METODE ODREĐIVANJA DOAK LIJEKOVA

Tečaj trajnog usavršavanja HKMB
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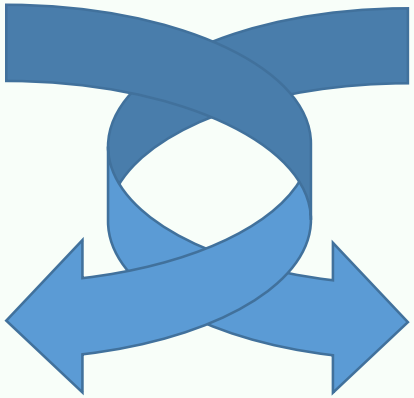
KBC Sestre milosrdnice
Zagreb, 4. listopad 2025.

METODE ODREĐIVANJA DOAK LIJEKOVA



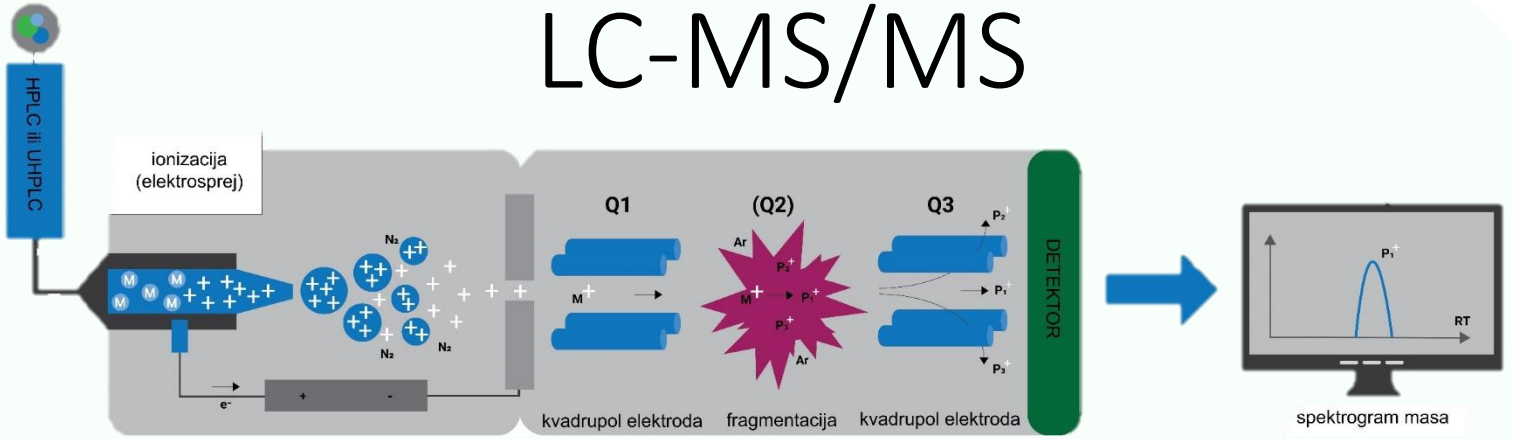
KVANTITATIVNE

- ✓ LC-MS/MS
- ✓ KOAGULACIJSKE
  - ✓ koagulometrijske
  - ✓ kromogene

KVALITATIVNE/ SEMIKVANTITATIVNE

- ✓ POC
  - ✓ DOAK dipstick
  - ✓ TEM
  - ✓ TEG
- ✓ OSTALE METODE
  - ✓ TGA

LC-MS/MS



- ✓ referentna metoda
- ✓ svi DOAK lijekovi
- ✓ serum ili plazma
- ✓ niska granica kvantifikacije (0,025 do 3 ng/mL)*
- ✓ široki mjerni raspon (5 do 500 ng)*

- ✓ oprema
- ✓ znanje
- ✓ dugotrajna
- ✓ zahtjevna
- ✓ cijena

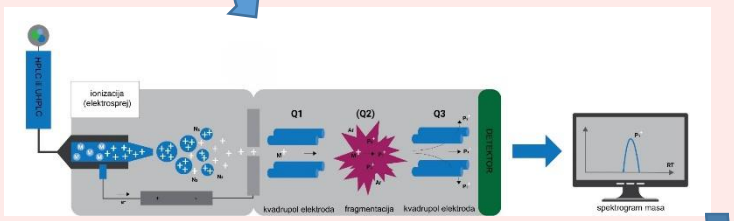
- ✓ nije pogodna za rutinsku uporabu

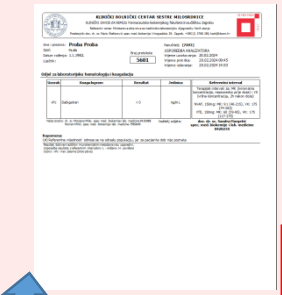
*ovisi o metodi i proizvođaču

LC-MS/MS iz suhe kapi krvi

- ✓ budućnost?
- ✓ alternativa stanjima kada nije potrebno hitno odrediti koncentraciju lijeka: npr. ambulantni pacijenti
- ✓ nanosi sam pacijent

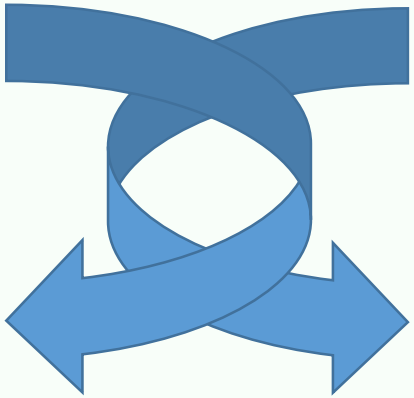
- ✓ dostava poštom
- ✓ kvantifikacija do 52 dana od uzorkovanja
- ✓ izvrsno slaganje s koncentracijama u plazmi





- ✓ oprema
- ✓ hematokrit
- ✓ nanosi sam pacijent – edukacija?

ALTERNATIVA?



DABIGATRAN

RIVAROKSABAN
APIKSABAN
EDOKSABAN

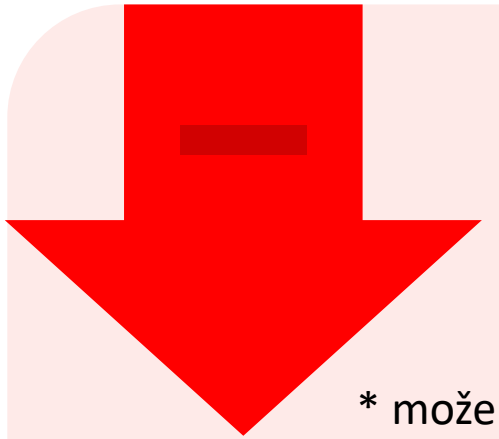
- ✓dTT - diluirano trombinsko vrijeme
- ✓ECT - ekarinski koagulometrijski test
- ✓ECA - ekarinska kromogena metoda
- ✓anti - FIIa test

- ✓anti-Xa test

- ✓ 3,2% Na-citrat plazma

DILUIRANO TROMBINSKO VRIJEME (dTV)

- ✓ dabigatran
- ✓ jednostavan
- ✓ komercijalna ili in-house metoda
- ✓ usporediv s ECA/ECT i anti-FIIa



