Lab Protocols

Hemocoagulation tests
Blood groups

v. 0.2

physiology.lf1.cuni.cz

Labs aim: Explore biology in context through brain and hands

DRAFT

Blood Coagulation tests

BRIEFLY:

- *Hemocoagulation* is one of (three major) *hemostatic* (stopping bleeding) mechanisms.
- During coagulation blood becomes solid - principally due to the transformation of plasma protein fibrinogen into polymerized insoluble fibrin.
- *Blood Coagulation Tests* determine the speed of coagulation. They are necessary before starting surgery and during *anticoagulation therapy* (e.g. of thrombosis)

AIM of the lab

- Understand *coagulation* and *anticoagulation* mechanisms
- Know *coagulation tests* and usage
- Know *anticoagulation* procedures

REQUIRED KNOWLEDGE (major topics)

Hemostasis, hemocoagulation, clotting factors (principle), coagulation pathways (principles), vitamin K, anticoagulation, Heparin, thrombus, thrombosis, plasma calcium

TASKS

1. Anticoagulation tests
   1.1. INR
   1.2. APTT
   1.3. capillary fragility test

WHAT

- Blood Coagulation:
  - PubMed: The process of the interaction of BLOOD COAGULATION FACTORS that results in an insoluble FIBRIN clot. (MeSH tree#
  - Wikipedia: The **coagulation** of blood is a complex process during which blood forms solid clots. It is an important part of hemostasis (the cessation of blood loss from a damaged vessel) whereby a damaged blood vessel wall is covered by a fibrin clot to stop hemorrhage and aid repair of the damaged

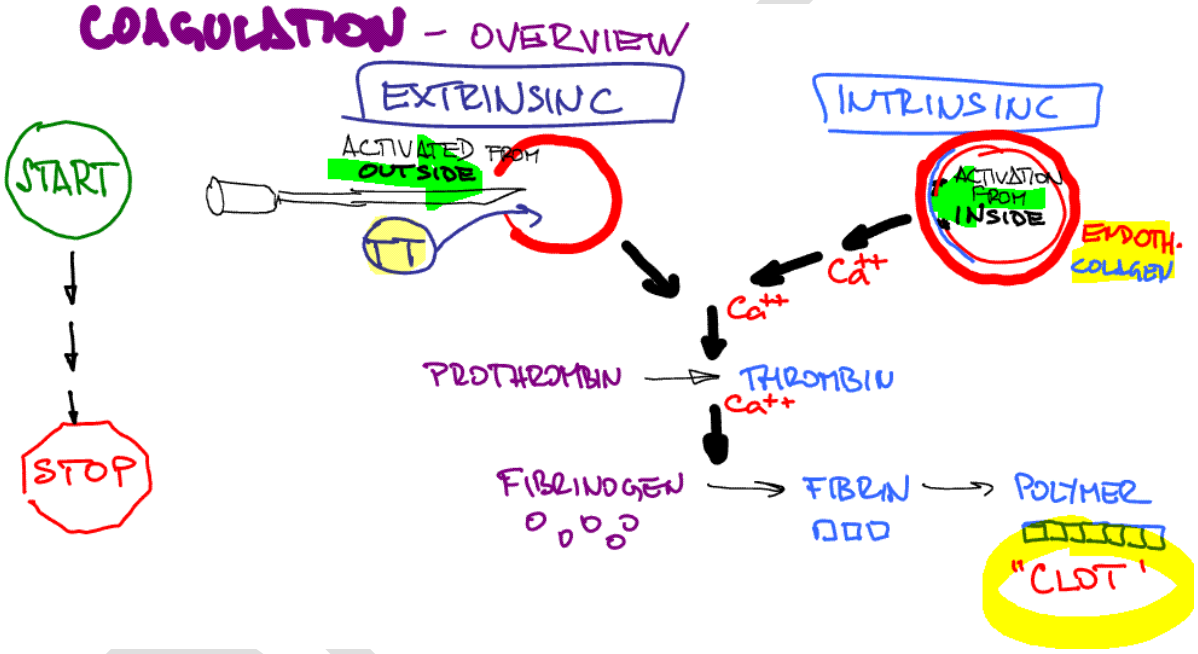
vessel. Disorders in coagulation can lead to increased hemorrhage and/or thrombosis and embolism.

