Molekularna tipizacija eritrocitnih (RBC), leukocitnih (HLA) i trombocitnih (HPA) antigena te detekcija antitrombocitnih antitijela

kao doprinos sigurnoj transfuziji krvi

Marko Lilić, mag. biol. mol.
Sveučilište u Osijeku, Osijek, Hrvatska

Sarajevo, 7.12.2018.

SADRŽAJ

1. Metode molekularne tipizacije i detekcije protutijela
2. Eritrocitni antigeni (RBC)
3. Leukocitni antigeni (HLA)
4. Trombocitni antigeni (HPA)

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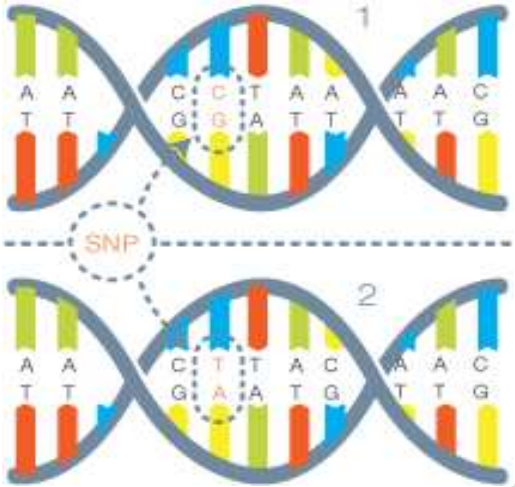
Što je molekularna tipizacija?

Određivanje slijeda nukleotida DNA koji diktira ekspresiju antigena

na razini proteina: **fenotip**
(ekspresija antigena)

polimorfizam / SNP (single nucleotide polymorphism)

na razini DNA / gena: **genotip**
(slijed nukleotida)

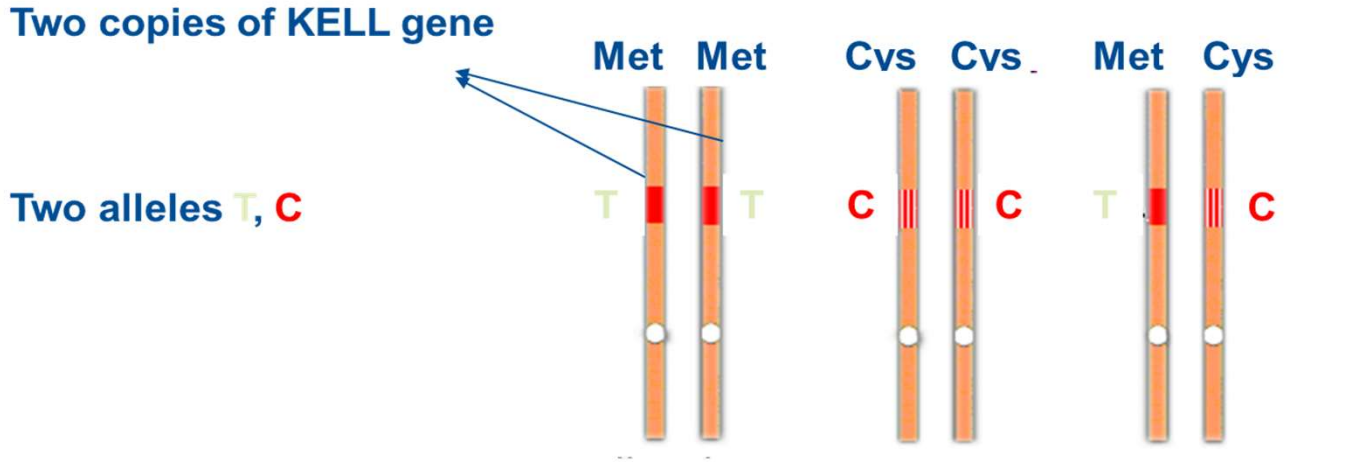


Diego B/A. ekson 19 2461C>T
*DI*A, DI*B*

Primjer

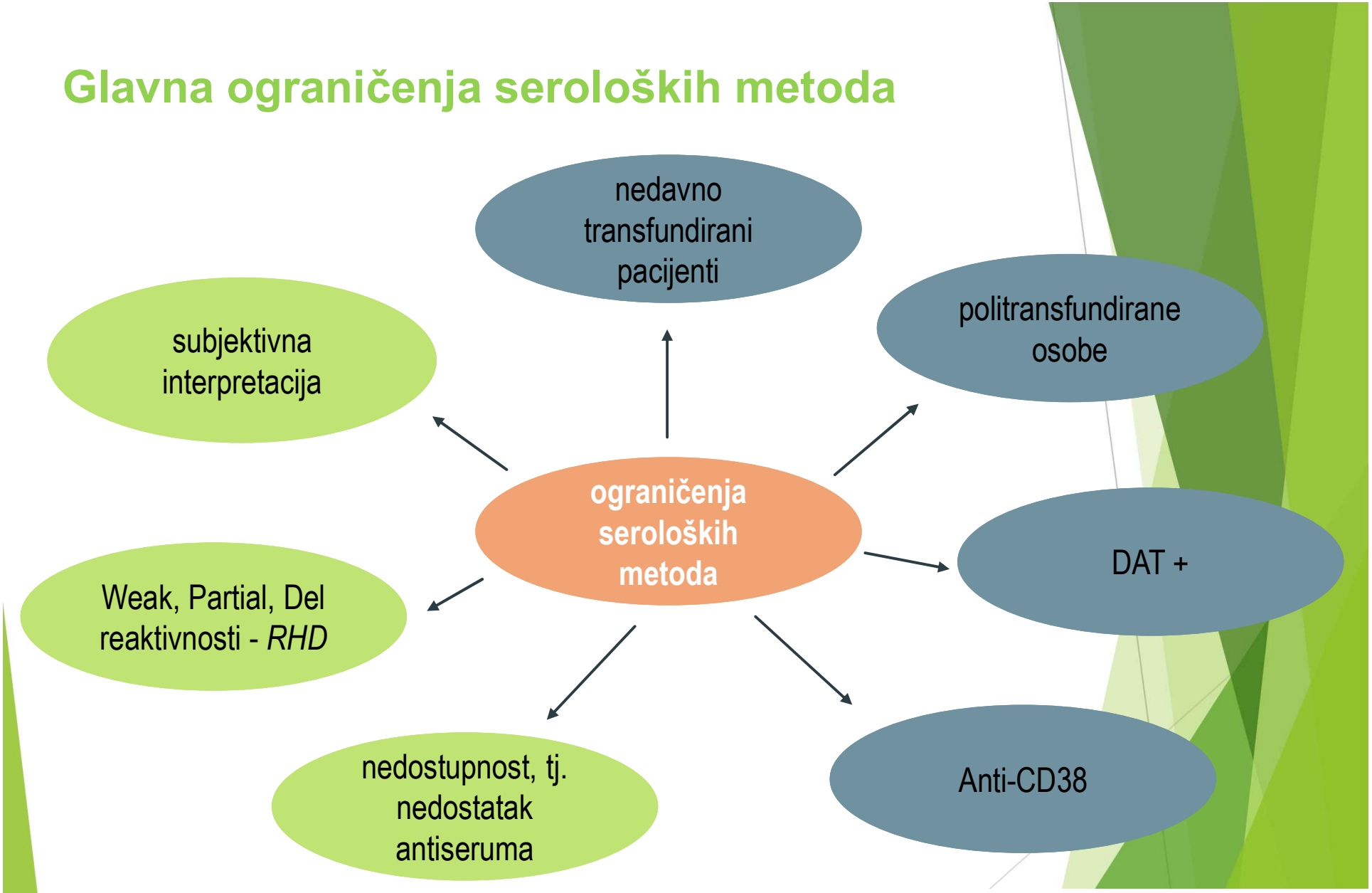
ALLELE, GENOTYPE, PHENOTYPE

(KELL K/k: Exon 6, 698T>C)



	Homozygous 698 TT	Homozygous 698 CC	Heterozygous 698 TC
Three genotypes:	KK	kk	Kk
Three phenotypes:	K+k-	K-k+	K+k+

Glavna ograničenja seroloških metoda



ograničenja serologije zbog dostupnosti reagenasa ili varijabilnosti jačina reakcija

ograničenja serologije zbog interferencije s protutijelima ili donorskim RBC

Pomoć molekularnih metoda

ograničenja serologije zbog dostupnosti reagenasa ili varijabilnosti jačina reakcija

- DNA omogućuje proučavanje bilo koje regije genoma
- neovisno o dostupnosti rijetkih protutijela
- jačina reakcije Ag-Ab nema efekta jer proučavamo slijed nukleotida u DNA
- metode molekularne tipizacije daju objektivan rezultat, koji ne ovisi o subjektivnoj evaluaciji

ograničenja serologije zbog interferencije s protutijelima ili donorskim RBC

- DNA iz krvi potječe iz leukocita
- nema interferencije protutijela iz uzorka

Ograničenja molekularnih metoda

predviđeni fenotip (predicted phenotype) – ograda u nazivu zbog posttranskripcijskih događaja

nove mutacije u veznim mjestima za primere ili probe korištene u testu, a koje još nisu opisane u literaturi ili uključene u test


FLUORESCENCIJA ?
Waiting for the perfect method
is like waiting for the perfect man.....