- ✓ dugotrajno mjerenje
- ✓ uži mjerni raspon (100-400 ng/mL)*

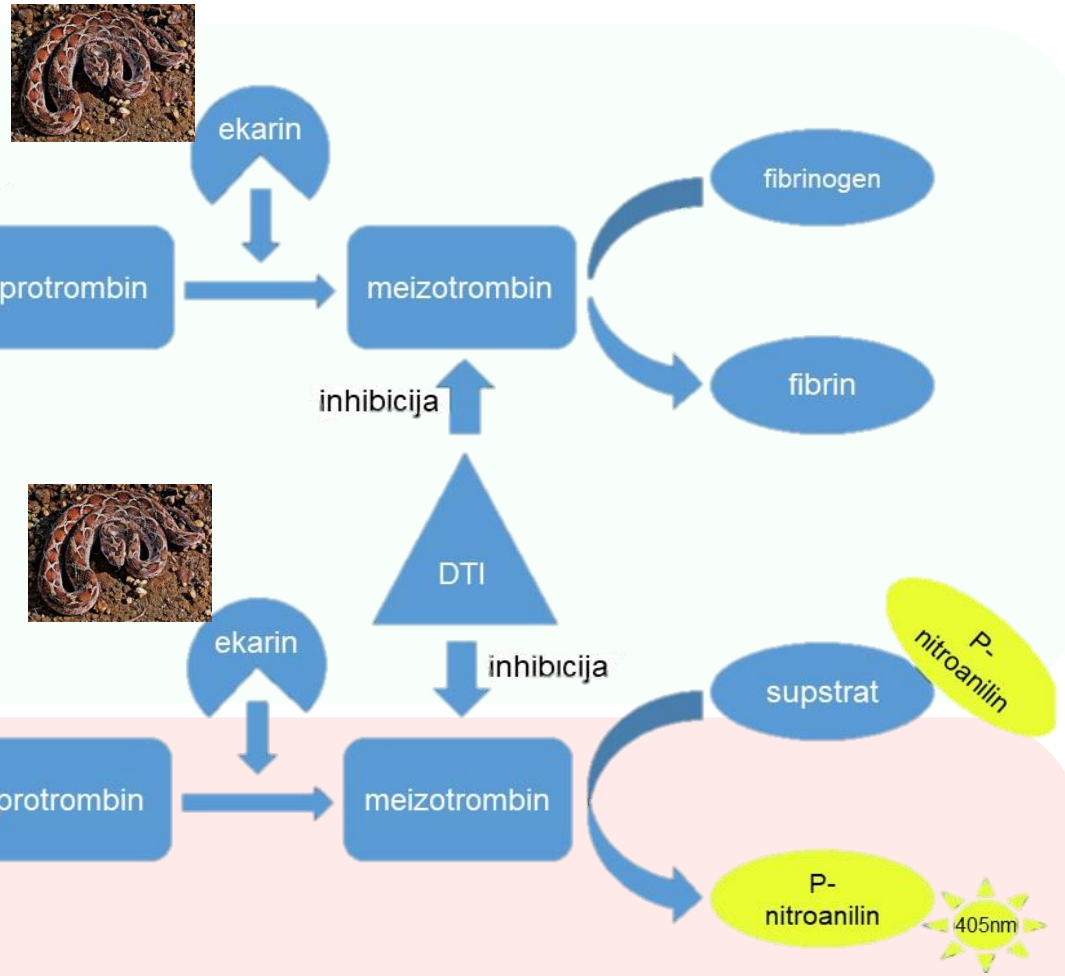
- ✓ TV nije pogodan – preosjetljivost
- ✓ modifikacija trombinskog vremena
  - ✓ dilucija pacijentove plazme 1:8
- ✓ TV test – pročišćeni trombin
- ✓ koagulometrijska detekcija

* može se povećati upotrebom krivulje u niskom/visokom području

EKARINSKI TESTOVI

- ✓ dabigatran
- ✓ detekcija klinički značajnih koncentracija (<30 ng/mL)
- ✓ širok mjerni raspon (20 – 500 ng/mL)**
- ✓ dobro slaganje s LC–MS/MS

- ✓ nedostatak protrombina i fibrinogena utječe na rezultate ECT



- ✓ ECT – ekarinski koagulometrijski test (A)
- ✓ ECA – ekarinska kromogena metoda (B)

*DTI - dabigatran
**ovisi o metodi i proizvođaču

anti-FIIa

- ✓ inkubacija pacijentove plazme s kromogenim supstratom specifičnim za aktivirani čimbenik zgrušavanja II
- ✓ dodatak trombinskog reagensa
- ✓ mjeri se ostatna aktivnost trombina u prisutnosti dabigatrana

- ✓ dabigatran
- ✓ niža granica detekcije od ekarinskih metoda (oko 15 ng/mL, ovisno o proizvođaču)
- ✓ izvrsno slaganje s LC-MS/MS

COMPARISON OF TWO QUANTITATIVE METHODS FOR DETERMINATION OF DABIGATRAN CONCENTRATION: DILUTED THROMBIN TIME TEST AND INNOVANCE DABIGATRAN TEST IN REAL LIFE PATIENTS TREATED WITH DABIGATRAN

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[1]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia
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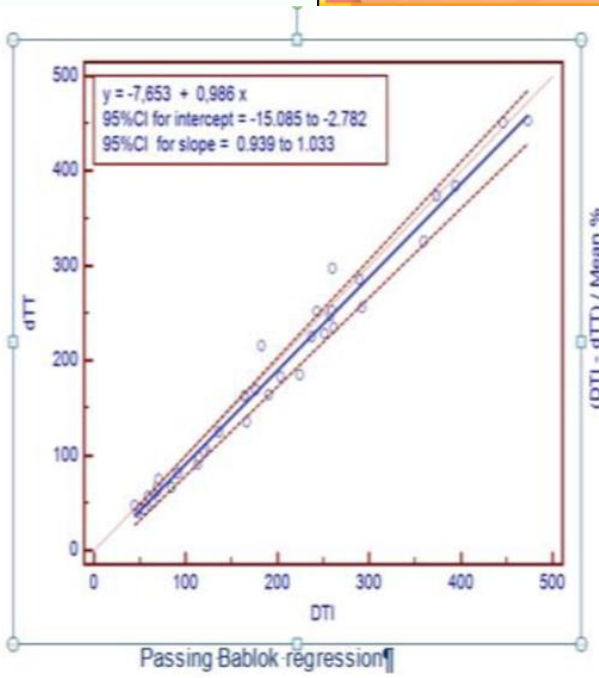
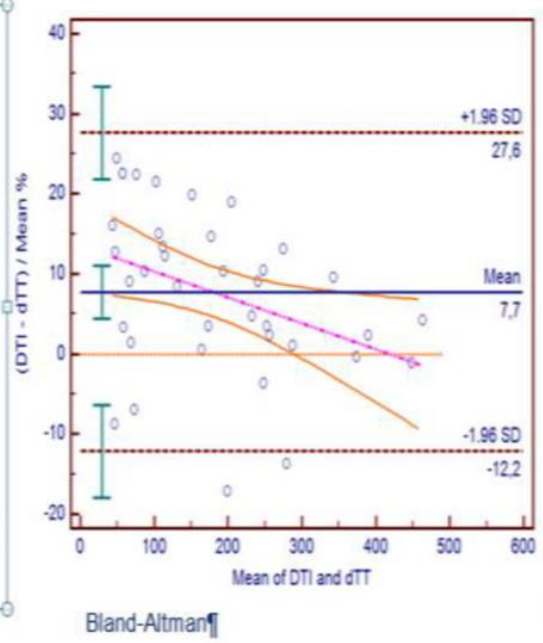