WHY

- Why do we perform the lab:
  - Understand common coagulation tests
  - Know why and when to test coagulation
    - Surgery. **Why?**
    - Delivery. **Why?**
    - Anticoagulation therapy. **Why?**
    - …
  - Discuss and see anticoagulation methods.

HOW does that work?

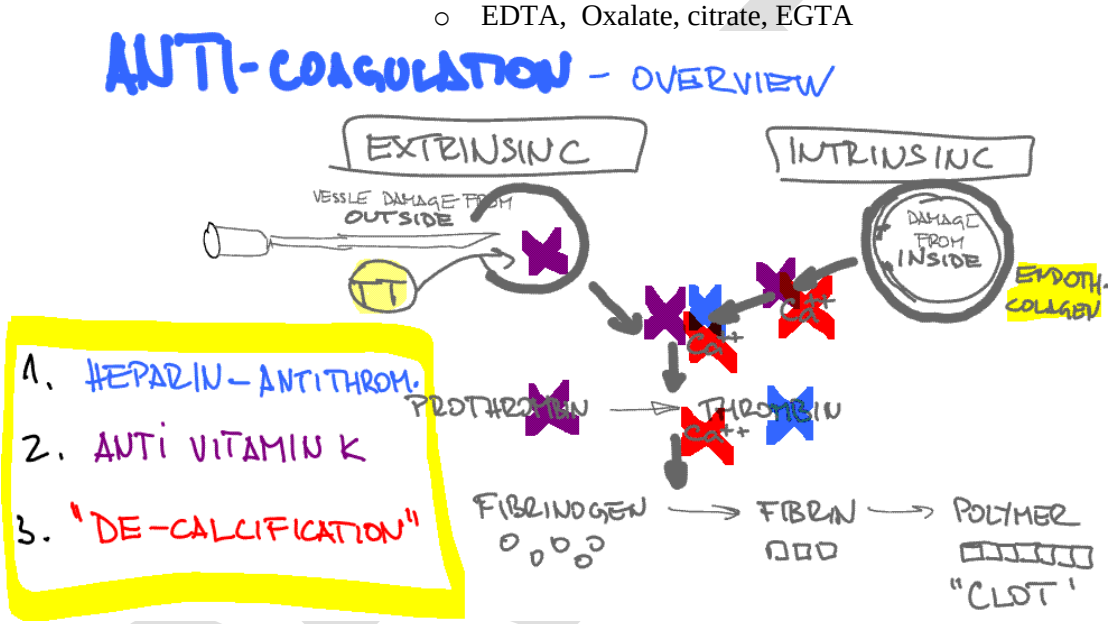
1. **COAGULATION**



  - Hemocoagulation has one endpoint - formation of fibrin polymer.
  - It has two distinct starting points:
    - Stimulation from inside a vessel. Ttypically endothelial damage and exposition of blood to collagen (intrinsinc pathway)
    - Stimulation from outside a vessel. Contact of blood with tissue substance *Tissue thromboplastin –TT* (extrinsinc pathway)
  - Between the starting and end points, a cascade of proteolytic reactions occurs. The actors of the reactions are called clotting factors. Most factors are plasma proteins (others represent: Ca^{++} and *phospholipids*)

2. **ANTICOAGULATION**
   - ***Physiology***
     - At any time, blood contains everything necessary for clotting
     - Thus it might clot even in vessels, that could be fatal.
     - Thus spontaneous unwanted coagulation must be prevented by effective and reliable mechanisms, such as:
       - **Blood flow** – reduces the chance of interaction of factors
       - **Intact endothelium –** prevents from activation of factors
       - **Anticoagulation factors** (endogenous)– inhibit ongoing coagulation process. E.g. Heparin – Antithrombin, …
   - ***Pharmacology***

- Anticoagulation is an important therapeutical strategy in some diseases where *thrombosis* (intravascular coagulation) appears. E.g.: stroke, coronary heart disease, deep venous thrombosis, implants, etc. short
- Major anticoagulation procedures:
  - **Heparin –** with Antithrombin (AT) deactivates some factors (mainly *thrombin* and *factor X*) already activated during coagulation.
  - **Anti-vitamin K (AVK)** e.g. coumarin (Warfarin, Macumar) interfere with the *production* of certain coagulation factors (II, VII, IX, X) Consequently factors are less effective and coagulation times are prolonged.
  - **"de-calcification"** Ca^{++} (ionized, free) is necessary for many steps of coagulation cascade. If Ca^{++} is unavailable, clotting does not occur. The easiest way to let Ca^{++} interact with substances that form insoluble molecules/complexes. E.g.:
    - EDTA, Oxalate, citrate, EGTA