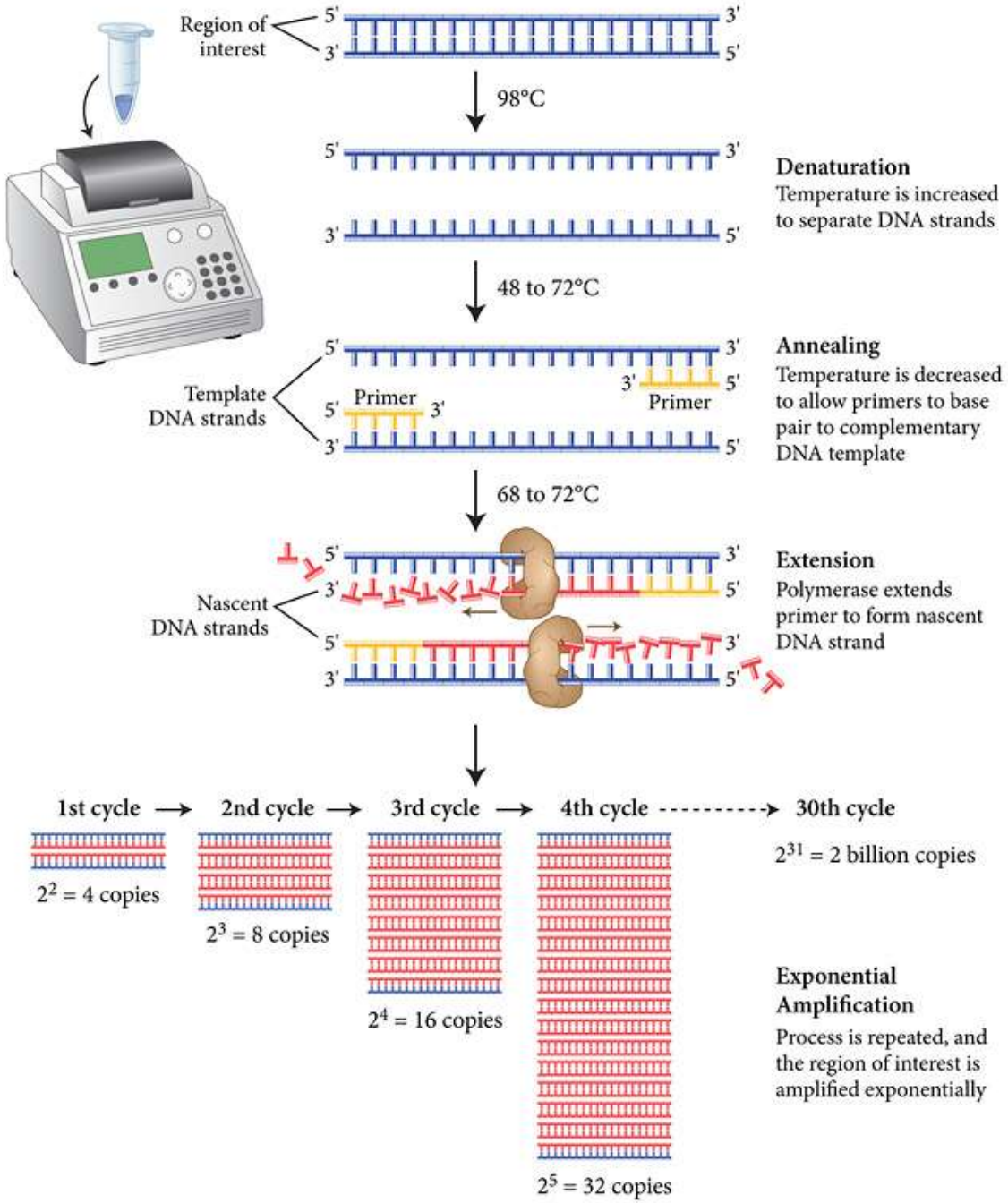
PCR

polymerase chain reaction
lančana reakcija polimerazom

umnožavanje (amplifikacija) DNA:

- pogodno za određivanje varijanti u genomu

vizualizacija ??



METODE

1. PCR-SSP s fluorometrijskom detekcijom signala - FluoVista
2. PCR u realnom vremenu – FluoQube
3. Ab deteckija / PCR-SSO - metodom mikrosfera - Luminex

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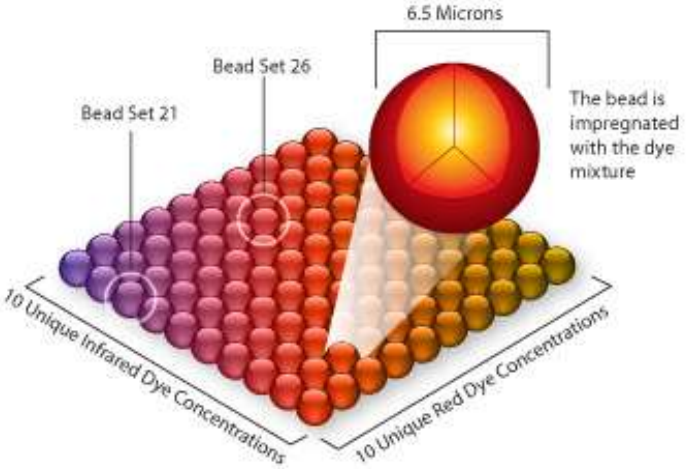
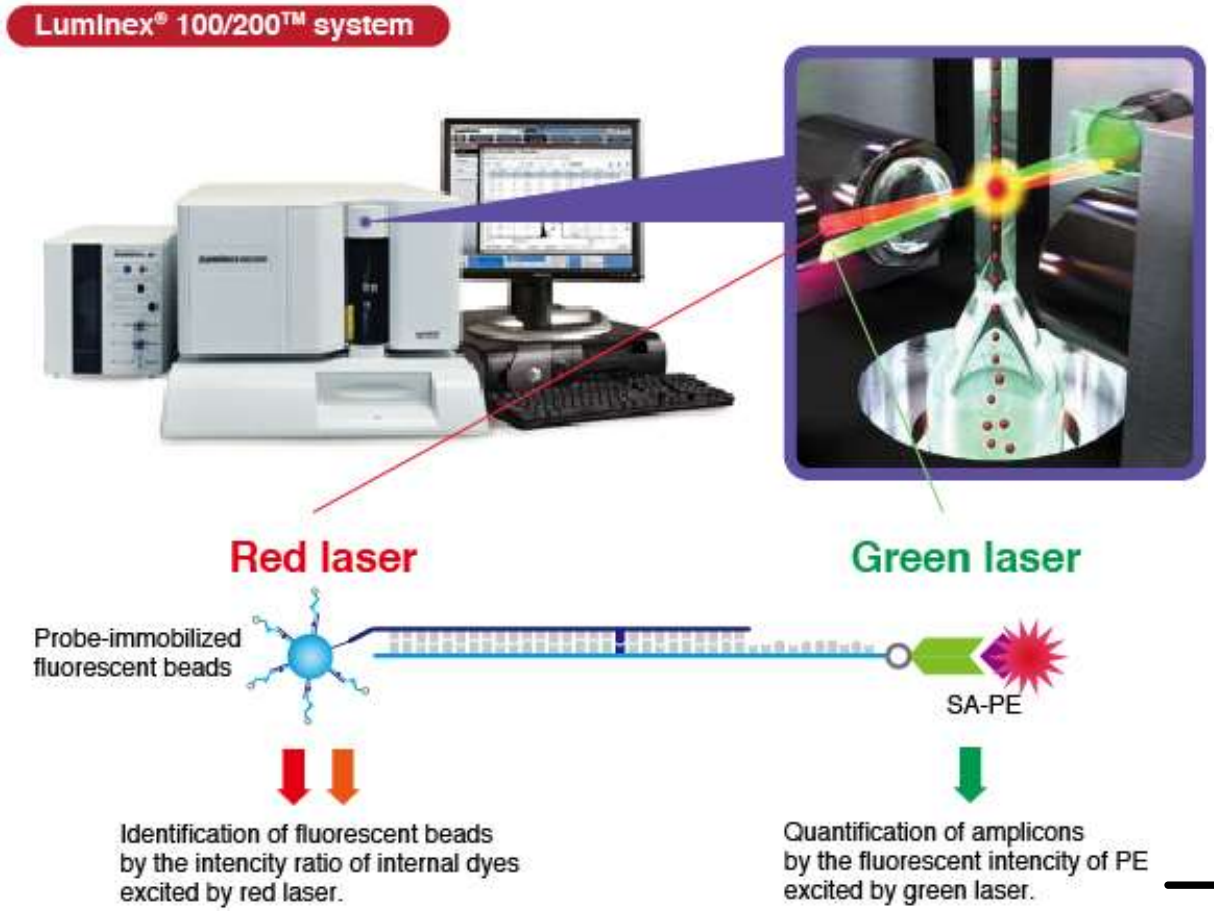
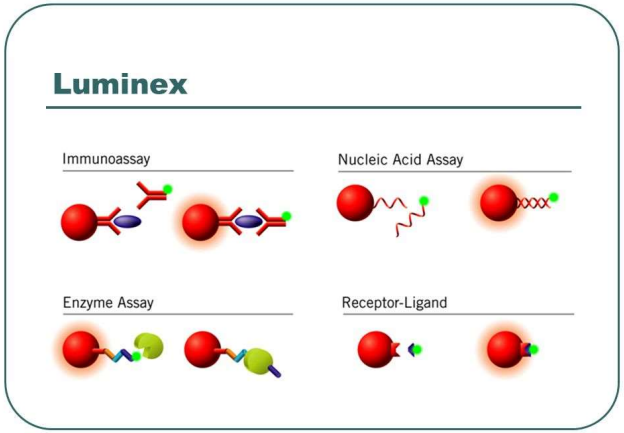
1. PCR-SSP s fluorometrijskom detekcijom signala - FluoVista
2. PCR u realnom vremenu – FluoQube
3. Ab deteckija / PCR-SSO - metodom mikrosfera - Luminex







