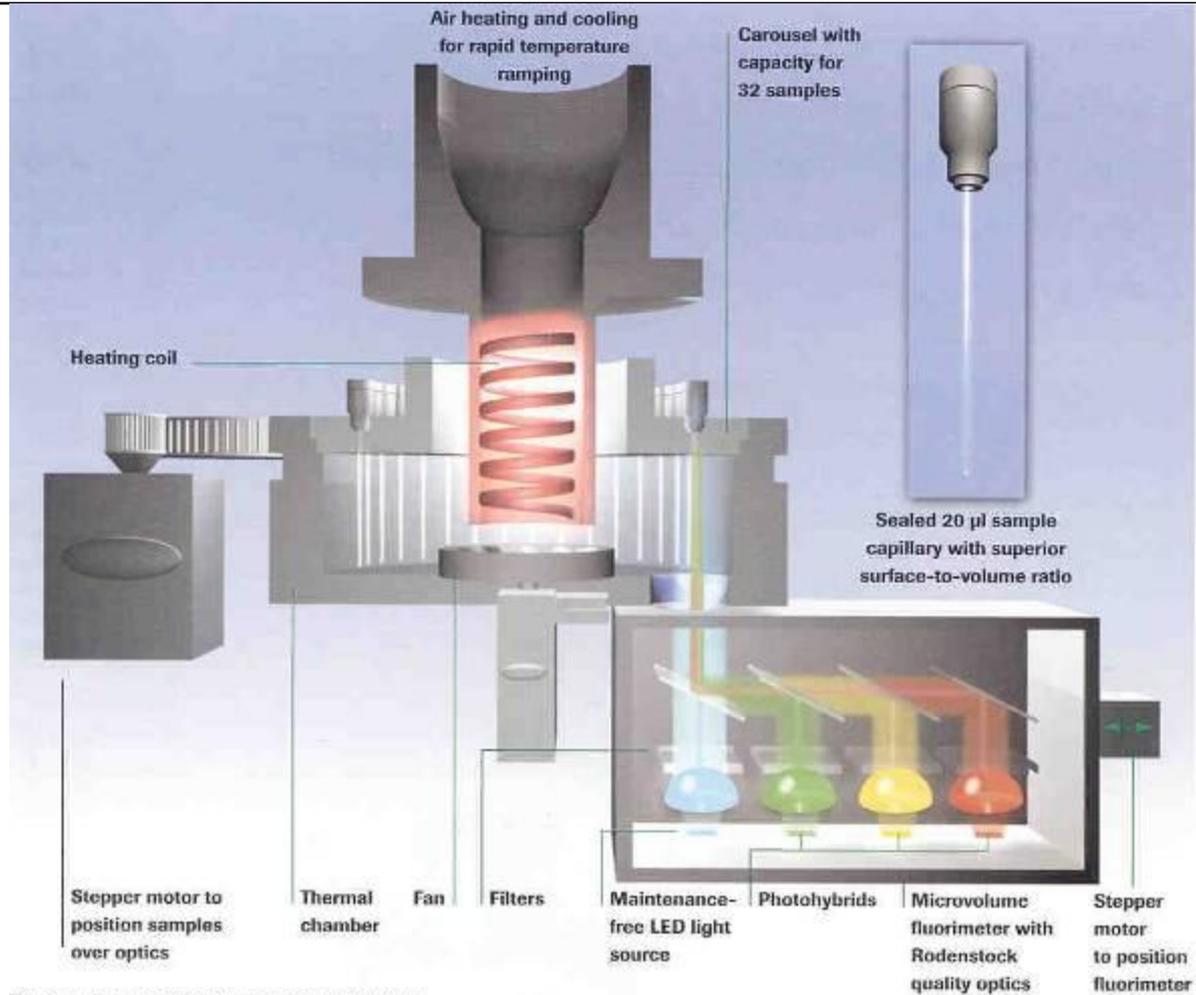
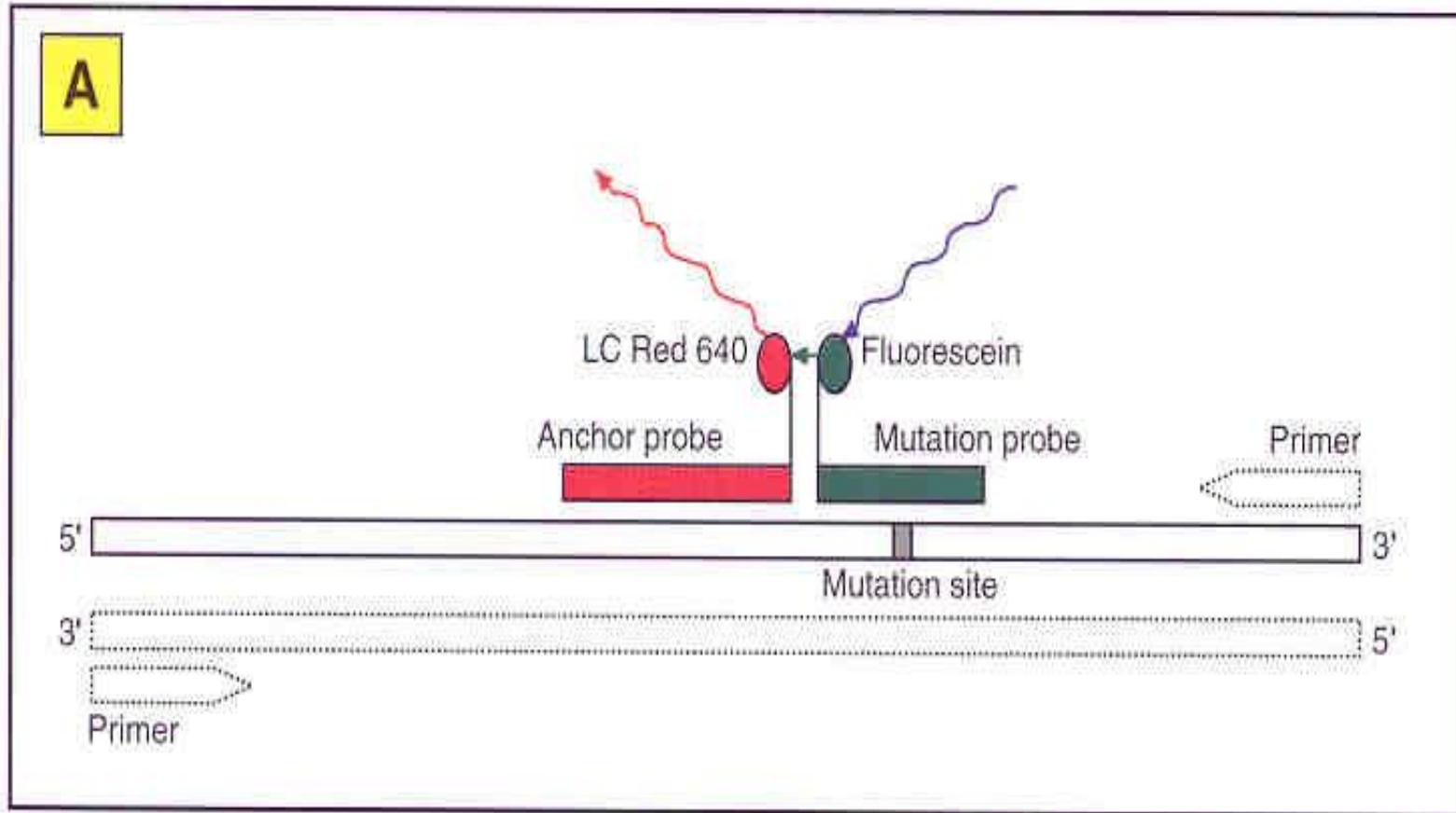