3. COAGULATION TESTS

- There are many tests, just two most common are mentioned.
- The ones we perform (APTT, INR) evaluate the *time* of coagulation *after* specific *activation* (i.e. "how long it takes to clot")
- The simplest test would be just to wait until blood spontaneously clots in a test tube (cca 5-10 minutes)
- "Our" tests are just slightly smarter (more sophisticted)
  - Use just plasma. **Why?**
  - Use specific activator:

Activator	Test name	pathway	Normal result
TT	Quick INR	extrinsic	15 s 0.9 – 1.1
PT	APTT	intrinsic	40 – 50 s

  - TT – tissue Thromboplastin

- INR - international normalized ratio

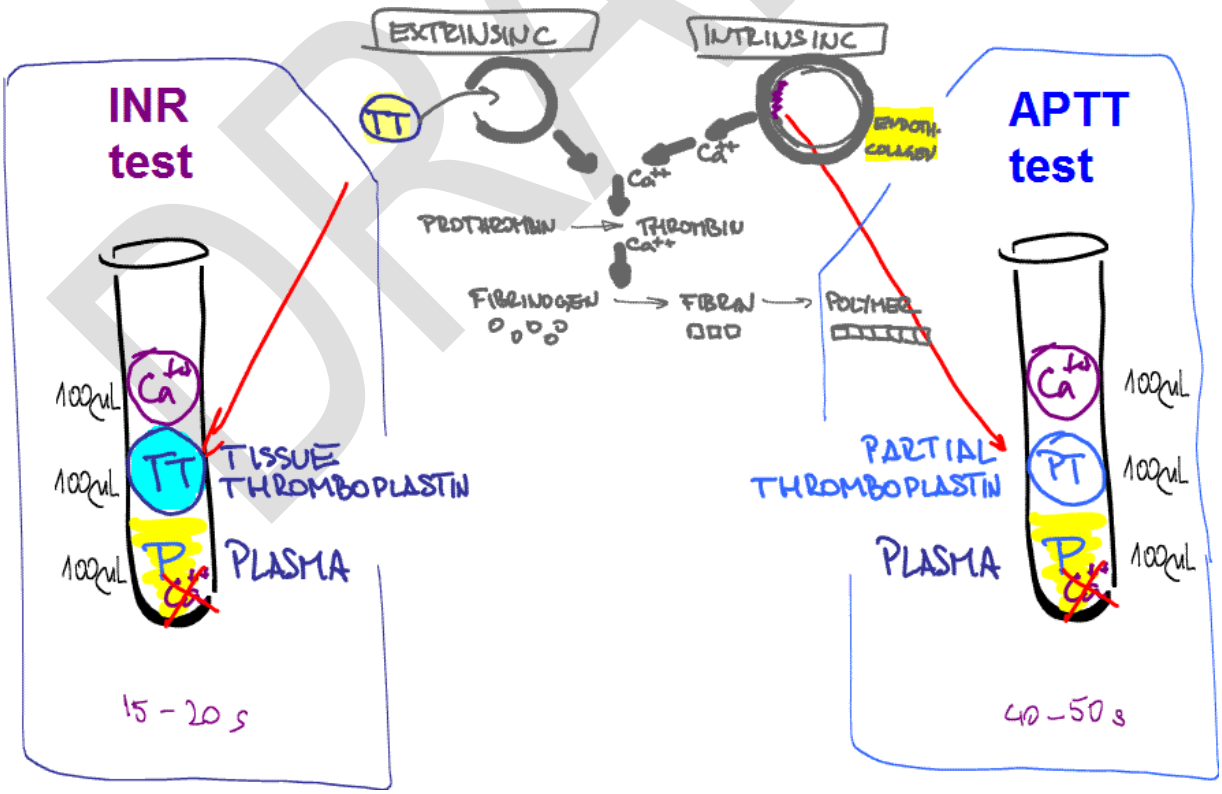
$$INR = \frac{Quick(patient)}{Quick(norm)}$$