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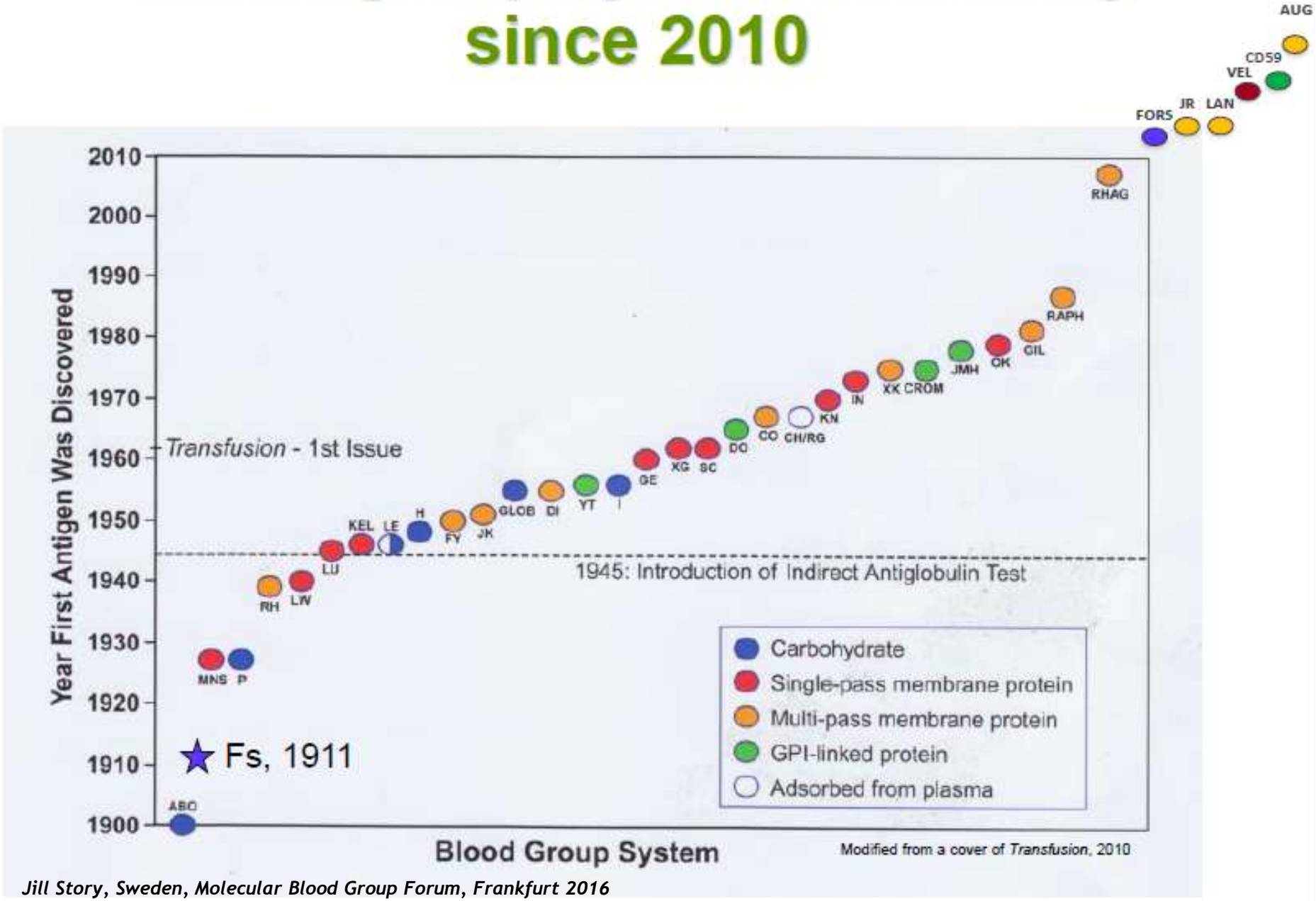
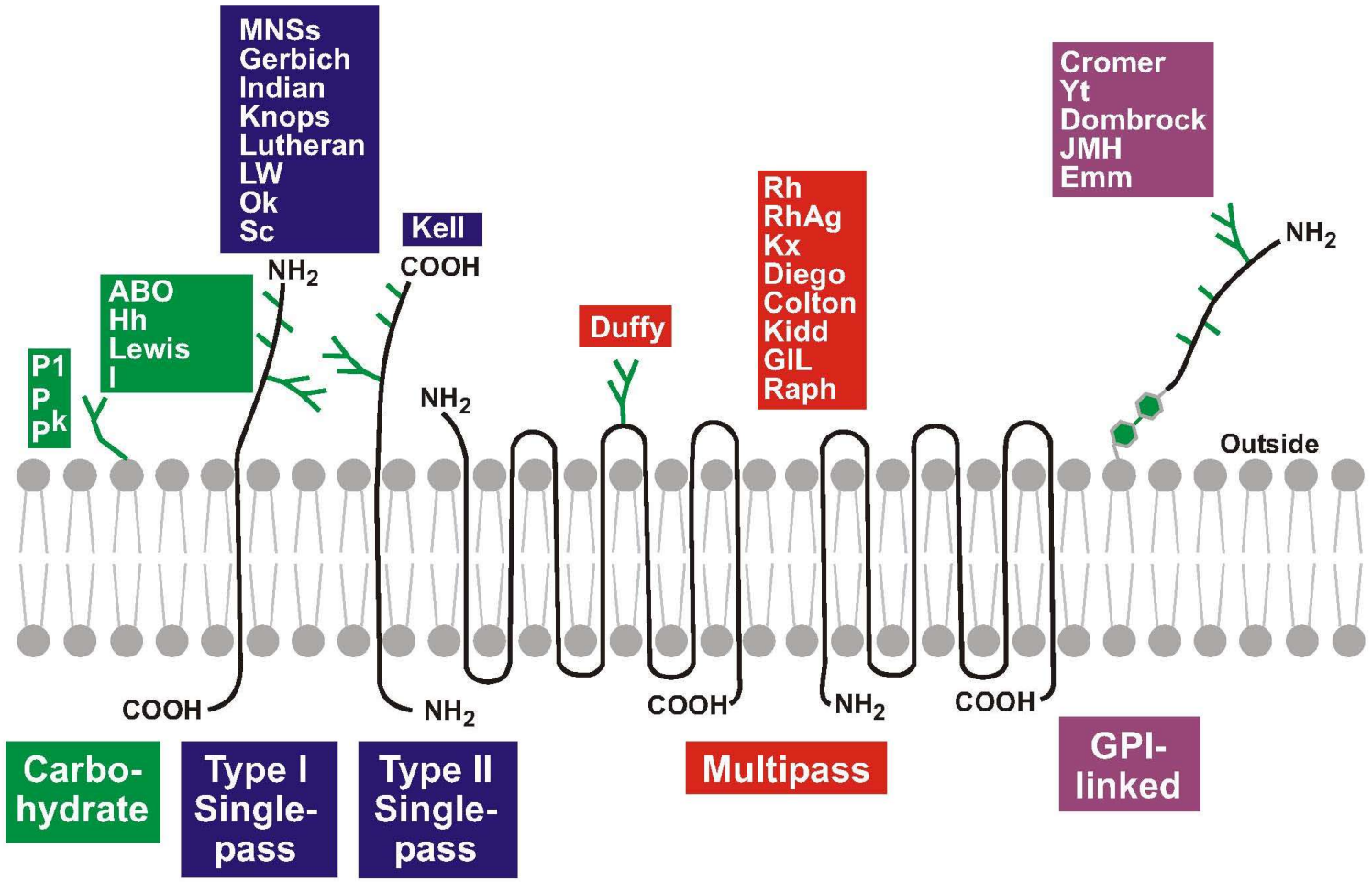
Blood group system discovery since 2010



Jill Story, Sweden, Molecular Blood Group Forum, Frankfurt 2016

ERITROCITNI ANTIGENI (RBC)



ERITROCITNI ANTIGENI (RBC)

ZAŠTO MOLEKULARNI (DNA) TEST?
ograničenja hemaglutinacije

- nedavno / učestalo politransfundirane osobe teško serološki tipizirati
  - potrebni eritrociti za testiranje
  - transfundirane stanice imaju fenotip donora

- teško odrediti fenotip nekih DAT+ uzoraka
  - serološka tipizacija ovisi o (monoklonskim) reagensima koji se vežu na antigene eritrocita – rezultat testa ovisan o korištenim reagensima
  - (auto) protutijela na eritrocitima utječu na rezultat

- slabi ili nedostupni reagensi – trošak?
  - pr. nedostupni reagensi za Dombrock, Colton, Cartwright

- subjektivnost aglutinacije

ERITROCITNI ANTIGENI (RBC)

KORISTI MOLEKULARNE TIPIZACIJE DAVATELJA KRVI

- probir davatelja u rutini
- identifikacija davatelja negativnih za učestale antigene = identifikacija davatelja s rijetkim tipovima antigena
  - pr. homozigota za rijetke alele / antigene
  - mogu se koristiti kao reagensi za detekciju protutijela
- pomoć u identifikaciji davatelja za aloimunizirane pacijente, odnosno identifikaciji antigen negativne krvi (krvi negativne na specifične antigene na koje pacijent može imati stvorena protutijela)
- potvrdna tipizacija seroloških rezultata
- molekularna tipizacija može koristiti različit izvor materijala za testiranje (DNA): osim pune krvi, *buffy coat*, može se uzeti i bris bukalne sluznice, sediment urina, amniociti ili sl.