Passing Bablok regression

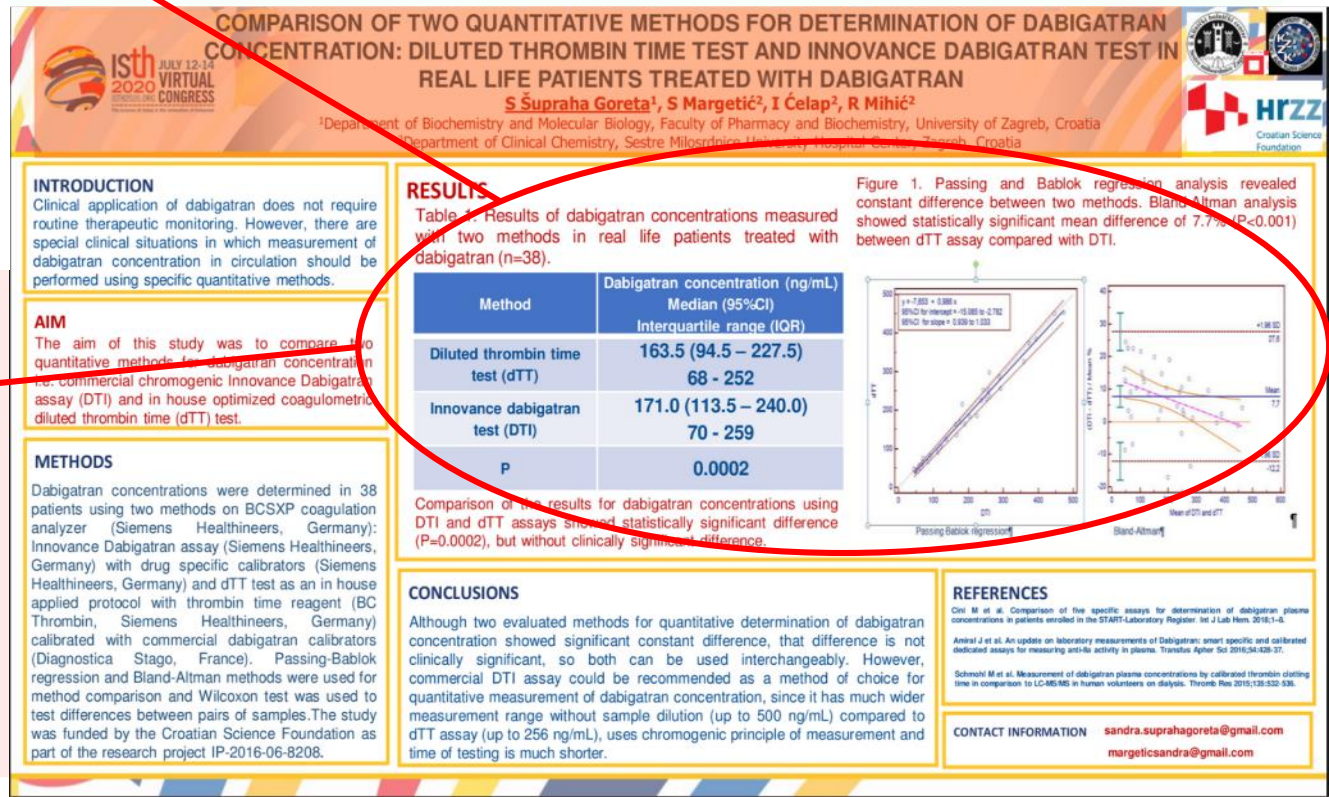


Bland-Altman

Siemens Innovance DTI (anti-FIIa) vs in-house dTV

- ✓ kromogena vs koagulometrijska metoda
- ✓ N=38
- ✓ statistički značajna razlika uz konstantno odstupanje
- ✓ klinička razlika nije bila značajna
- ✓ manji mjerni raspon (500 ng/mL DTI vs 256 ng/mL dTV)
- ✓ dugotrajnije mjerenje dTV

Method	Dabigatran concentration (ng/mL) Median (95%CI) Interquartile range (IQR)
Diluted thrombin time test (dTT)	163.5 (94.5 – 227.5) 68 - 252
Innovance dabigatran test (DTI)	171.0 (113.5 – 240.0) 70 - 259
P	0.0002



COMPARISON OF TWO QUANTITATIVE METHODS FOR DETERMINATION OF DABIGATRAN CONCENTRATION: DILUTED THROMBIN TIME TEST AND INNOVANCE DABIGATRAN TEST IN REAL LIFE PATIENTS TREATED WITH DABIGATRAN

S Šupraha Goreta[1], S Margetić[2], I Ćelap[2], R Mihić[2]

INTRODUCTION
Clinical application of dabigatran does not require routine therapeutic monitoring. However, there are special clinical situations in which measurement of dabigatran concentration in circulation should be performed using specific quantitative methods.

AIM
The aim of this study was to compare two quantitative methods for dabigatran concentration i.e. commercial chromogenic Innovance Dabigatran assay (DTI) and in house optimized coagulometric diluted thrombin time (dTT) test.

METHODS
Dabigatran concentrations were determined in 38 patients using two methods on BCSXP coagulation analyzer (Siemens Healthineers, Germany): Innovance Dabigatran assay (Siemens Healthineers, Germany) with drug specific calibrators (Siemens Healthineers, Germany) and dTT test as an in house applied protocol with thrombin time reagent (BC Thrombin, Siemens Healthineers, Germany) calibrated with commercial dabigatran calibrators (Diagnostica Stago, France). Passing-Bablok regression and Bland-Altman methods were used for method comparison and Wilcoxon test was used to test differences between pairs of samples.The study was funded by the Croatian Science Foundation as part of the research project IP-2016-06-8208.

RESULTS
Table 1. Results of dabigatran concentrations measured with two methods in real life patients treated with dabigatran (n=38).

Method	Dabigatran concentration (ng/mL) Median (95%CI) Interquartile range (IQR)
Diluted thrombin time test (dTT)	163.5 (94.5 – 227.5) 68 - 252
Innovance dabigatran test (DTI)	171.0 (113.5 – 240.0) 70 - 259
P	0.0002

Comparison of the results for dabigatran concentrations using DTI and dTT assays showed statistically significant difference (P=0.0002), but without clinically significant difference.

Figure 1. Passing and Bablok regression analysis revealed constant difference between two methods. Bland-Altman analysis showed statistically significant mean difference of 7.7% (P<0.001) between dTT assay compared with DTI.

CONCLUSIONS
Although two evaluated methods for quantitative determination of dabigatran concentration showed significant constant difference, that difference is not clinically significant, so both can be used interchangeably. However, commercial DTI assay could be recommended as a method of choice for quantitative measurement of dabigatran concentration, since it has much wider measurement range without sample dilution (up to 500 ng/mL) compared to dTT assay (up to 256 ng/mL), uses chromogenic principle of measurement and time of testing is much shorter.

REFERENCES

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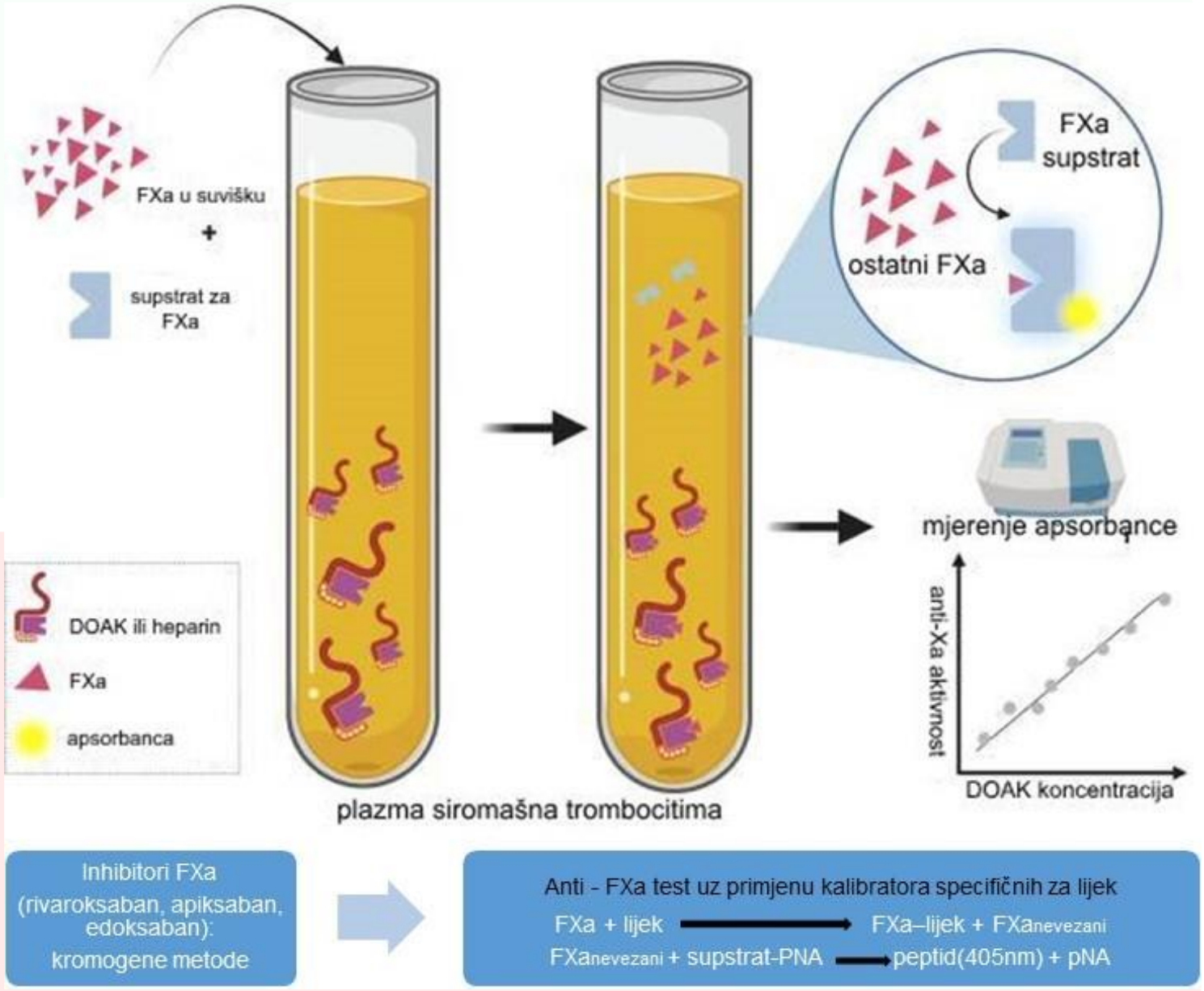
anti-Xa

