Evaluation of global coagulation tests and their implications in haemophilia

Instutitionen för Translationell medicin, klinisk koagulationsforskning

Marcus Ljungkvist, Biomedicinsk analytiker / doktorand

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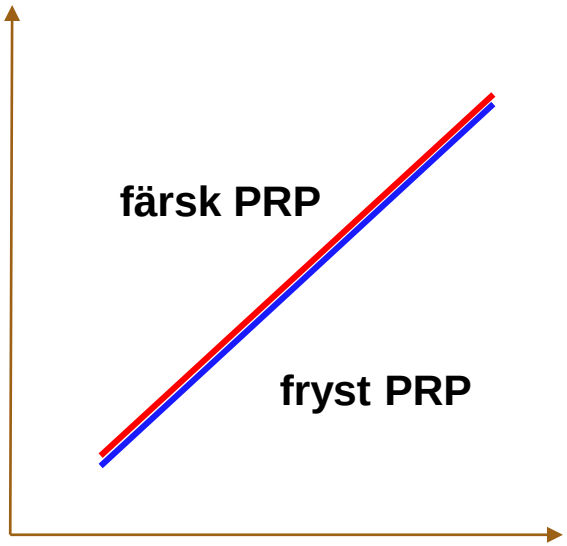
Correlation or agreement statistics?



God korrelation
God överensstämmelse

PRP – platelet-rich plasma

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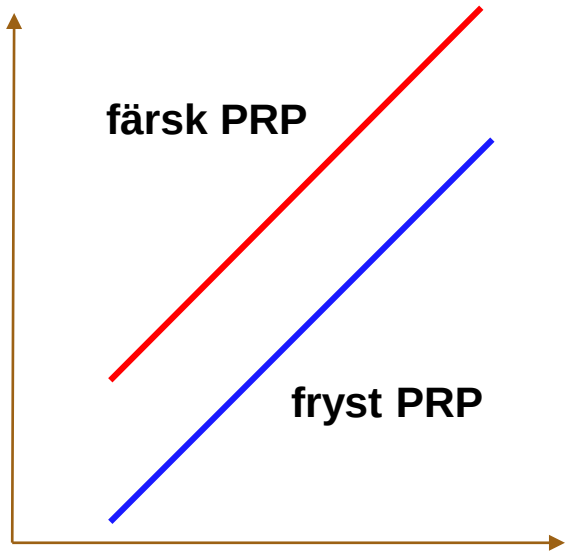
Correlation or agreement statistics?



God korrelation
Dålig överensstämmelse

PRP – platelet-rich plasma

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Evaluation of a standardized protocol for thrombin generation using the calibrated automated thrombogram - A Nordic study.

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Laboratory protocol

Laboratory protocol

Ver. 1.07

Start the analyzer 1 h before beginning the with step 1. if you want to start the moment you arrive at your laboratory make sure you have left the analyzer on over night.

Gather all material on the checklist(below) before you start the test run. Make and print a plate set-up using the Thrombinoscope software.

Prime the analyzer(at volume to 15000) with 3775 distilled water(50 ml tube).

Ver. 1.07

a 50 min countdown.

led ones.

Checklist

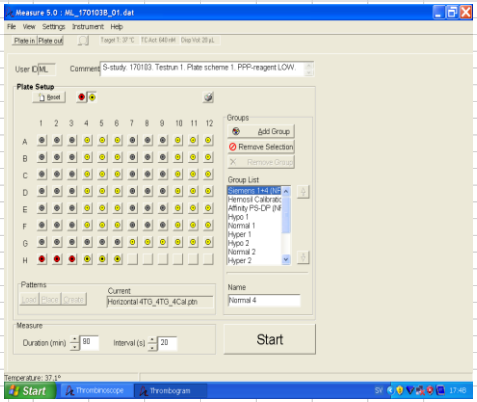
1 pipette set on 20 µl
1 pipette set on 80 µl
1 pipette set on 1000 µl
1 pipette set on 5000 µl
pipette tips for each pipette model
2 plastic tubes 50 ml(stand)
2 U-bottom plastic tubes 14 ml
a vortex mixer
a container for used pipette tips etc.
a screw cap flash 10ml (distilled water)
tube racks (for cryovials 0,5ml and U-bottom plastic tubes 14ml)

The pipettes have to be recently calibrated.
The analyzer´s latest maitenance service must not be older than a year at the time of the main study start.
Below a screenshot of the testrun setup, make sure you have the same parameters set, 640nM, 37°C, 20µl, 90min, 20s etc.