ERITROCITNI ANTIGENI (RBC)

RBC-FluoGene KITS

ARTICLE NO.	ARTICLE	TESTS/PLATE	TESTS/KIT
001 086 040 / 10	**RBC-FluoGene ABO basic** 01, 02, B, A, A2	4 / 1	40 / 10
001 081 030 / 10	**RBC-FluoGene vERYfy** RHD: exons 3, 5, 10, psi; RHCE: C, C^{w}, c, E, e; KEL1(K), KEL2(k), JK1(Jk^{a}), JK2(Jk^{b}), FY1(Fy^{a}), FY2(Fy^{b}), FYnull(Fy^{a-}, Fy^{b-}), FYX(Fy^{bweak}), MNS1(M), MNS2(N), MNS3(S), MNS4(s), U+var(P2), U+var(NY), DO1(Do^{a}), DO2(Do^{b})	3 / 1	30 / 10
001 082 040 / 10	**RBC-FluoGene CDE** RHCE: C, C^{w}, c, E, e, 733G, 1006T RHD: exons 1-7, 9, 10, psi, DNB, D cat VII, DHMi, DAU, 697A, 697C	4 / 1	40 / 10
001 083 040*/ 10*	**RBC-FluoGene D weak/variant** weak D type 1, 1.1, 2, 3, 4.0/ 4.1, 4.2 (DAR), 5, 11 (M295I), 14, 15, 17, K409K, IVS3+1G>A	4 / 1	40 / 10
001 085 010	**RBC-FluoGene Rare** DI1(Di^{a}), DI2(Di^{b}), DI3(Wr^{a}), DI4(Wr^{b}), YT1(Yt^{a}), YT2(Yt^{b}), LU1(Lu^{a}), LU2(Lu^{b}), KEL3(Kp^{a}), KEL4(Kp^{b}), KEL6(Js^{a}), KEL7(Js^{b}), CO1(Co^{a}), CO2(Co^{b}), KN1(Kn^{a}), KN2(Kn^{b})	1	10
001 087 048	**RBC-FluoGene D-Screen** For screening of RHD exons 3/5 and 10 in a single reaction.	12	48
001 088 048	**RBC-FluoGene Vel-Screen** For screening of Vel+ and Vel-.	12	48
001 089 010	**RBC-FluoGene KKD** KEL1(K), KEL2(k), KEL3(Kp^{a}), KEL4(Kp^{b}), KEL6(Js^{a}), KEL7(Js^{b}), JK1(Jk^{a}), JK2(Jk^{b}), FY1(Fy^{a}), FY2(Fy^{b}), FYnull(Fy^{a-}, Fy^{b-}), FYX(Fy^{bweak})	1	10
001 090 010	**RBC-FluoGene MNS** MNS1(M), MNS2(N), MNS3(S), MNS4(s), U+var(P2), U+var(NY)	1	10

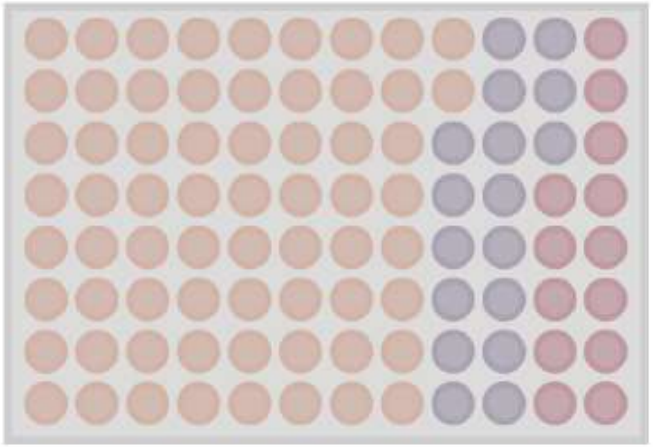
*The purchase price of this product includes limited, non-transferable rights under European Patent EP 1 047 777 B1.

ERITROCITNI ANTIGENI (RBC)

Easy, fast and flexible

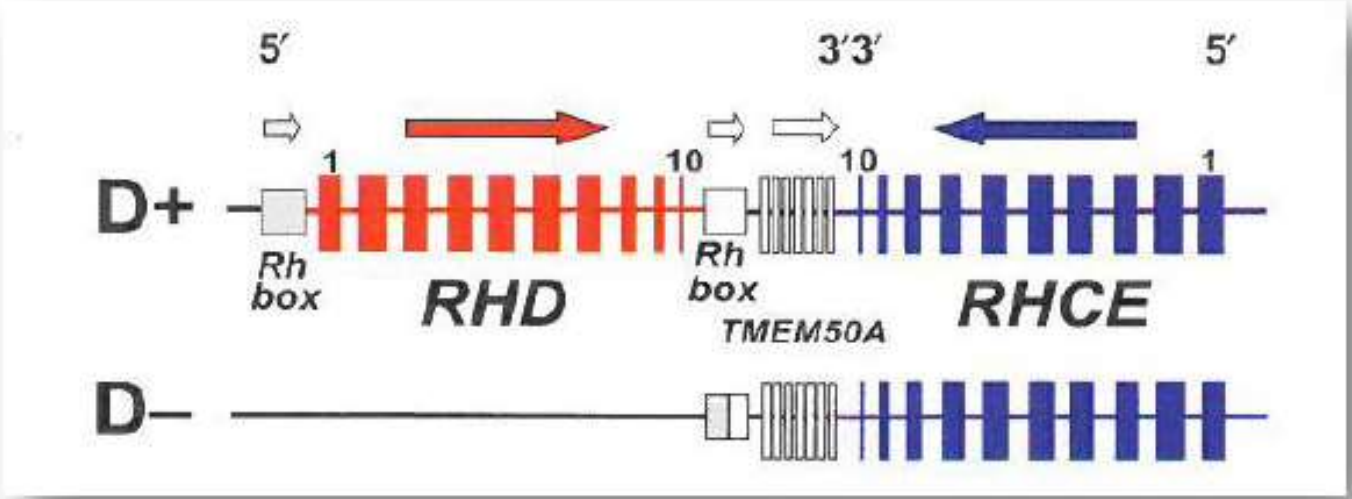
Flexible

- From 1 to 96 tests per run
- Multiple product batch; ID CORE XT, ID HPA XT and ID RHD XT can be performed in the same run
- Open technology: standard Luminex™ equipment can be used for other products



ID CORE XT
ID HPA XT
ID RHD XT

GENETICS OF *RHD* AND *RHCE*



Source: "Human Blood Groups" by Geoff Daniels

RHD gene deletion as a result of unequal crossing over. This might happen in homologous regions, e.g. Rh box.

The Rh boxes are ~9000 bp each and 98,6% homologuous. The deletion occurs within a 1463 bp region which is in both boxes identical.

inno-train RBC-FluoGene D-Screen

CE LOT R987077 Seite 1 von 1

Spezifitätentabelle / *Specificity Table*

IVD 2018-12

System	Position	Mix Name	Spezifität / *Specificity*	SNP	Intron / Exon	Aminosäure / *Amino acid*	ISBT Phänotyp / *ISBT phenotype*	ISBT Allelnamen / *ISBT allele names*	RBC marker [Color]	IC marker [Color]
RHD-Exon Screening	B1, B2, B3, B4, B5, B6, F1, F2, F3, F4, F5, F6	D-Screen-D-E10	Exon 10	+1054	3'UTR	-	RH:1 (D) vs. RHD negative	RHD*01 vs. RHD*01N.01	1	3
		D-Screen-D-E3/5	Exon 3 Exon 5	455A 676G 787G	3 5	Ile172 Ala226 Gly263			2	

SNPs and nomenclature according to ISBT Blood Group Allele Tables "004_RHD_alleles_v4.0" and "Names for RHD (ISBT 004) negative null blood group alleles v3.0 160713" (http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/).

Plattenansicht / Plate view:

Die Graphik zeigt die Positionen der vorgetropften Primer-Sonden-Mix
The graph shows the positions of the probemixes.