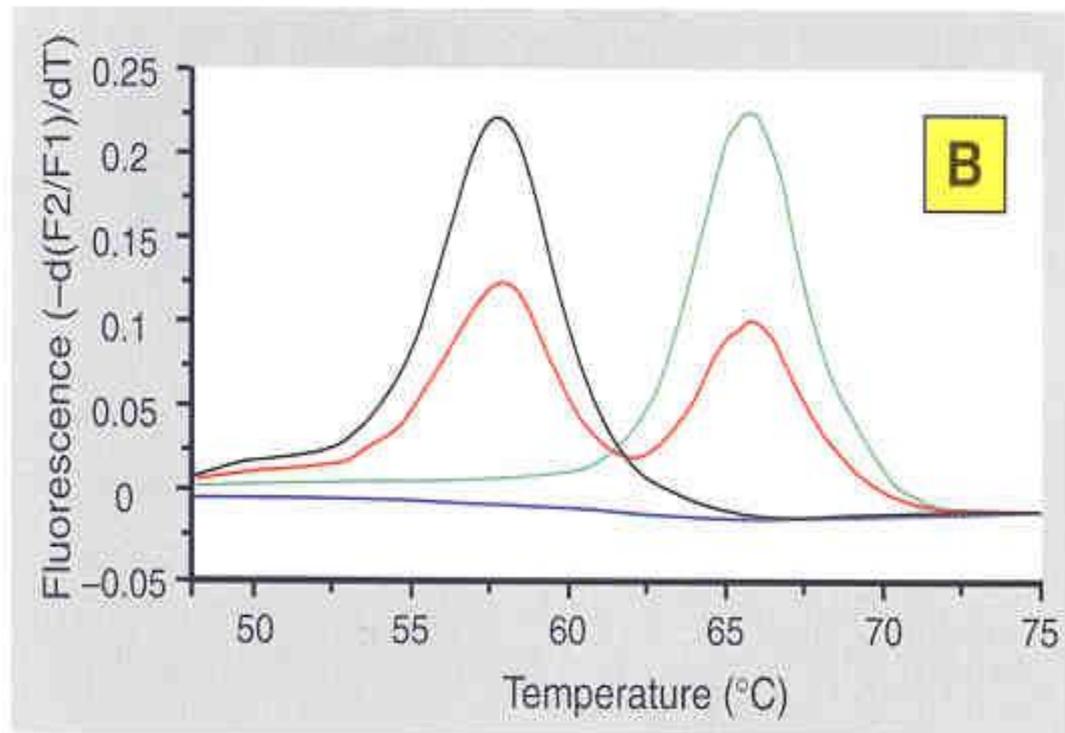