Picture 1.

Picture 2.

Picture 3.



Last but not least make sure you have registered the test run in the "Test run log" file.

nt and position the tip at the bottom
n the bottom and press to the first
touching the wall.
pipette tip as close to the surface
llow the pipette tip to slide against
agent/plasma formed on the outside

a, the tip of the syringe should be
l pressure should be applied from

ork swiftly and to try keep 37°C by
s (picture 3.).

brator, PPP-Reagent or
sIL Calibration plasma,
d now start the 50 min countdown.
m plastic tube, then cap it and

for reconstitution of reference

them in a 37°C water bath.
g 5ml distilled water. ● Reconstitute
ding 1ml distilled water.
ully between your hands to make

) or Advate 1-5 and Adynovate 1-5

g to the provided plate set-up
● Check that all wells are filled.
e wells with plasma/reference plasma
using reversed pipetting technique.

n incubation.
strate preparation by setting 65 µl.
least 5ml)
µl Fluo-Buffer (37°C) and

delay to keep the dispensing system

e use a Kleenex to wipe it dry and
e hole marked "M" and press start.
4 ml U-bottom plastic tube with
ge. ● Put the pin back to the hole
plastic tube with distilled water.

Ver 1.03

DAY 1 and 2

	First run of the day												Second run of the day											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
A	Siemens						HemosIL						A	Siemens						HemosIL				
B	Affinity PS-DP						Hypo PL						B	Affinity PS-DP						Hypo PL				
C	Normal PL						Hyper PL						C	Normal PL						Hyper PL				
D	Hypo PL						Normal PL						D	Hypo PL						Normal PL				
E	Hyper PL						Hypo PL						E	Hyper PL						Hypo PL				
F	Normal PL						Hyper PL						F	Normal PL						Hyper PL				
G	Hypo PL						Normal PL						G	Hypo PL						Normal PL				
H	Hyper PL												H	Hyper PL										

Wells in column 1-3 + 7-9, 20 µl PPP-reagent LOW.
Wells in column 4-6 + 10-12, 20 µl Thrombin Calibrator.

Wells in column 1-3 + 7-9, 20 µl PPP-reagent LOW.
Wells in column 4-6 + 10-12, 20 µl Thrombin Calibrator.

DAY 3

	First run of the day												Second run of the day											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
A	Siemens						HemosIL						A	Siemens						HemosIL				
B	Affinity PS-DP						Hypo PL						B	Affinity PS-DP						Hypo PL				
C	Normal PL						Hyper PL						C	Normal PL						Hyper PL				
D	Hypo PL						Normal PL						D	Hypo PL						Normal PL				
E	Hyper PL						Hypo PL						E	Hyper PL						Hypo PL				
F	Normal PL						Hyper PL						F	Normal PL						Hyper PL				
G	Hypo PL						Normal PL						G	Hypo PL						Normal PL				
H	Hyper PL												H	Hyper PL										

Wells in column 1-3 + 7-9, 20 µl PPP-reagent LOW.
Wells in column 4-6 + 10-12, 20 µl Thrombin Calibrator.

Wells in column 1-3 + 7-9, 20 µl **PPP-reagent.**
Wells in column 4-6 + 10-12, 20 µl Thrombin Calibrator.