O	*Rh neg*	*C-	*E+	*c+	*e+	*K-	Fya+

	1	2	3	4	5	6	7	8	9	10
A										
B	1	3	5	7	9	11				
C										
D										
E										
F	2	4	6	8	10	12				
G										
H										

Detailed Run Information

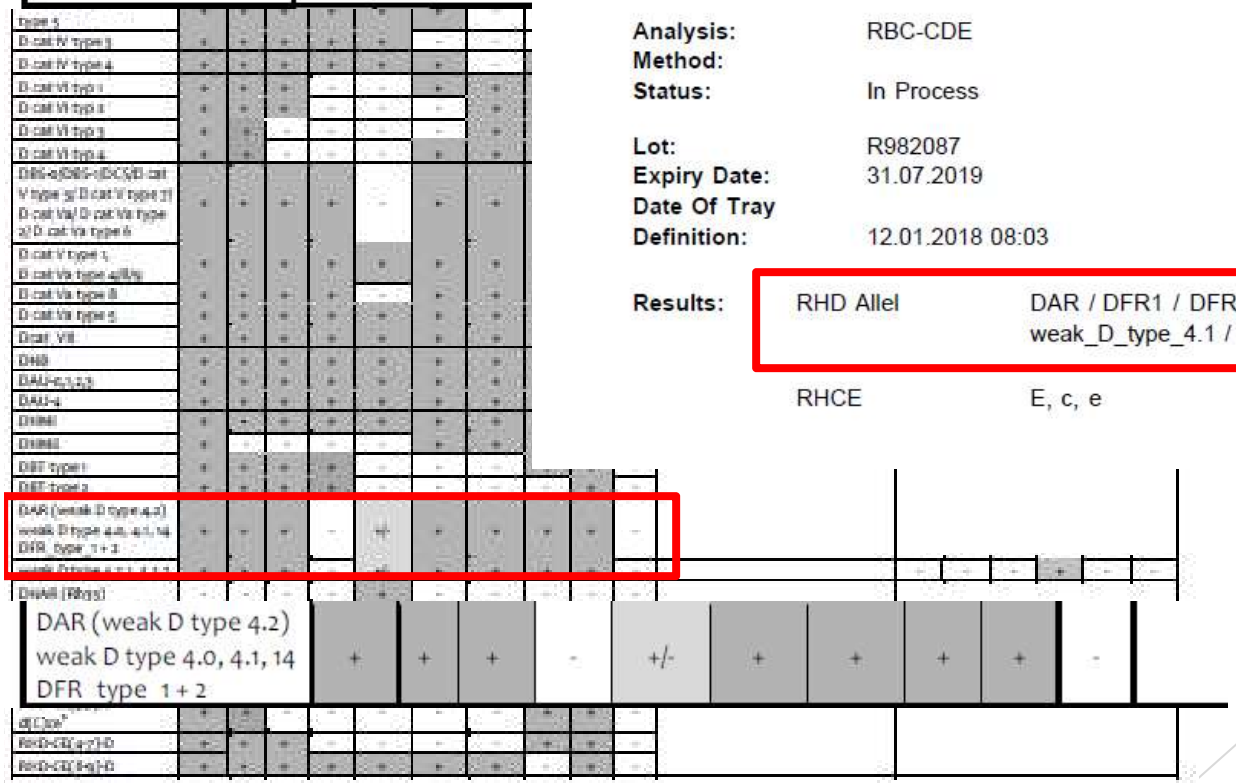
Position	Control	Mix	Score
F4	+	D-Screen-D-E10	+
		D-Screen-D-E3/5	+

Hinweis: die Software belegt die Platten spaltenweise von oben nach unten. Daher muss die Auftragung der DNA-FluoMix Gemische ebenfalls von oben nach unten und von links nach rechts erfolgen (entsprechend der Nummerierung in der Graphik).
***Note:** the software assigns the plate column by column from top down. Therefore the DNA-FluoMix solutions have to be applied accordingly from top down and from left to right (see numbers in graph).*

inno-train RBC-FluoGene CDE CE LOT F982064 / F982065

Auswertetabelle / Reaction pattern IVD 2015-11

Position	H2/D2	G2	F2	E2	A2/D2	C2/H3	B2	G2	A2	E1/E3	E1/E3	G1/G3	F1/F3	G1/G3	F1/F3	D1/D3	C1/C3	A1/A3	D1/D3	A1/A3	B1/B3
Mix	D-Ex1-1/2	D-Ex 2	D-Ex3	D-Ex4	D-Ex5-1/2	D-Ex6-1/2	D-Ex7	D-Ex9	D-Ex10	D-psi-1/2	W-1/2	C-1/2	c-1/2	E-1/2	e-1/2	D-DHMi-1/2	D-catVII-1/2	D-697A-1/2	D-697C-1/2	D-DNB-1/2	D-DAU-1/2
Specificity	D_1	D2	D3	D_4	D_5	D_6	D_7	D_9	D_{10}	Dpsi	"W"	C	c	E	e	DHMi	D^{VII}	697A	697C	DNB	DAU
	D Exon Block									CcEe Block						CATPA Block					
D, #[1]	+	+	+																		
d	-	-	-																		
d, C	-	+	-																		
C^w																					
c^w (rare)																					
c																					
E																					
e																					



FluoGene v1.5.0.0

inno-train DIAGNOSTIK GMBH

Person ID: FLUO-61705286

Order No.: 61705286 **Order Date:** 12.01.2018

Analysis: RBC-CDE
Method:
Status: In Process

Lot: R982087
Expiry Date: 31.07.2019
Date Of Tray Definition: 12.01.2018 08:03

Results:

RHD Allel	DAR / DFR1 / DFR2 / weak_D_type_14 / weak_D_type_4.0 / weak_D_type_4.1 / weak_D_type_4.2.1 / weak_D_type_4.2.2
RHCE	E, c, e

RBC-FluoGene D weak/variant

Spezifitätentabelle / *Specificity table*

Reaktion / Reaction	Position	Spezifität / Specificity	S
Dweak_type_1-1.1	A2	weak_D_type_1	80
Dweak_type_1.1		weak_D_type_1.1	52
Dweak_type_2-1, Dweak_type_2-2*	A1, B2	weak_D_type_2	115
Dweak_type_3	B1	weak_D_type_3	8
Dweak_type_4.2*	D2	weak_D_type 4.2.1/4.2.2	95 102
Dweak_type_4-14**	C1	weak_D_type_4.0/4.1/4.2.1/4.2.2/DAR (weak_D_type_4.2)/4.3/14	60 (5
Dweak_type_4**	C2	weak_D_type_4.0/4.1/4.2.1/4.2.2/DAR (weak_D_type_4.2)/4.3	60 (5
Dweak_type_5	D1	weak_D_type_5	44
Dweak_type_11	E1	weak_D_type_11	88
Dweak_type_15-1, Dweak_type_15-2	F1, E2	weak_D_type_15	84
Dweak_type_17*	F2	weak_D_type_17	34
Dweak_type_K409K	G2	K409K	122
Dweak_type_IVS3-1, Dweak_type_IVS3-2	G1, H2	IVS3+1G>A	486

SNPs und Nomenklatur nach / *SNPs and nomenclature*
*Names for alleles encoding weak D phenotypes v4.0
"004 RHCE alleles v3.0 20160728" (http://www.isbtwe

FluoGene v1.5.0.0

inno-train DIAGNOSTIK GMBH

Person ID: FLUO-61705286

Order No.: 61705286 **Order Date:** 12.01.2018

Analysis: RBC-Dweak
Method:
Status: In Process

Lot: R983117V
Expiry Date: 31.10.2019
Date Of Tray Definition: 12.01.2018 09:38

Results: RHD weak_D_type_14

Detailed Run Information

Position	Control	Mix	Score	Edition	Value / Cutoff
A1	+	Dweak_type_2-1			- / 8
		Dweak_type_4.1-1	-		- / 22
B1	+	Dweak_type_3			/ 10
C1	+	Dweak_type_4-14	+		25 / 8
D1	+	Dweak_type_5	-		- / 14
E1	+	Dweak_type_11	-		- / 6
F1	+	Dweak_type_15-1	-		- / 6
G1	+	Dweak_type_IVS3-1	-		- / 6
H1	-				
A2	+	Dweak_type_1-1.1	-		4 / 5
		Dweak_type_1.1	-		- / 14
B2	+	Dweak_type_2-2	-		- / 8
		Dweak_type_4.1-2	-		- / 22
C2	+	Dweak_type_4	-		- / 12
D2	+	Dweak_type_4.2	-		- / 5
E2	+	Dweak_type_15-2	-		- / 6
F2	+	Dweak_type_17	-		- / 8
G2	+	Dweak_type_K409K	-		- / 4
H2	+	Dweak_type_IVS3-2	-		- / 6

ERITROCITNI ANTIGENI (RBC)

ERITROCITNI ANTIGENI (RBC)

RBC-FluoGene vERYfy eXtend

Combining RHD, RHCE, KEL, JK, FY, MNS, DO, LU, YT, DI, VEL, CO and KN typing in one Assay

- Fastest automated molecular technology with the lowest manual hands-on-time
- Unique composition with the highest number of detected human erythrocyte antigens

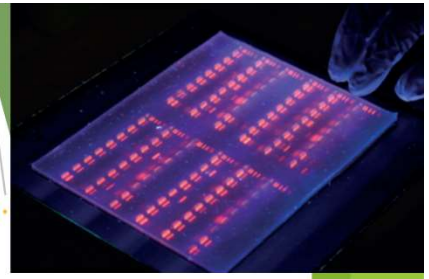
RHD/RHCE	RHD exons 1, 2, 3, 4, 5, 6, 7, 9, 10, psi, DNB, D cat VII, DHMi, DAU, 697A, 697C weak D type 1, 1.1, 2, 3, 4.1, 4.0/4.2/4.3, 5, 11 (M295I), 14, 15, 17, K409K, IVS3+1G>A C, C^{W}, c, E, e, 733G, 1006T
KEL	KEL1(K), KEL2(k), KEL3(Kp^a), KEL4(Kp^b), KEL6(Js^a), KEL7(Js^b)
JK	JK1(Jk^a), JK2(Jk^b)
FY	FY1(Fy^a), FY2(Fy^b), FYnull(Fy^{a-}, Fy^{b-}), FYX(Fy^{bweak})
MNS	MNS1(M), MNS2(N), MNS3(S), MNS4(s), U+var(P2), U+var(P3), U+var(NY)
DO	DO1(Do^a), DO2(Do^b), Hy+, Hy-, Jo^{a+}, Jo^{a-}
LU	LU1(Lu^a), LU2(Lu^b)
YT	YT1(Yt^a), YT2(Yt^b)
DI	DI1(Di^a), DI2(Di^b), DI3(Wr^a), DI4(Wr^b)
VEL	Vel+, Vel-
CO	CO1(Co^a), CO2(Co^b)
KN	KN1(Kn^a), KN2(Kn^b)