Figure 1. Schematic of the LightCycler System.

# FV Leiden Mutation: Hybridization Probe with Fluorescence Resonance Energy Transfer (FRET)



## FV Leiden Mutation: Melting Curve Analysis



- Sample 1 No template control
- Sample 2 Homozygous wild type
- Sample 3 Heterozygous
- Sample 4 Homozygous mutant

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- On to brief information on other causes of hypercoagulation....

# Prothrombin Gene Mutation

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- Single base pair substitution at nucleotide position 20210 in chromosome 11, guanine-> adenine (G20210A). This results in relatively high prothrombin level with increased risk for venous thrombosis (132% vs 105% of normal)
- Autosomal dominant. 1-3% of Caucasian population; risk increased 3 fold (heterozygote)
- 5-18% of hypercoagulation cases. Tx: heparin, coumadin
- Laboratory: PCR testing for G20210A, Factor II assay (optional)

# Protein C Deficiency

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- Protein C: a vitamin K-dependent coagulation inhibitor; synthesized in the liver; inactivating F Va and F VIIIa
- Protein C deficiency: autosomal dominant; 0.14-0.5% of population; risk increased 6.5-8 fold; 6-10% of hypercoagulation cases
- Clinical manifestation: recurrent deep vein thrombosis, pulmonary embolism, neonatal purpura fulminans (in homozygote). Tx: heparin, Coumadin
- Laboratory: immunological, functional assays, no mutation testing (>160 mutations)

# Protein S Deficiency

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- Protein S: a vitamin K-dependent protein; synthesized in the liver and megakaryocytes; cofactor of protein C
- Protein S deficiency: autosomal dominant; 0.7% of population; risk increased 1.6-11.5 fold; 5-10% of hypercoagulation cases
- Clinical manifestation: recurrent deep vein thrombosis, pulmonary embolism, neonatal purpura fulminans. Tx: heparin, coumadin
- Laboratory: immunological assay, functional assay, no mutation testing (>70 mutations)