DAY 4 and 5

	First run of the day												Second run of the day											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
A	Siemens						HemosIL						A	Siemens						HemosIL				
B	Affinity PS-DP						Hypo PL						B	Affinity PS-DP						Hypo PL				
C	Normal PL						Hyper PL						C	Normal PL						Hyper PL				
D	Hypo PL						Normal PL						D	Hypo PL						Normal PL				
E	Hyper PL						Hypo PL						E	Hyper PL						Hypo PL				
F	Normal PL						Hyper PL						F	Normal PL						Hyper PL				
G	Hypo PL						Normal PL						G	Hypo PL						Normal PL				
H	Hyper PL												H	Hyper PL										

Wells in column 1-3 + 7-9, 20 µl **PPP-reagent.**
Wells in column 4-6 + 10-12, 20 µl Thrombin Calibrator.

Wells in column 1-3 + 7-9, 20 µl **PPP-reagent.**
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Trombingenering och NOAK

“Large inter-individual variation of the pharmacodynamics effect of anticoagulant drugs on thrombin generation” Hemker et al. 2012.
“Laboratory monitoring of direct oral anticoagulants” av Tuukka Helin 2017.

NOAKs inhibitionseffekt varierar stort på individ nivå.

Koncentrations nivån av NOAK bestämmer inte ensamt den faktiska koagulationskapaciteten hos en individ.

↓

TGA-CAT

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Trombingenering och NOAK

TGA-CAT

tidskrävande (semiautomatisk)
låg standardiseringsnivå
dålig tillgänglighet

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Trombingenering och NOAK

TGA-CAT

tidskrävande (semiautomatisk)
låg standardiseringsnivå
dålig tillgänglighet

TGA

mindre tidskrävande (helautomatisk)
bra standardiseringsnivå
god tillgänglighet

helblods TGA-CAT (mindre tidskrävande, mer *in vivo* liknande analysförhållande)

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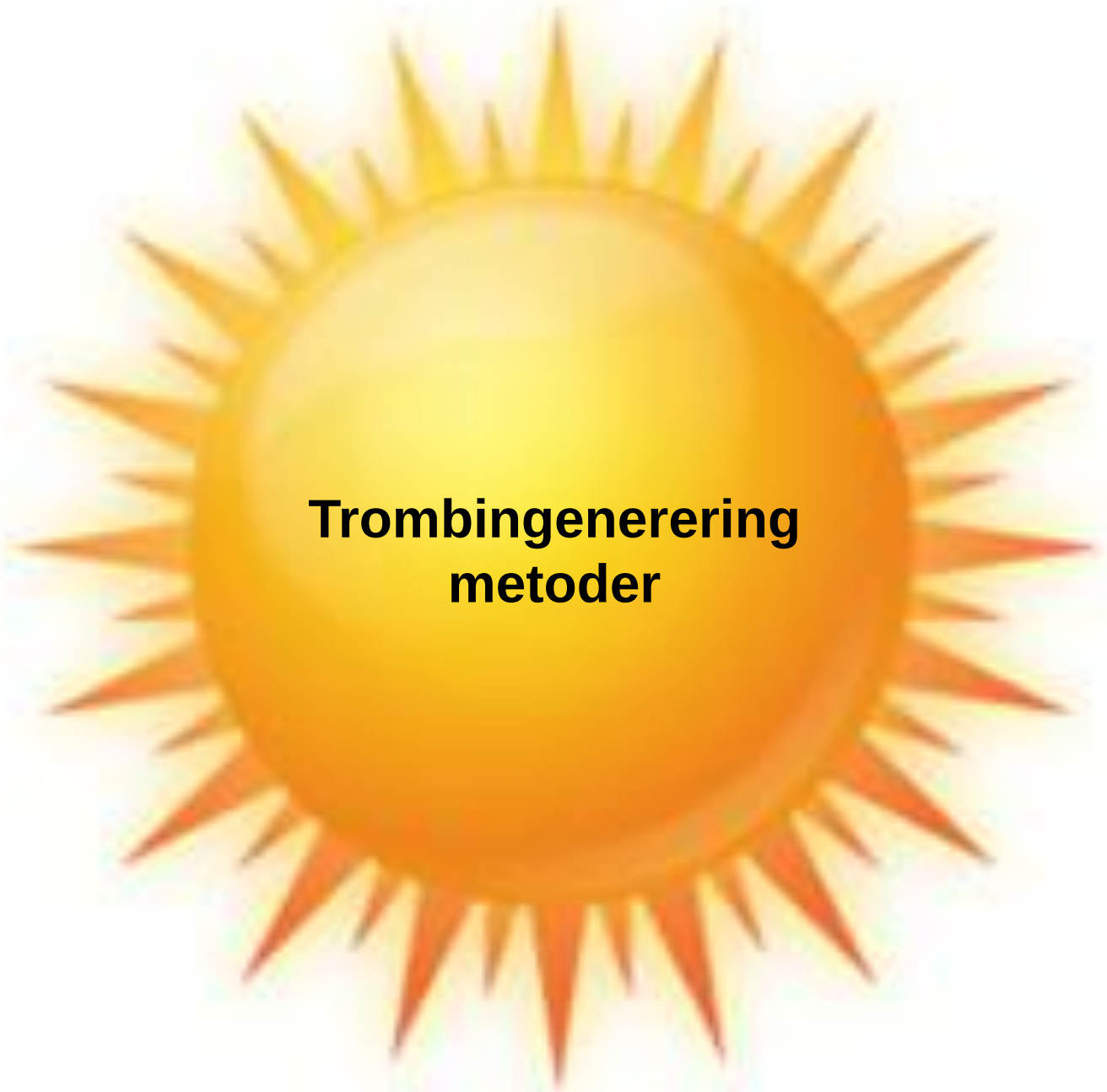
Trombingenerering
metoder