ERITROCITNI ANTIGENI (RBC)

ID CORE XT antigen list

Blood groups	Alleles assayed	PHENOTYPES (Antigens)
RhCE	*RHCE*ce, RHCE*Ce, RHCE*cE; RHCE*CE, RHCE*CeCW, RHCE*ceCW, RHCE*CECW, RHCE*ceAR, RHCE*CeFV , RHCE*CeVG , RHCE*cEFM, RHCE*ce[712G] , RHCE*ce[733G], RHCE*ce[733G,1006T], RHCE*CE-D[2, 5, 7]-CE, RHCE*cE[697G,712G,733G], RHD*r's-RHCE*ce[733G,1006T]*	**C** (RH:2), **E** (RH:3), **c** (RH:4) **e** (RH:5), **C^{W}** (RH:8), **V** (RH:10), **hrS** (RH:19) **VS** (RH:20) , **hrB** (RH:31)
Kell	*KEL*K_KPB_JSB, KEL*k_KPB_JSB KEL*k_KPA_JSB, KEL*k_KPB_JSA*	**K** (KEL:1), **k** (KEL:2), **Kpa** (KEL:3), **Kpb** (KEL:4) **Jsa** (KEL:6), **Jsb** (KEL:7)
Kidd	*JK*A, JK*B, JK*B_null(871C), JK*B_null(IVS5-1a)*	**Jka** (JK:1), **Jkb** (JK:2)
Duffy	*FY*A; FY*B, FY*B_GATA, FY*B[265T]_FY*X*	**Fya** (FY:1), **Fyb** (FY:2)
MNS	*GYPA*M, GYPA*N, GYPB*s, GYPB*S, GYPB*Mur, GYPB*deletion , GYPB*S_null(230T), GYPB*S_null(IVS5+5t)*	**M** (MNS:1), **N** (MNS:2), **S** (MNS:3), **s** (MNS:4) **U** (MNS:5), **Mia** (MNS:7)
Diego	*DI*A, DI*B*	**Dia** (DI:1), **Dib** (DI:2)
Dombrock	*DO*A, DO*B, DO*B_HY, DO*A_JO*	**Doa** (DO:1), **Dob** (DO:2) **Hy** (DO:4), **Joa** (DO:5)
Colton	*CO*A, CO*B*	**Coa** (CO:1),**Cob** (CO:2)
Cartwright	*YT*A, YT*B*	**Yta** (YT:1), **Ytb** (YT:2)
Lutheran	LU*A, LU*B	**Lua** (LU:1), **Lub** (LU:2)

ERITROCITNI ANTIGENI (RBC)



New Ready Gene kit available in October: RBC-Ready Gene D weak Screen

NEW

We are happy to announce our new RBC-Ready Gene D weak Screen kit (Art. No. 001 080 024). The new test system enables weak D type 1, 2 and 3 genotyping with only four reactions. Individuals with a weak D type 1, 2 or 3 are handled as RhD positive and can be treated with RhD positive blood products as recipients of transfusions. Pregnant women carrying one of these alleles do not need a Rhesus prophylaxis. This new product will be available for 24 tests per kit.

Spezifitätentabelle / *Specificity Table*

Reaktion / *Reaction*	1	2	3	4
PCR-Produkt/ *PCR product* (bp)	434	250 150	125	165
Spezifität / *Specificity*	negative control	weak D type 1.1 weak D type 1	weak D type 2	weak D type 3
SNP		52C>G 809T>G	1154G>C	8C>G
ISBT Allelname / *Allele name*		RHD*01W.1.1 (RHD*weak D type 1.1) RHD*01W.1/.1.1/.1.2 (RHD*weak D type 1/1.1/1.2) RHD*62	RHD*01W.2/.2.1/.2.2 (RHD*weak D type 2/2.1/2.2)	RHD*01W.3/.3.1 (RHD*weak D type 3/3.1)
ISBT Phänotyp / *Phenotype*		Type 1.1 Type 1/1.1/1.2 DNT (V270G)	Type 2/2.1/2.2	Type 3/3.1
Ergebnis / *Result*				
Reaktion / *Reaction*				
RHD*weak D type 1/1.2/ RHD*62	-	150	-	-
RHD*weak D type 1.1	-	250 150	-	-
RHD*weak D type 2/2.1/2.2	-	-	+	-
RHD*weak D type 3/3.1	-	-	-	+

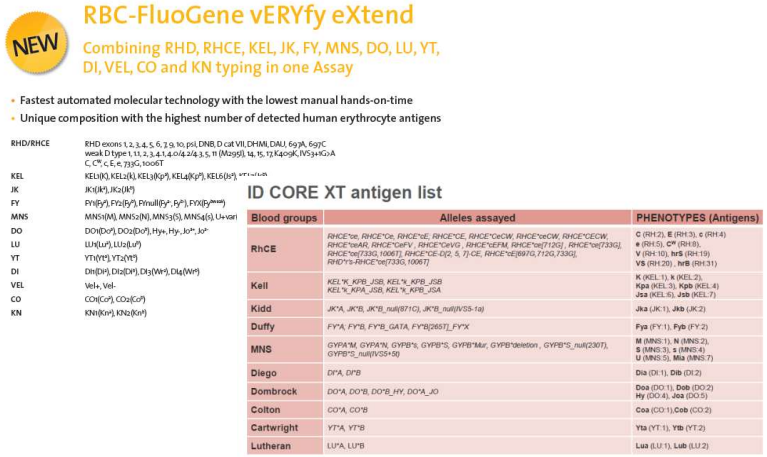
ERITROCITNI ANTIGENI (RBC)

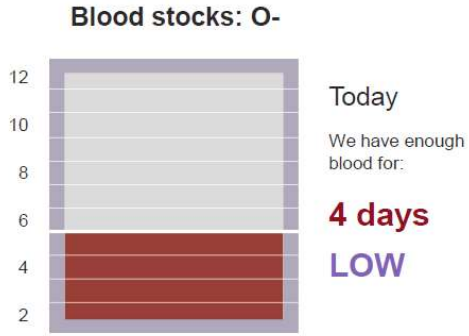
ID RHD XT variant list

Blood group system	Predicted Phenotype	Alleles assayed	ISBT name
Rh	Weak D Type 1	RHD*weak D type 1	RHD*01W.1
Rh	Weak D Type 2	RHD*weak D type 2	RHD*01W.2
Rh	Weak D Type 3	RHD*weak D type 3	RHD*01W.3
Rh	D-	RHD*Pseudogene	RHD*04N.01
Rh	D-	RHD*DIIIa-CE(3-7)-D	RHD*03N.01
Rh	D-	RHD deletion	RHD*01N.01
HPA-1	HPA-1a, HPA-1b	HPA1a, HPA1b	N.A.

paralelna tipizacija

ERITROCITNI ANTIGENI (RBC)





ID RHD XT variant list

Blood group system	Predicted Phenotype	Alleles assayed	ISBT name
Rh	Weak D Type 1	RHD*weak D type 1	RHD*01W.1
Rh	Weak D Type 2	RHD*weak D type 2	RHD*01W.2
Rh	Weak D Type 3	RHD*weak D type 3	RHD*01W.3
Rh	D-	RHD*Pseudogene	RHD*04N.01
Rh	D-	RHD*DIIIa-CE(3-7)-D	RHD*03N.01
Rh	D-	RHD deletion	RHD*01N.01
HPA-1	HPA-1a, HPA-1b	HPA1a, HPA1b	N.A.