# AT III Deficiency

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- AT III: inactivates thrombin and other factors (Xa, IXa, XIa, XIIa, kallikrein); accelerated by heparin
- AT III deficiency: autosomal dominant; 0.17% of population; risk increased 5-8.1 fold; 5-10% of hypercoagulation cases
- Clinical manifestation: recurrent deep vein thrombosis, pulmonary embolism. Tx: AT III, heparin, coumadin
- Laboratory: functional assay (chromogenic), immunologic assay, no mutation testing (>250 mutations)

# Lupus Anticoagulant

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- Immunoglobulins that prolong in-vitro phospholipid-dependent clotting times
- Found in various conditions; 30% of patients have thrombosis ; 10-20% of hypercoagulation cases
- Antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibody, antiphosphatidyl serine, anti Beta 2 Glycoprotein I, etc): 1-2% of population, 50% of SLE patients
- Clinical manifestation: variety of thrombotic diseases. Tx: Heparin, Coumadin, Aspirin & prednisone (to prevent fetal demise)
- Laboratory: aPTT, dilute Russell Viper venom time (dRVVT), Hexagonal Phospholipid Neutralization

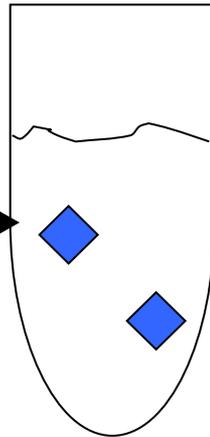
# Evaluation of lupus anticoagulant

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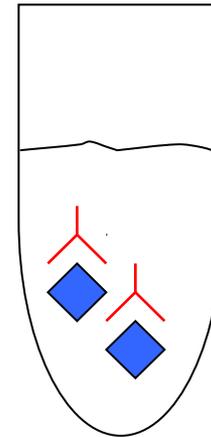
- Mixing studies
  - Mix equal parts patient and control plasma
  - aPTT will correct if prolongation due to factor deficiencies
  - If LA present will fail to correct aPTT
    - Usually immediate acting (before incubation)

## Dilute Russell Viper Venom Time

Phospholipid



Normal plasma  
dRVVT 36-42 sec



Plasma with lupus anticoagulant  
dRVVT > 43 sec

# Evaluation of lupus anticoagulant

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Neutralization study:

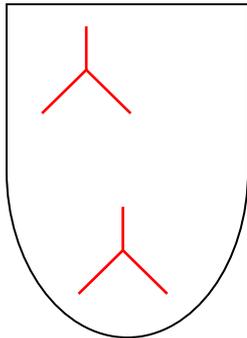
Addition of phospholipid will neutralize lupus anticoagulant

Two common neutralization tests:

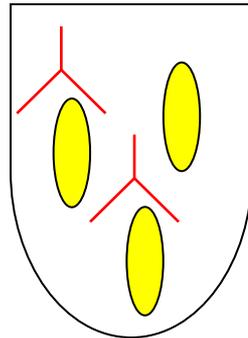
- Platelet neutralization:  
lysates of frozen, thawed and washed platelets
- Hexagonal phase phospholipid neutralization:  
hexagonal phospholipid with high affinity for lupus anticoagulant

# LA Confirmatory Tests

## Platelet Neutralization

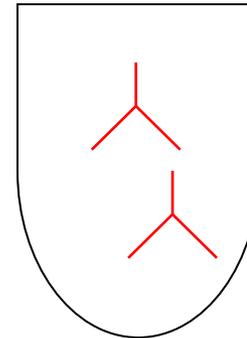


Prolonged aPTT

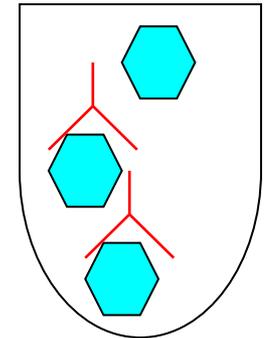


Shortened aPTT

## Sta Clot-LA



Prolonged aPTT



Shortened aPTT

Clotting time >8 seconds shorter after addition of PL = + for LA

# Anticardiolipin Antibodies

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- ACA: IgG, IGM, IgA
- Found in various conditions; thrombotic manifestations; in 5-10% of hypercoagulation cases. Tx: not well worked out, including Heparin, coumadin, and steroid
- Laboratory: ACA by ELISA; high levels are associated with high risks of thrombosis