- ✓ apiksaban, rivaroksaban, edoksaban, heparin
- ✓ specifični kalibrator za lijek
- ✓ niska granica kvantifikacije (10 – 30 ng/mL)*
- ✓ široki mjerni raspon (20 – 500 ng/mL)
- ✓ usporediva s LC-MS/MS

- ✓ Interferencija heparina – lažno ↑ vrijednosti DOAK lijekova

*ovisi o metodi i proizvođaču, može se povećati ili smanjiti upotrebom dodatnih kalibracijskih krivulja



Analytical verification and comparison of chromogenic assays for dabigatran, rivaroxaban and apixaban determination on BCSXP and STA Compact Max analysers

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Analyser	Assay	Measurement range (ng/mL)
BCSXP	Dabigatran	20 – 500
	Rivaroxaban	20 – 500
	Apixaban	< 500
STA Compact Max	Dabigatran	15 – 460
	Rivaroxaban	25 – 500
	Apixaban	23 – 500

Usporedba 2 analitička sustava:

- ✓ Siemens BCSXP
  - ✓ anti-Xa i anti-FIIa
- ✓ STAGO STA Compact Max
  - ✓ anti-Xa i ECT

Table 5. Passing Bablok regression analysis

	N	BCSXP mean conc. (ng/mL)	STA Compact Max. mean conc. (ng/mL)	Intercept 95%CI	Slope 95%CI	P (Cusum test)
Dabigatran	40	129.5 ± 81.3	112.5 ± 85.6	- 40.73 to -18.69	0.95 to 1.17	0.150
Rivaroxaban	40	139.8 ± 109.9	125.0 ± 93.8	2.94 to 11.83	0.80 to 0.89	0.97
Apixaban	40	133.5 ± 76.1	121.5 ± 69.8	- 0.85 to 13.37	0.83 to 0.93	0.300

- ✓ N=120
- ✓ značajan bias između rezultata dabigatrana i apiksabana
- ✓ rivaroksaban bez značajnog bias-a
- ✓ odstupanja značajnija pri nižim koncentracijama; <50 ng/mL
- ✓ oprez prilikom interpretacije rezultata s različitih sustava!

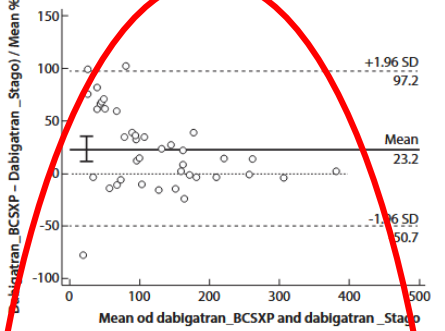

Figure 1. Relative bias between mean values of dabigatran measured on BCSXP and Stago Compact Max analyser using Bland-Altman analysis. Graph shows statistically significant bias between methods with the mean bias of 23.2% (continuous line). Vertical line represents confidence interval of the relative mean bias (11.18 to 35.31). SD – standard deviation (dashed lines).

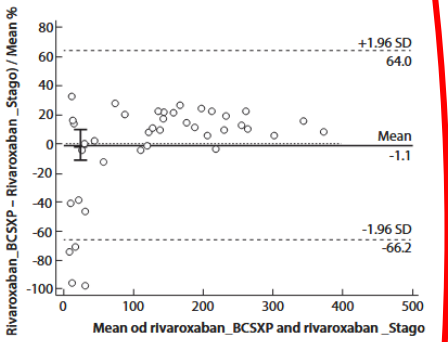

Figure 2. Relative bias between mean values of rivaroxaban measured on BCSXP and Stago Compact Max analyser using Bland-Altman analysis. Graph shows there is no statistically significant bias between methods with the mean bias of - 1.1% (continuous line). Vertical line represents confidence interval of the relative mean bias (-11.70 to 9.53). SD – standard deviation (dashed lines).

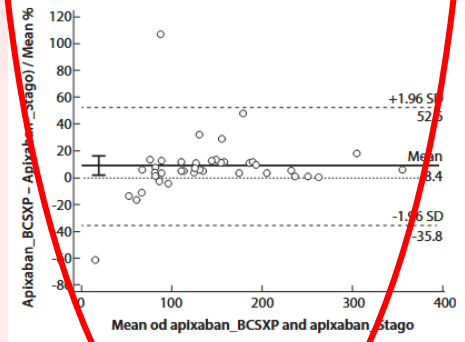

Figure 3. Relative bias between mean values of apixaban measured on BCSXP and Stago Compact Max analyser using Bland-Altman analysis. Graph shows statistically significant bias between methods with the mean bias of 8.4% (continuous line). Vertical line represents confidence interval of the relative mean bias (1.18 to 15.61). SD – standard deviation (dashed lines).

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Comparison of rivaroxaban concentrations measured by anti-Xa rivaroxaban calibrated assay and LC-MS/MS

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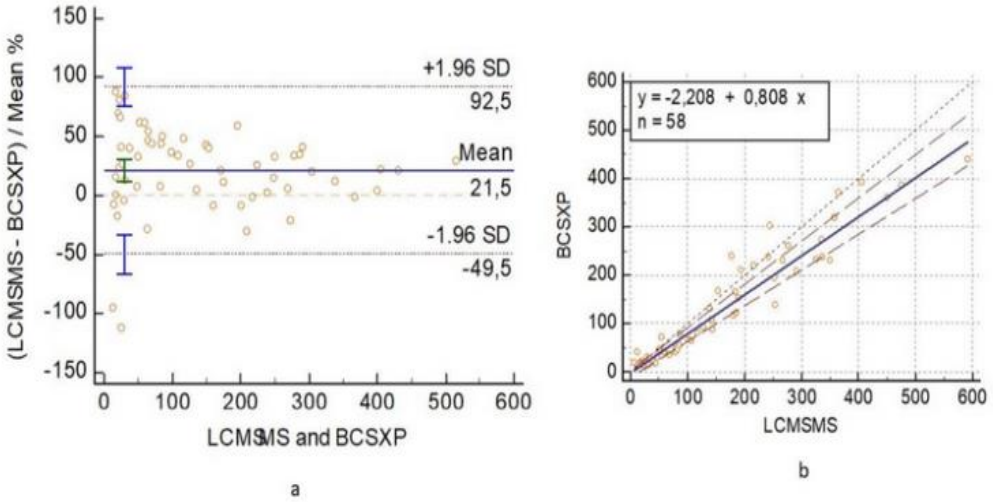


Figure 1. Comparison of rivaroxaban levels measured by LC-MS/MS and anti-Xa rivaroxaban calibrated assay on BCSXP (n=58). a) Bland-Altman analysis; b) Passing-Bablok regression analysis.