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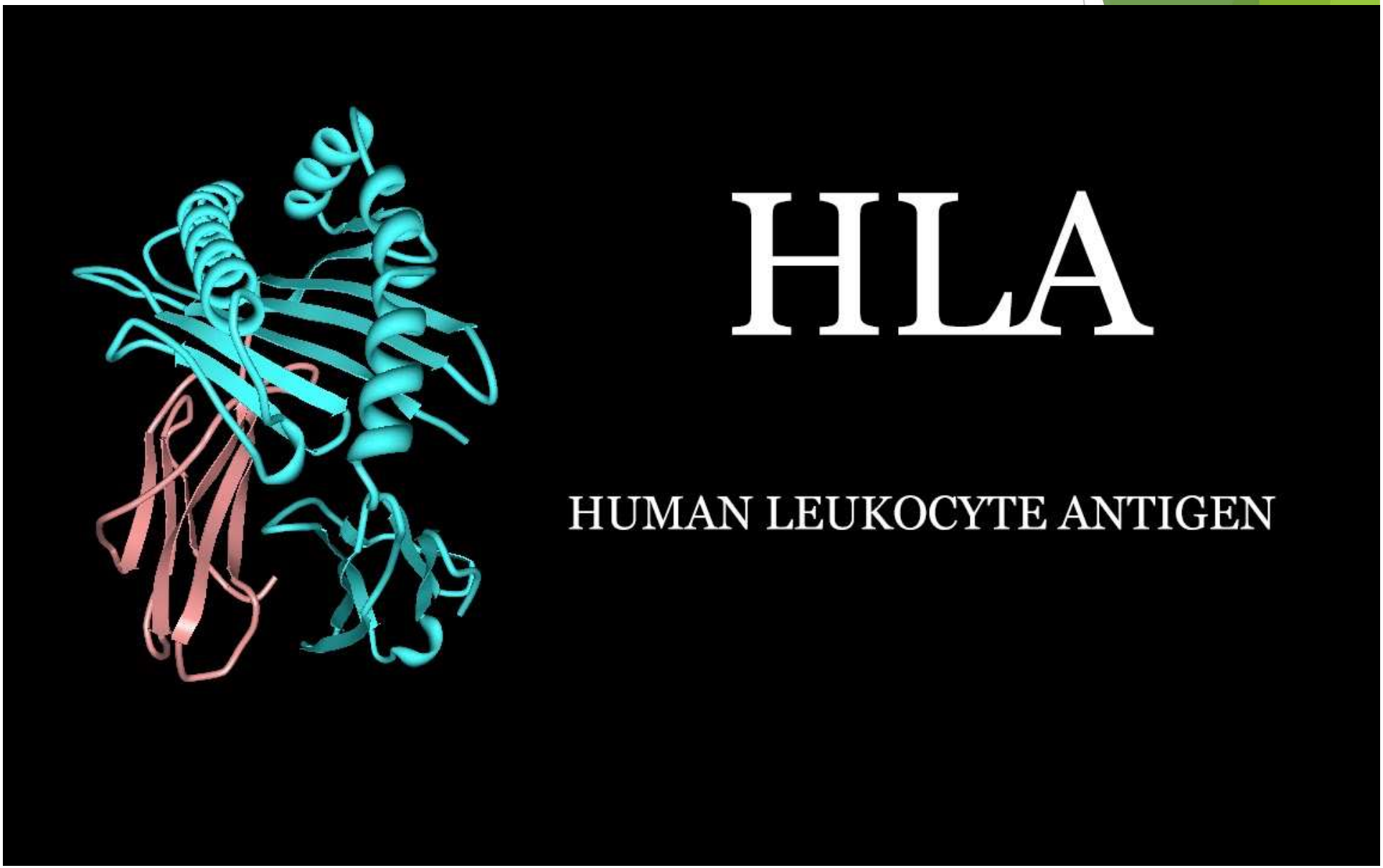
- odrediti prisutnost / odsutnost alela kod kronično i nedavno transfundiranih pacijenata
- probir davatelja u rutini
- odabir podudarnih davatelja za aloimunizirane pacijente
- nadopuna serološkog panela pomoću identifikacija dodatnih antigena
- tipizacija pacijenata koji uzimaju lijekove koji utječu na serološku tipizaciju (pr. daratumumab, terapeutsko anti-CD38 protutijelo na stanicama mijeloma, koji utječu na tipizaciju Kell antigena)

- tipizacija pacijenata sa slabim RhD antigenom za racionalniju uporabu D negativnih jedinica krvi
- tipizacija trudnica sa slabim RhD antigenom za racionalniju uporabu RhIG (paralelna tipizacija sustava HPA-1)
- potvrda D negativnih davatelja

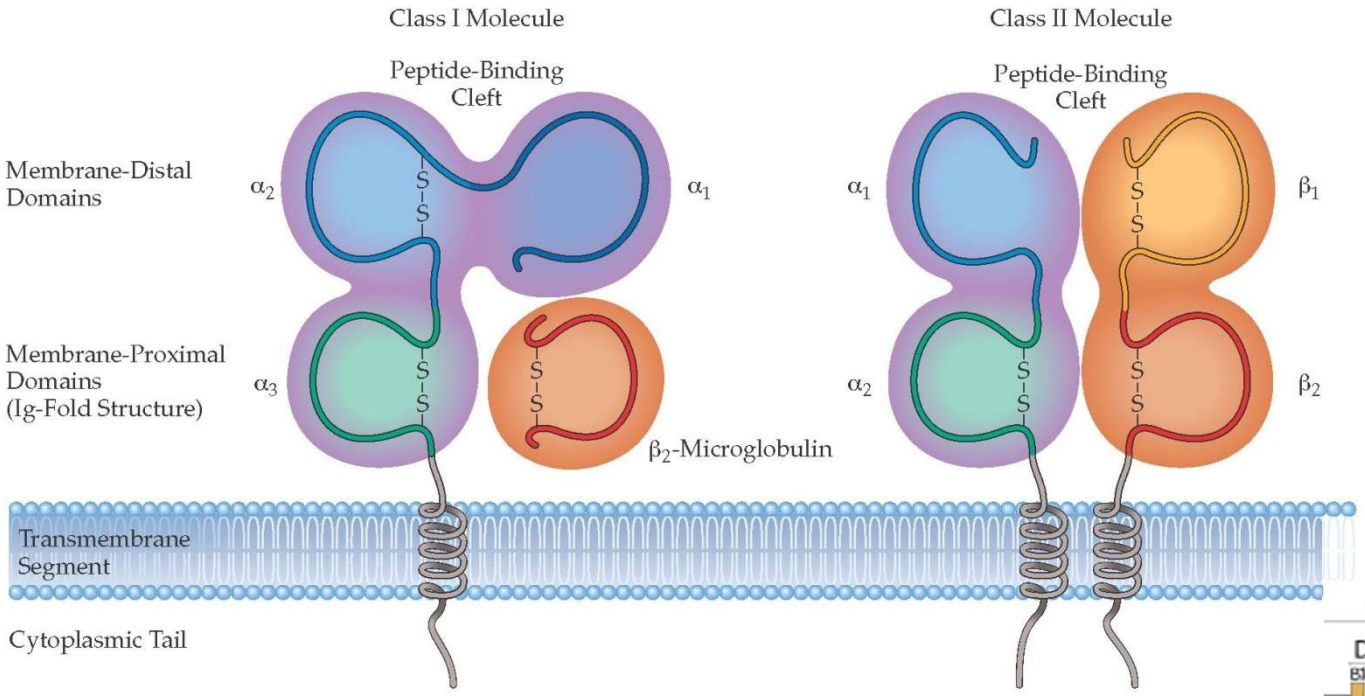
SADRŽAJ

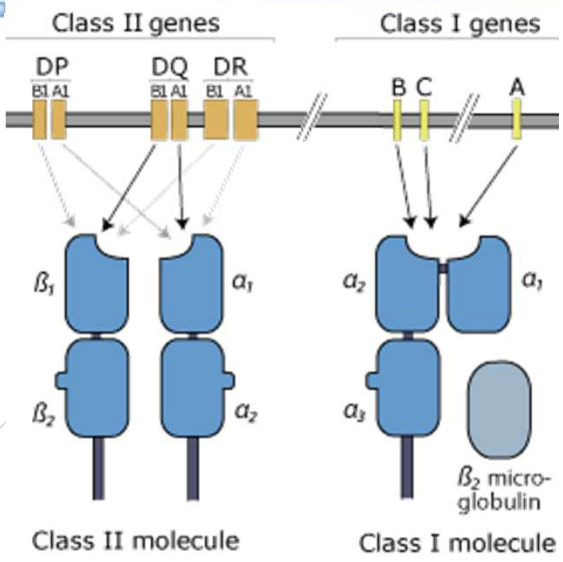
1. Metode molekularne tipizacije i detekcije protutijela
2. Eritrocitni antigeni (RBC)
3. Leukocitni antigeni (HLA)
4. Trombocitni antigeni (HPA)

LEUKOCITNI ANTIGENI (HLA)

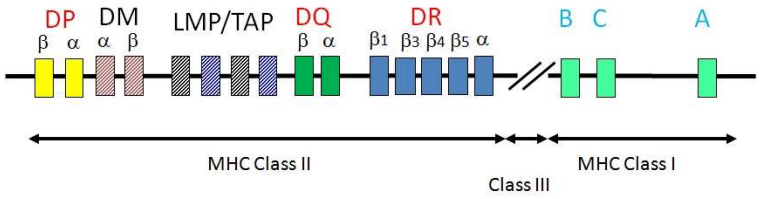


LEUKOCITNI ANTIGENI (HLA)