- PT – partial thromboplastin
  - PT = diluted thromboplastin + clay
  - "pretends" endothelial damage
  - also called KK = kefalin + kaolin

SETUP:
Test tubes, water-bath, little hook to pull the fiber, stopwatch (on your phone;-) Really so simple ☺

Procedure

1. Get ready
   a. Chck that all reagents are available and heated to 37 °C. **Why?**
   b. Chck that test tubes are clean. Preheat them. **Why?**
   c. Make sure to use proper pipettes and fresh tips
2. Add reagents (100 µL of each, but always fresh tips!)
   a. Decalcified plasma. **Why decalcified?**
   b. Activator (TT or PT)
3. Start timing once $CaCl_2$ was added. Still keep in the bath. **Why?**
4. Use hook to pull the fiber
5. Stop timing once the first fiber is seen



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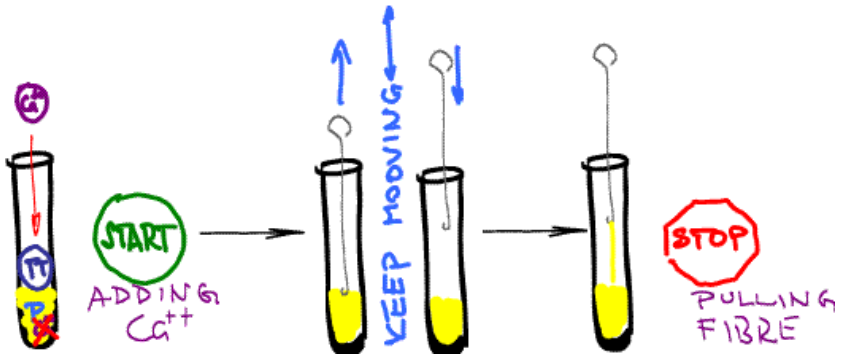

TASKS:

- APTT and Quick (INR) test for provided plasma(s) – marked P1, P2, … Perform as many tests (equal number of APTT and INR) as possible from given plasma.

RESULTS:

Sample	APTT [s]	Quick [s]	Rem.
P1			
P1			
P1			
P2			
P2			
P2			

Blood Groups

AIM of the lab

- Understand blood group evaluation
- Understand importance of blood group testing

REQUIRED KNOWLEDGE

- Blood, blood groups, antigen, antibody, antibody class, agglutination, cross-match, fetal erythroblastosis,

WHAT?

- Blood group is **antigen** determination of **erythrocytes**
- There are many antigens on each ery,
- thus there are many systems of blood groups.
  - Most important are: AB0, Rhesus, MN, …

WHY?

… is it important to know the group?

1. because of potentially fatal complications in case of incompatibility, typically:
   a. during transfusion and organ transplantations
   b. during certain pregnancies (of some Rh-neg. mothers)

   Knowledge of groups can completely prevent the complications

HOW?

… does that work?

- Erythrocytes have antigens on their surface
- Only **sometimes** plasma contains antibodies against non-self group. Why?

system	group	Antigen on RBC	Antibody in plasma
ABO	A	A	Anti B
AB0	B	B	Anti A
ABO	AB	A and B	None of above
AB0	0	None of above	Anti A and Anti B

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Rhesus	POS	D	usually none
Rhesus	NEG	D	usually none
MN	M	M	usually none
MN	N	N	usually none
…			usually none

- The problem is if erythrocytes and antibodies against them (e.g. *A* and *anti-A*) meet in bloodstream. Why? RBC – AB form complexes, stimulate immune system, and systemic shock can develop. This can be fatal in tens of minutes. Also hemolysis, renal failure and other complications occur.

HOW? … do we test the blood group?

- By mixing blood with specific monoclonal antibodies against RBC group antigens
- For AB0 group we need:
  - 2 drops of blood (e.g. from disinfected!!!!! finger)
  - 2 types of antibodies (Anti-A and Anti-B)
  - Something to work on and mix with
- Agglutination (clumping) occurs if RBCs are mixed with specific antibodies targeted against them.
- AGGLUTINATION IS NOT CLOTTING! ☺
- WHICH GROUP IT IS?
  - In the picture below showing the procedure, try to estimate which group it is (step 6)! Write your result.