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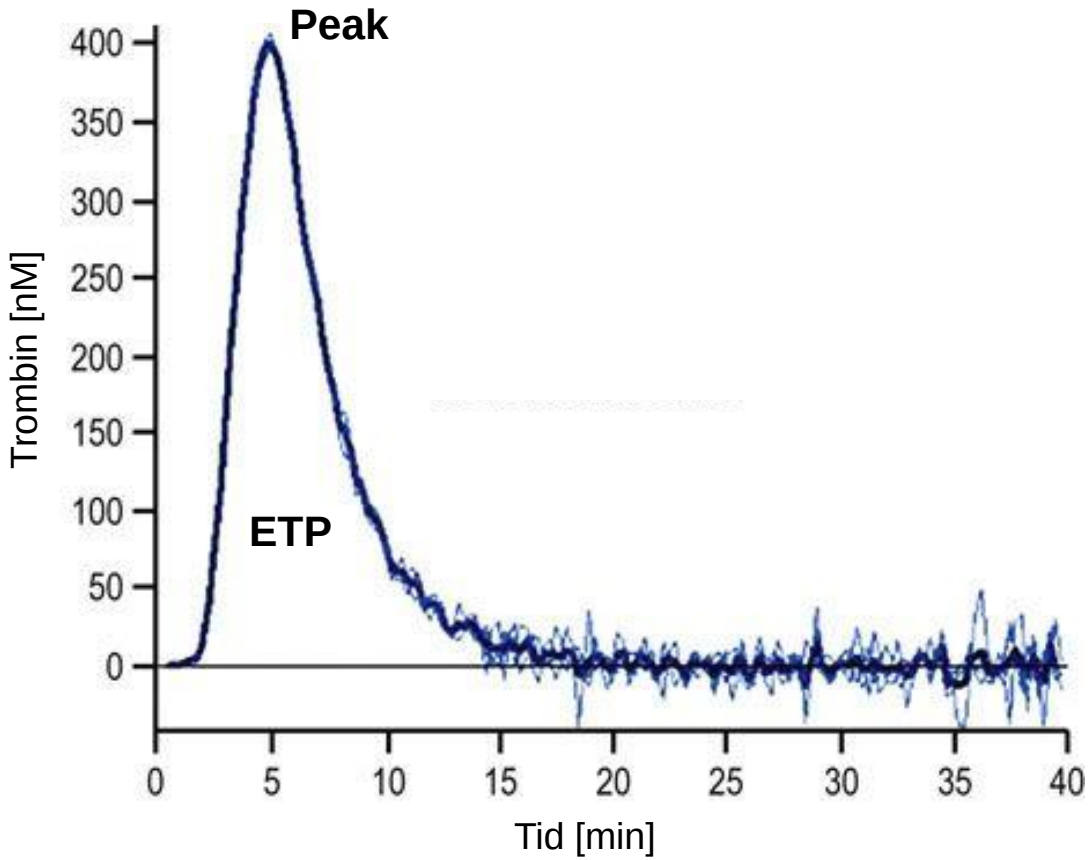
TGA-CAT