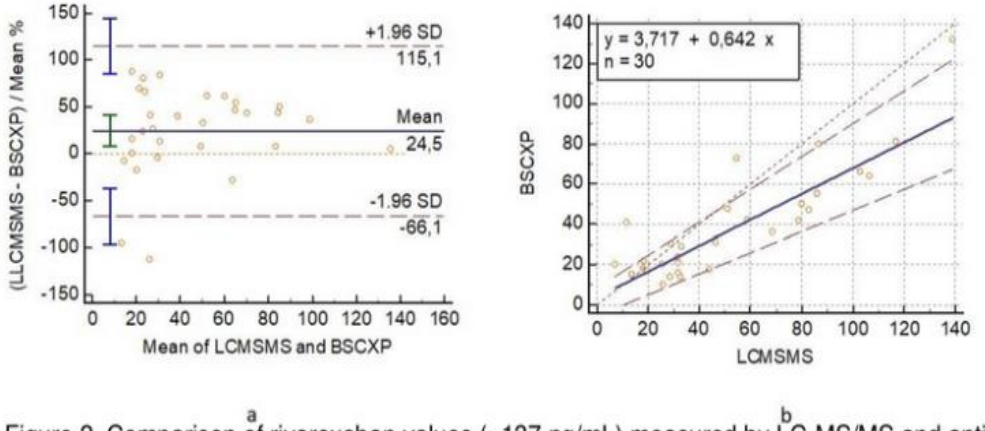
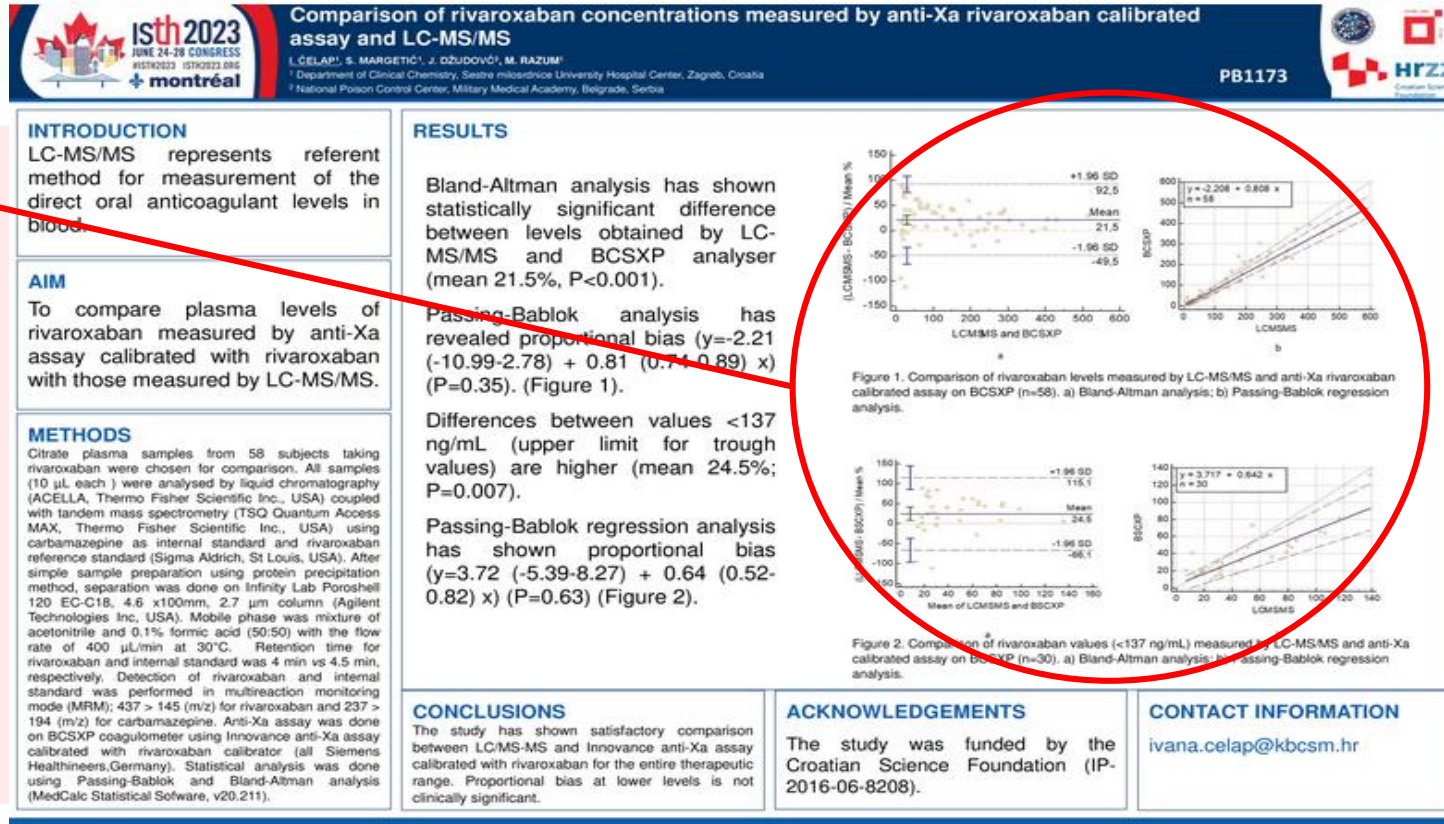


Figure 2. Comparison of rivaroxaban values (<137 ng/mL) measured by LC-MS/MS and anti-Xa calibrated assay on BCSXP (n=30). a) Bland-Altman analysis; b) Passing-Bablok regression analysis.

- ✓ LC-MS/MS vs Siemens anti-Xa
- ✓ N=30 uzoraka
- ✓ rivaroksaban

- ✓ statistički značajna razlika uz proporcionalan bias
- ✓ razlika u nižim vrijednostima nije klinički značajna



ISTH 2023 JUNE 24-28 CONGRESS montréal

Comparison of rivaroxaban concentrations measured by anti-Xa rivaroxaban calibrated assay and LC-MS/MS

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PB1173

INTRODUCTION
LC-MS/MS represents referent method for measurement of the direct oral anticoagulant levels in blood.

AIM
To compare plasma levels of rivaroxaban measured by anti-Xa assay calibrated with rivaroxaban with those measured by LC-MS/MS.

METHODS
Citrate plasma samples from 58 subjects taking rivaroxaban were chosen for comparison. All samples (10 µL each) were analysed by liquid chromatography (ACELLA, Thermo Fisher Scientific Inc., USA) coupled with tandem mass spectrometry (TSQ Quantum Access MAX, Thermo Fisher Scientific Inc., USA) using carbamazepine as internal standard and rivaroxaban reference standard (Sigma Aldrich, St Louis, USA). After simple sample preparation using protein precipitation method, separation was done on Infinity Lab Poroshell 120 EC-C18, 4.6 x100mm, 2.7 µm column (Agilent Technologies Inc, USA). Mobile phase was mixture of acetonitrile and 0.1% formic acid (50:50) with the flow rate of 400 µL/min at 30°C. Retention time for rivaroxaban and internal standard was 4 min vs 4.5 min, respectively. Detection of rivaroxaban and internal standard was performed in multireaction monitoring mode (MRM); 437 > 145 (m/z) for rivaroxaban and 237 > 194 (m/z) for carbamazepine. Anti-Xa assay was done on BCSXP coagulometer using Innovance anti-Xa assay calibrated with rivaroxaban calibrator (all Siemens Healthineers,Germany). Statistical analysis was done using Passing-Bablok and Bland-Altman analysis (MedCalc Statistical Sofware, v20.211).

RESULTS
Bland-Altman analysis has shown statistically significant difference between levels obtained by LC-MS/MS and BCSXP analyser (mean 21.5%, P<0.001).

Passing-Bablok analysis has revealed proportional bias (y=-2.21 (-10.99-2.78) + 0.81 (0.74-0.89) x) (P=0.35). (Figure 1).

Differences between values <137 ng/mL (upper limit for trough values) are higher (mean 24.5%; P=0.007).

Passing-Bablok regression analysis has shown proportional bias (y=3.72 (-5.39-8.27) + 0.64 (0.52-0.82) x) (P=0.63) (Figure 2).

Figure 1. Comparison of rivaroxaban levels measured by LC-MS/MS and anti-Xa rivaroxaban calibrated assay on BCSXP (n=58). a) Bland-Altman analysis; b) Passing-Bablok regression analysis.

Figure 2. Comparison of rivaroxaban values (<137 ng/mL) measured by LC-MS/MS and anti-Xa calibrated assay on BCSXP (n=30). a) Bland-Altman analysis; b) Passing-Bablok regression analysis.

CONCLUSIONS
The study has shown satisfactory comparison between LC/MS-MS and Innovance anti-Xa assay calibrated with rivaroxaban for the entire therapeutic range. Proportional bias at lower levels is not clinically significant.

ACKNOWLEDGEMENTS
The study was funded by the Croatian Science Foundation (IP-2016-06-8208).

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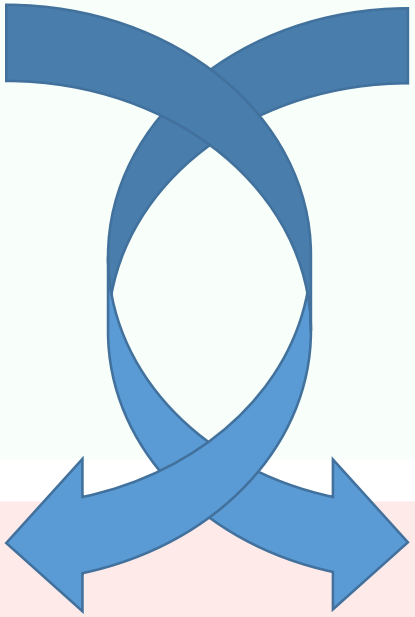
OSTALE METODE?