## Anticardiolipin Antibodies (cont'd)

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	Normal range	Clinically insignificant	Moderate risk	High risk
IgG	< 15 GPL	15-20	20-80	> 80
IgM	< 12.5 MPL	12.5-20	20-80	> 80
IgA	< 15 APL	15-20	20-80	> 80

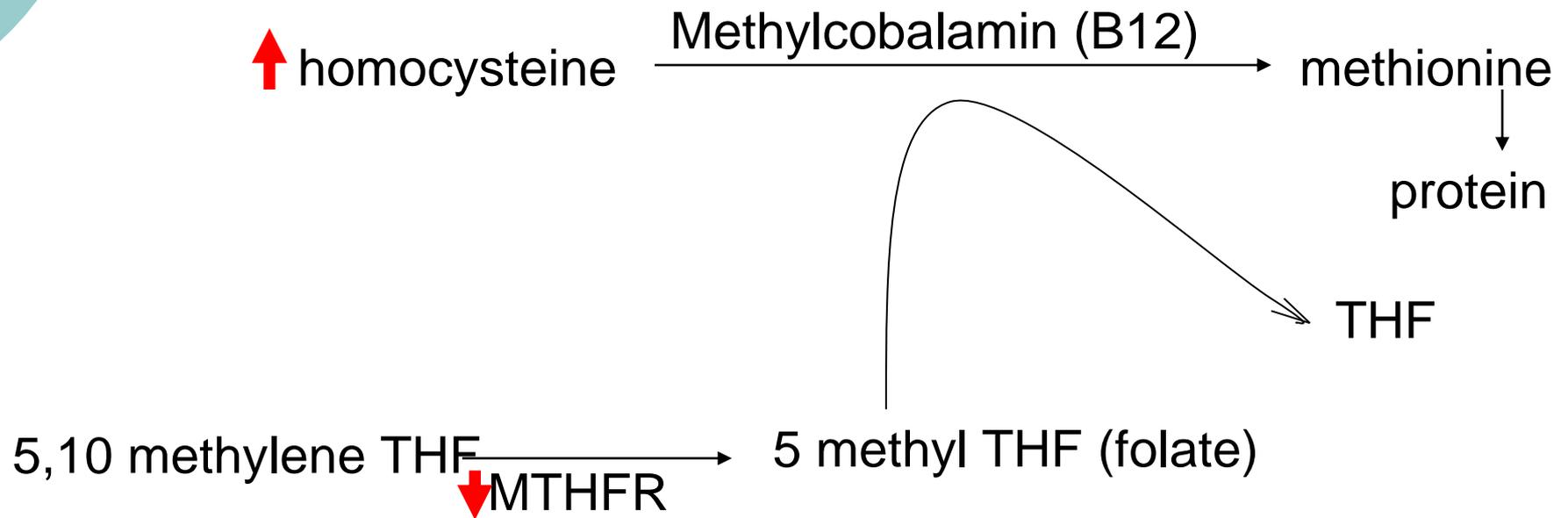
# Methylenetetrahydrofolate reductase (MTHFR) mutation

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- MTHFR is an enzyme in the folate-dependent homocysteine remethylation, catalyzing the reduction of 5,10 methylenetetrahydrofolate -> 5 methyltetrahydrofolate
- Single base pair substitution at nucleotide location 677 in chromosome 1, Cytosine->Thymine (C677T). This results in Alanine-> Valine at location 223 (A223V), decreased MTHFR, causing hyperhomocysteinemia and subsequent thrombophilia (mechanism: blood vessel injury, coagulation activation, fibrinolysis inhibition, platelet activation)
- Autosomal recessive, 11% of Caucasian population (homozygote); increased risk 3 fold (homozygote). Tx: folate with or without vitamin B6 (pyridoxine) and vitamin B12
- Laboratory: homocysteine assay; PCR testing (currently not fully accepted as part of the routine battery)