Thrombin generation assay – automated calibrated thrombogram



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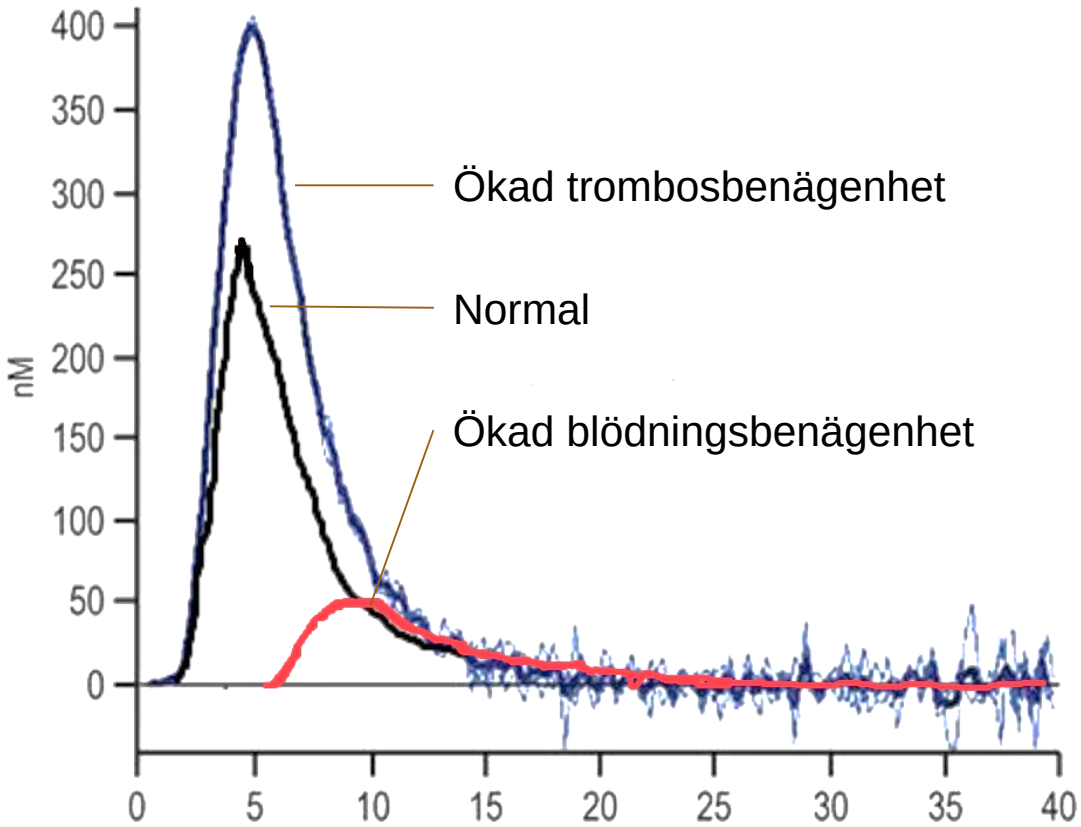
Trombogram



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Trombingenering och NOAK

“Non-VKA Oral Anticoagulants: Accurate Measurement of Plasma Drug Concentrations” *Douxfils et al. 2015.*

NOAK - “One-dose-fits-all”

nedsatt njurfunktion
läkemedelsinteraktion

blödningar och tromboser under pågående behandling
akut kirurgi och invasiva ingrepp
misstänkt NOAK överdos

koncentrationsbestämning

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