KVALITATIVNE

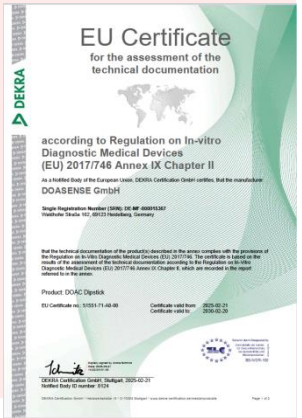
SEMIKVANTITATIVNE

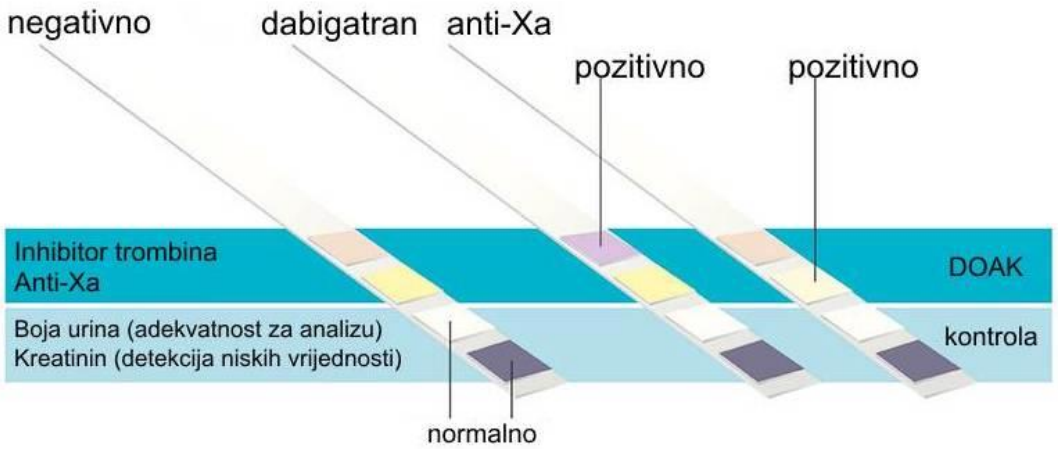
- ✓ ↑ DOAK lijekova – brze metode?
- ✓ isključenje klinički značajne koncentracije (>30 ng/mL)
- ✓ POC metode

DOAK Dipstick (*Doasense GmbH, Heidelberg Njemačka)*

- ✓ kvalitativna metoda
- ✓ detekcija dabigatrana i anti-Xa lijekova
- ✓ urin
- ✓ brza ~10 min
- ✓ isključenje klinički značajne koncentracije (>30 ng/mL)
- ✓ mogućnost automatskog očitanja
- ✓ IVDR za in vitro primjenu kao POC u hitnim stanjima

- ✓ ne možemo razlikovati o kojem se anti-Xa DOAK-u radi
- ✓ pozitivan rezultat -> određivanje u laboratoriju


EU Certificate
for the assessment of the technical documentation
according to Regulation on In-vitro Diagnostic Medical Devices (EU) 2017/746 Annex IX Chapter II
DOASENSE GmbH



DOAC Dipstick Testing Can Reliably Exclude the Presence of Clinically Relevant DOAC Concentrations in Circulation

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Sandra Šupraha Goreta[3] Anesa Čajević Glojnarić[1] Diana Delić Brkljačić[4] Pavao Mioč[4]
Job Harenberg[5,6] Svetlana Hetjens[7] Christel Weiss[7]

Thromb Haemost 2022;122:1542–1548.

DOAC	*n*	Mean (95% confidence interval) (ng/mL)		*p*-Values
		Plasma	Urine	
Dabigatran	44	37.8 (33.1–42.5)	3,954.3 (3,213.3–4,695.3)	<0.001
Apixaban	31	44.2 (37.8–50.6)	1,169.9 (883.8–1,456.0)	<0.001
Rivaroxaban	53	32.6 (27.8–37.5)	1,214.8 (853.0–1,576.5)	<0.001

DOAK	AUC ROC	Osjetljivost %	Specifičnost %	PPV %	NPV %
Xa	0,859	100	21,4	71,8	100
DTI	0,814	100	5,9	62,8	100

- ✓ N=128
- ✓ kvalitativno: DOAK Dipstick
- ✓ kvantitativno: anti-Xa i anti-FIIa (Siemens)

- ✓ pouzdano se može isključiti klinički značajna koncentracija (>30 ng/mL) DOAK lijeka
- ✓ anti-FIIa i anti-Xa, ali ne i koji anti-Xa
- ✓ brza metoda (10minuta)

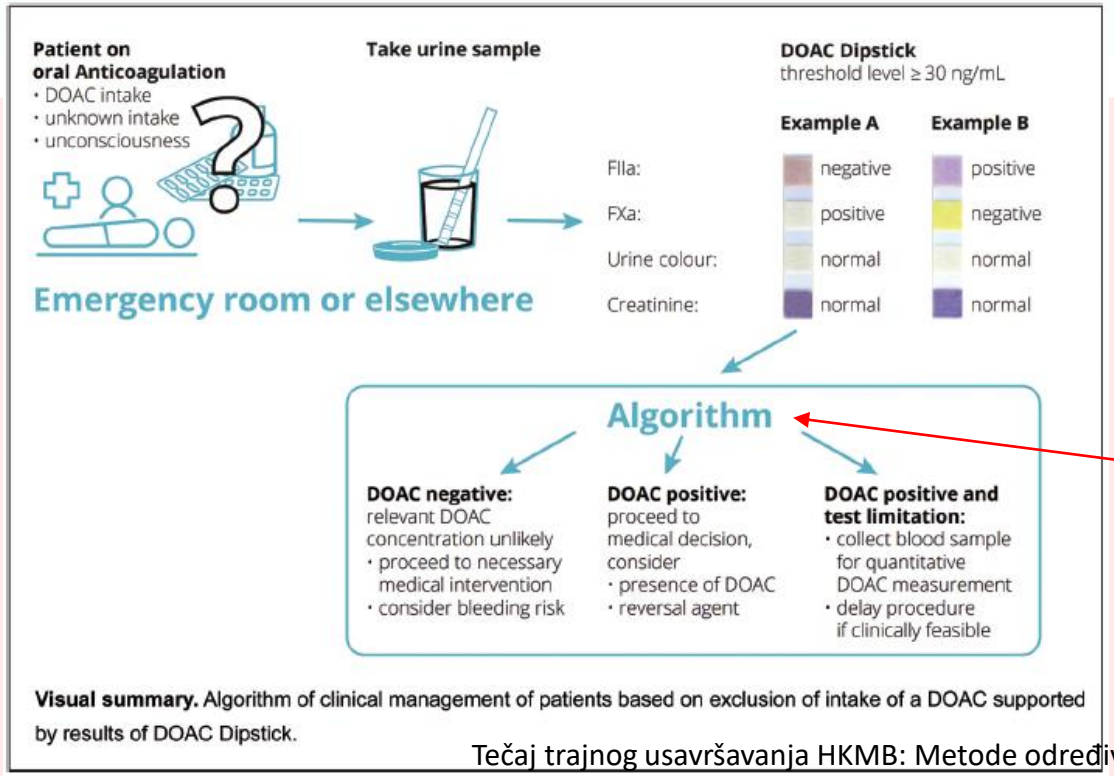
Algorithm for Rapid Exclusion of Clinically Relevant Plasma Levels of Direct Oral Anticoagulants in Patients Using the DOAC Dipstick: An Expert Consensus Paper

Job Harenberg[1,2] Robert C. Gosselin[3] Adam Cuker[4] Cecilia Becattini[5] Ingrid Pabinger[6]
Sven Poli[7,8] Jeffrey Weitz[9,10] Walter Ageno[11] Rupert Bauersachs[12] Ivana Celap[13,14]
Philip Choi[15,16] James Douketis[10] Jonathan Douxfils[17,18] Ismail Elalamy[19,20]
Anna Falanga[21,22] Jawed Fareed[23] Emmanuel J. Favaloro[24,25] Grigorios Gerotziafas[26,27]
Harald Herkner[28] Svetlana Hetjens[29] Lars Heubner[30] Robert Klamroth[31] Forian Langer[32]
Gregory Y. H. Lip[33,34] Brian Mac Grory[35] Sandra Margetić[12,36] Anne Merrelaar[28]
Marika Pikta[37,38] Thomas Renne[39,40] Sam Schulman[20,41] Michael Schwameis[28]
Daniel Strbian[42] Alfonso Tafur[43,44] Julie Vassart[17] Francesco Violi[45] Jeanine Walenga[46]
Christel Weiss[29]

Thromb Haemost 2024; 124:770–777.