# MTHFR Deficiency

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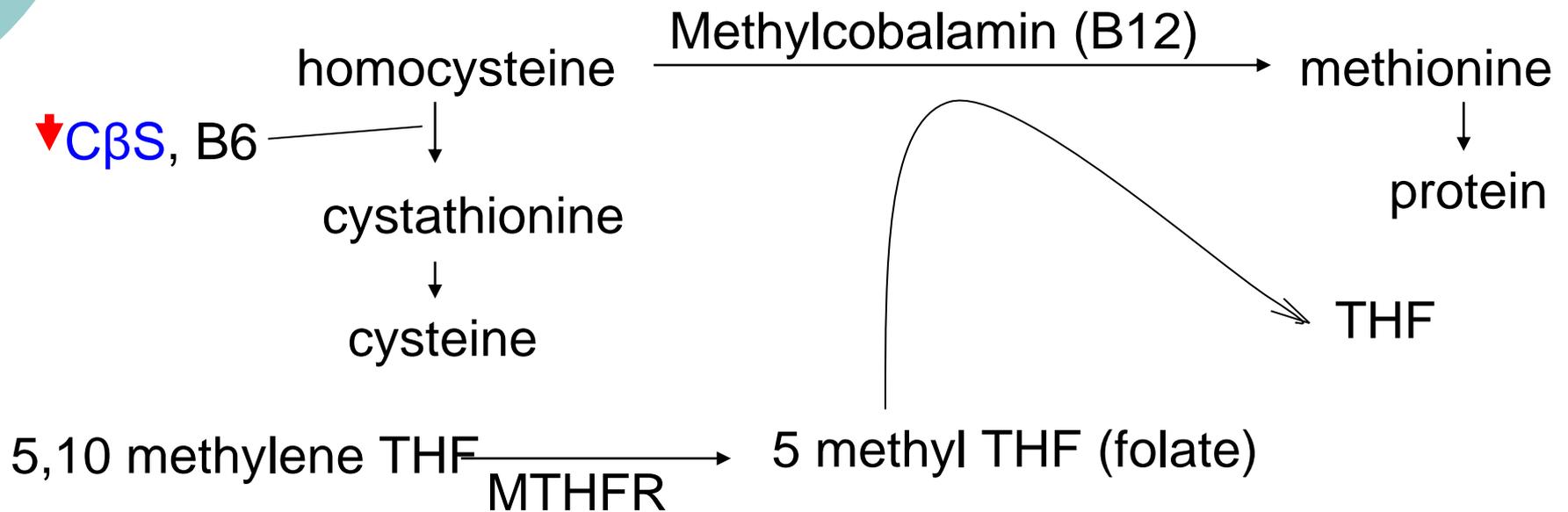
# Methylenetetrahydrofolate reductase (MTHFR) mutation (cont'd)

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- Other causes of hyperhomocysteinemia:
  - B12, folate, B6 deficiency
  - Renal failure
  - Hypothyroidism
  - Meds: methotrexate, phenytoin, theophylline
  - Malignancy
  - SLE
  - Rare homozygous genetic disease:  
Cystathionine  $\beta$ -synthase (C $\beta$ S) deficiency-> homocysteinuria

## Cystathionine $\beta$ -synthase (C $\beta$ S) deficiency-

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# Genetic and Environmental Factors

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- Combination of risks: genetic abnormality + environment factor (trauma, surgery, immobility, pregnancy, oral contraceptive, etc.)
- Multiple genetic abnormalities lead to synergistic effect:  
Example: Factor V Leiden heterozygote (risk 3-6 fold) +  
Prothrombin gene mutation heterozygote (risk 3 fold)-> risk 25 fold
- Hypercoagulation is relatively expensive since the panel includes all significant tests

# Interference in hypercoagulation tests

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- Patients in active DVT/PE may have low levels of: AT, Protein C, protein S (normal levels are still useful)
- Heparin affects: APC Resistance, lupus anticoagulant
- Coumadin affects: protein C, protein S
  
- For baseline testing of the above:
  - Get pre-anticoagulant samples
  - Test patients after discharge (follow-up visit)
  - Patients off heparin for one day
  - Patients off coumadin for 2 weeks