DOAK	Osjetljivost %	Specifičnost %	PPV %	NPV %
Xa	97,8	50,0	87,2	86,6
DTI	98,3	91,8	73,4	99,6

✓ skupna analiza 5 različitih istraživanja



Visual summary. Algorithm of clinical management of patients based on exclusion of intake of a DOAC supported by results of DOAC Dipstick.

- ✓ Algoritam za isključenje klinički značajne koncentracije DOAK lijeka
- ✓ hitna stanja
- ✓ urin

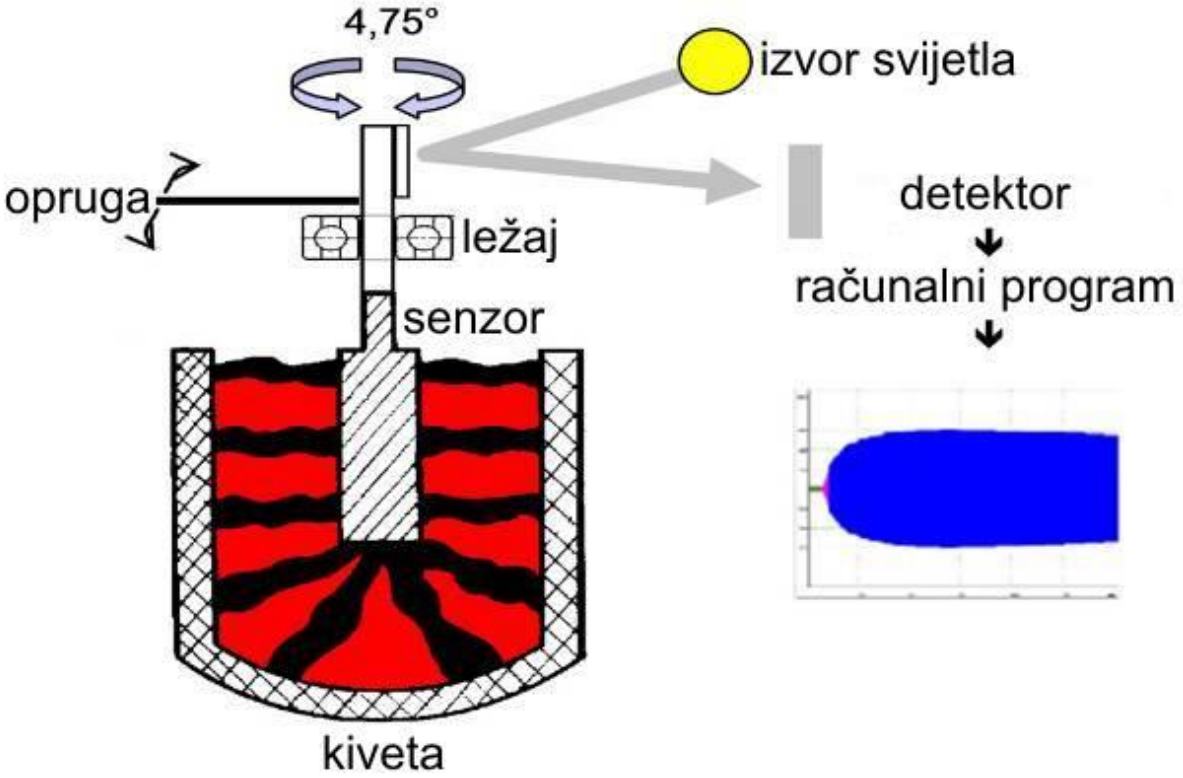
Tromboelastometrija (TEM) i tromboelastografija (TEG)

- ✓ procjena globalnog koagulacijskog i fibrinolitičkog puta
- ✓ mjerenje viskoelastometrijskih svojstava ugruška nakon aktivacije zgrušavanja

TEM
- ✓ senzor oscilira unutar kivete

TEG
- ✓ rotacija kivete oko senzora

- ✓ POC metode kod akutnih krvarenja
- ✓ brzi izbor terapije

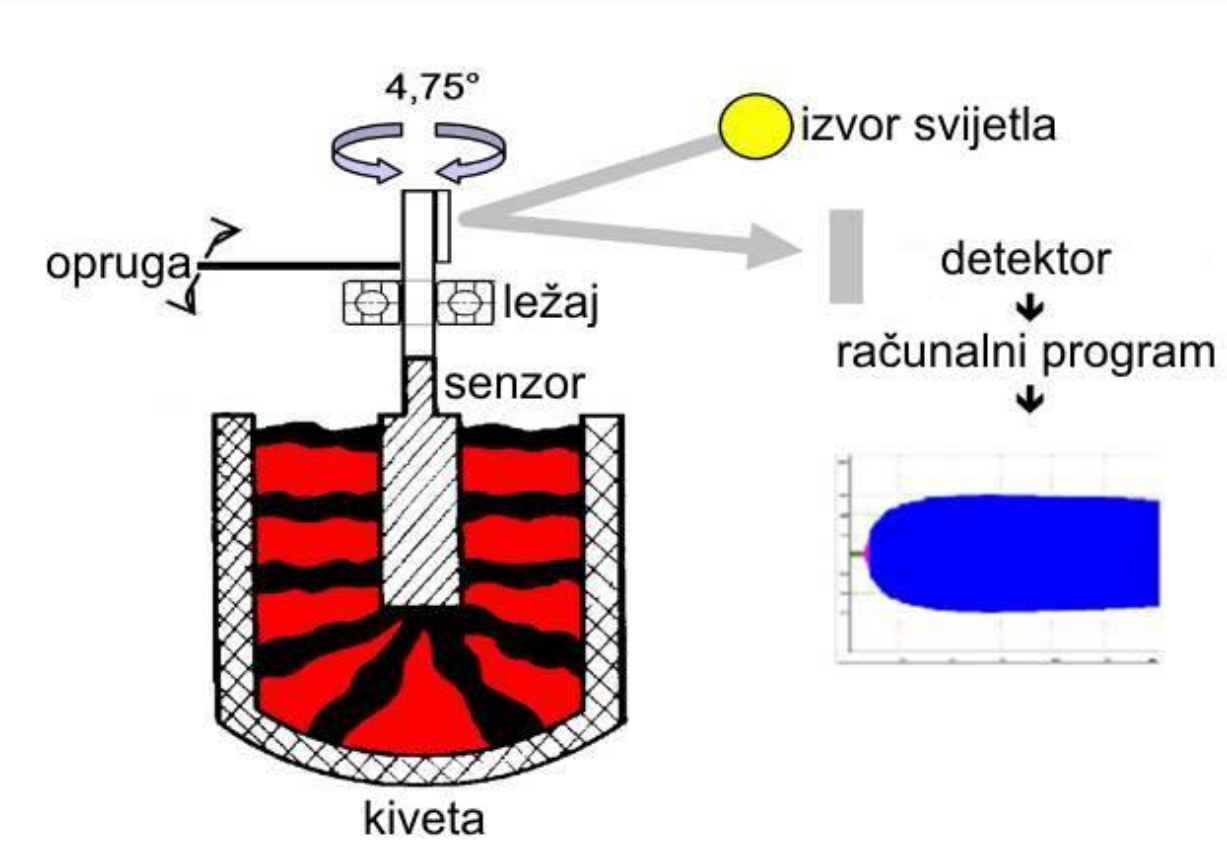


Princip rada ROTEM-a

ROTEM

ROTEM
- ✓ smanjenje rotacije prilikom nastanka ugruška

- ✓ EXTEM, INTEM, FIBTEM, HEPTEM nedovoljno osjetljivi i specifični
- ✓ modificirane metode ROTEM-a:
  - ✓ TFTEM – tkivni faktor u niskim koncentracijama
  - ✓ ECATEM – ekarinski test
- ✓ same nisu dovoljno specifične i osjetljive: kombinacija testova



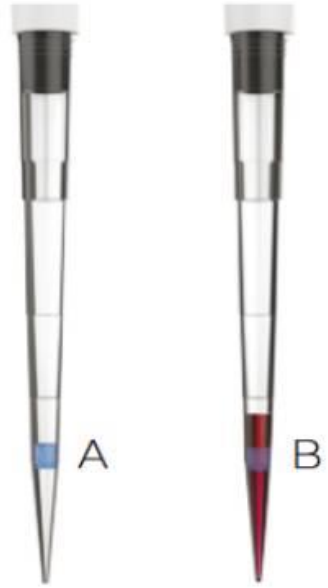
Princip rada ROTEM-a

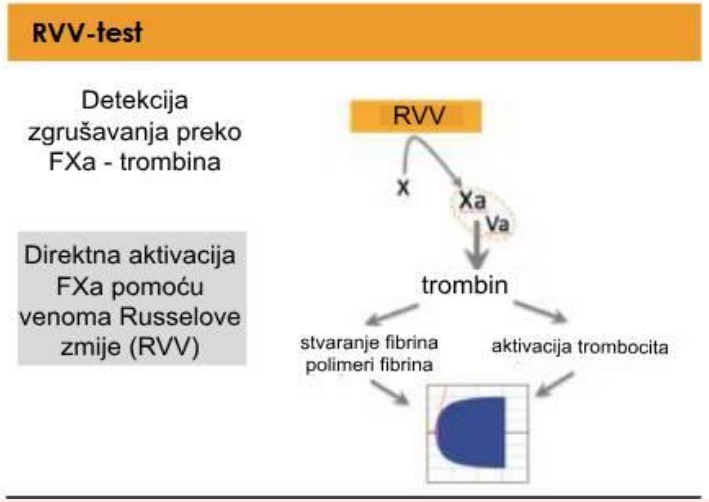
TEM - ClotPro (*Haemoview Diagnostics, Milton, Australia)*

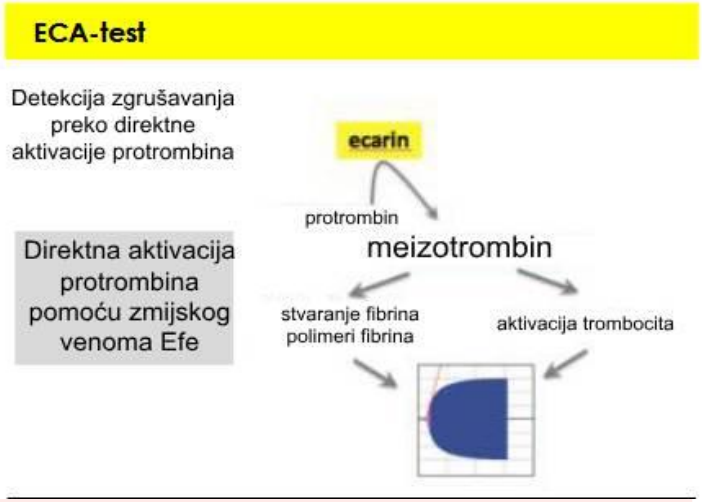
- ✓ TEM na principu suhe kemije
- ✓ istodobno izvođenje do 6 testova (ovisno o nastavaku)
- ✓ DOAK lijekovi:
  RVV i ECA
- ✓ brza, jednostavna
- ✓ POC za isključivanje u hitnim stanjima

Interferencije

- ✓ RVV
  - ✓ heparin
  - ✓ antagonisti vitamina K
- ✓ ECA
  - ✓ antagonisti vitamina K
- ✓ nedostatak fibrinogena
- ✓ hemodilucija



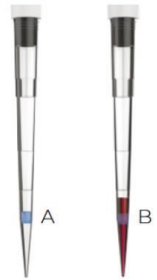


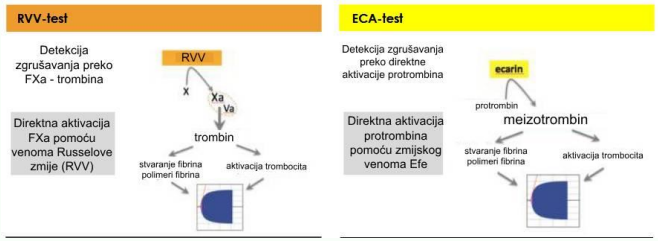


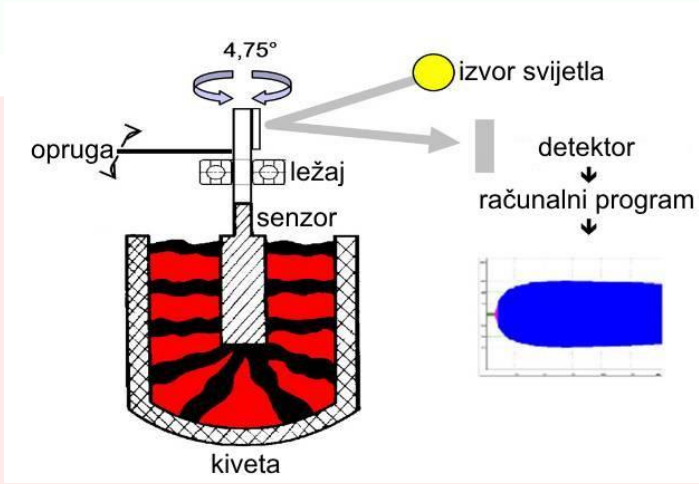
Teg6s (*Haemonetics Corporation. Haemonetics, SAD)*

- ✓ umetci s predefiniranim reagensima
- ✓ POC u hitnim stanjima
- ✓ DOAK umetak – klinička ispitivanja
- ✓ zadovoljavajuća osjetljivost
- ✓ razlikuje anti-FXa i anti-FIIa

- ✓ potencijal u budućnosti
- ✓ POC
- ✓ hitna stanja



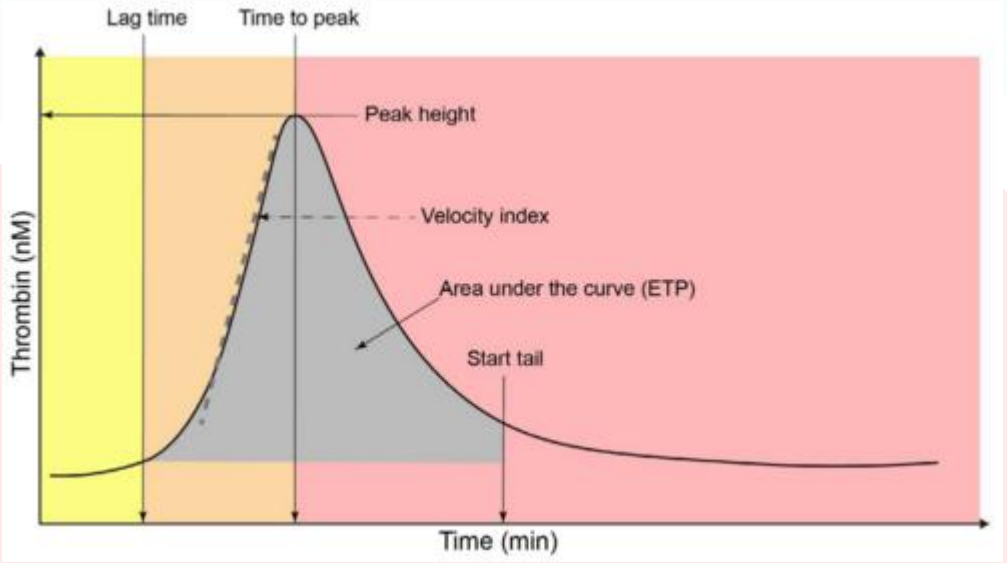




- ✓ mali broj pacijenata, mali broj studija
- ✓ nestandardizirani
- ✓ nisu preporučeni za kliničku upotrebu

Test stvaranja trombina (TGA)

- ✓ detekcija anti-FXa skupine DOAK lijekova
- ✓ temelji se na mjerenju kinetike nastanka trombina nakon aktivacije FX
- ✓ krivulja: maksimalna koncentracija i ukupna količina stvorenog trombina



- ✓ slaba korelacija s anti-FXa metodom za mjerenje rivaroksabana, apiksabana i edoksabana
- ✓ nije prikladna za kliničku primjenu