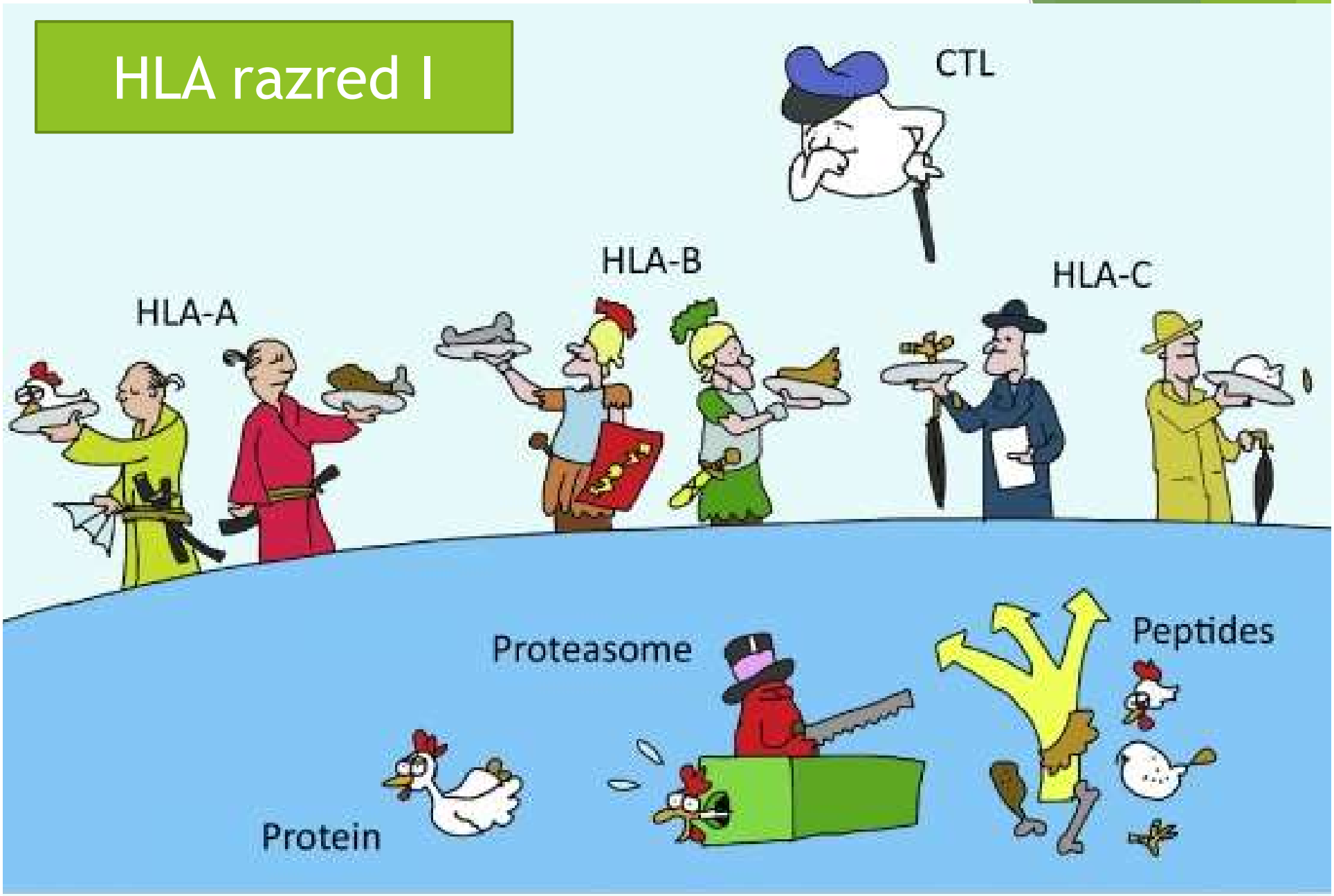




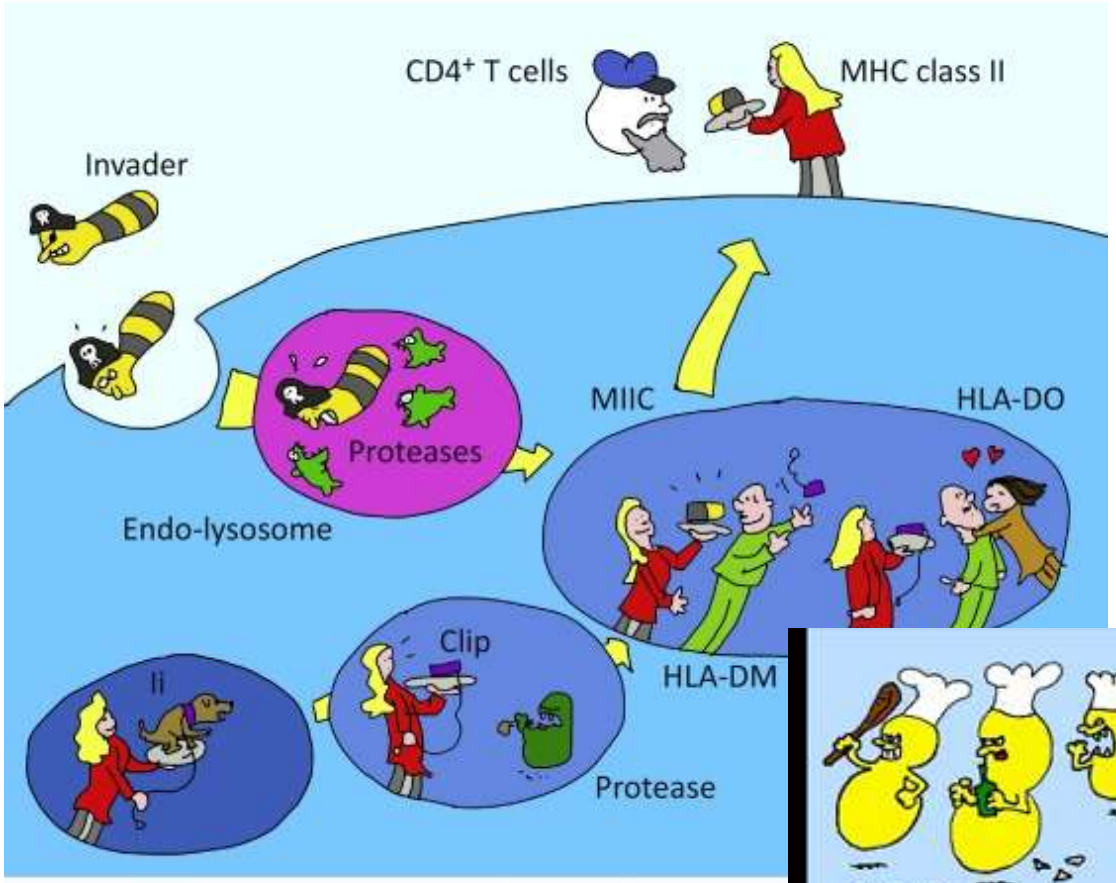
Simplified map of the HLA region



HLA razred I



Trends in Immunology

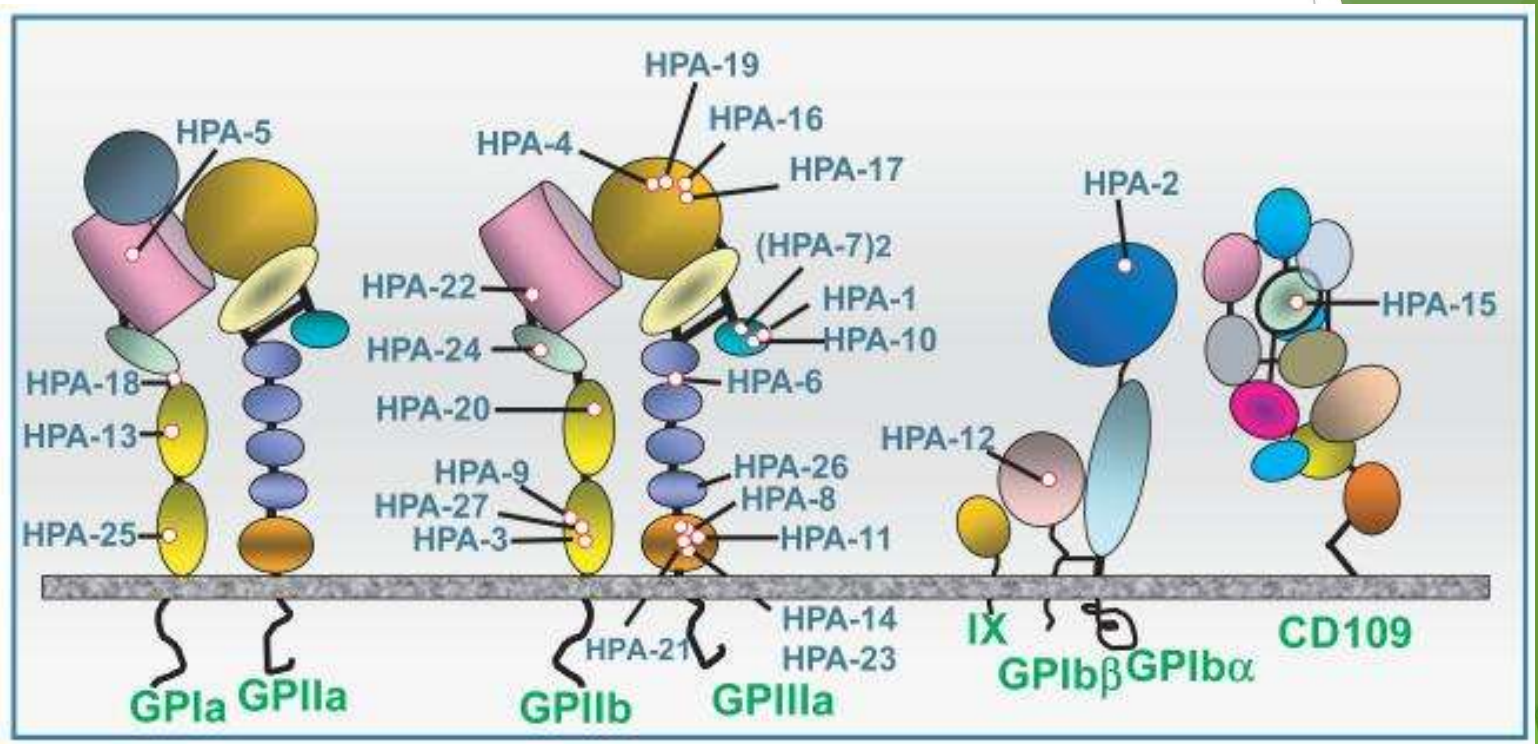


HLA razred II

SADRŽAJ

1. Metode molekularne tipizacije i detekcije protutijela
2. Eritrocitni antigeni (RBC)
3. Leukocitni antigeni (HLA)
4. Trombocitni antigeni (HPA)

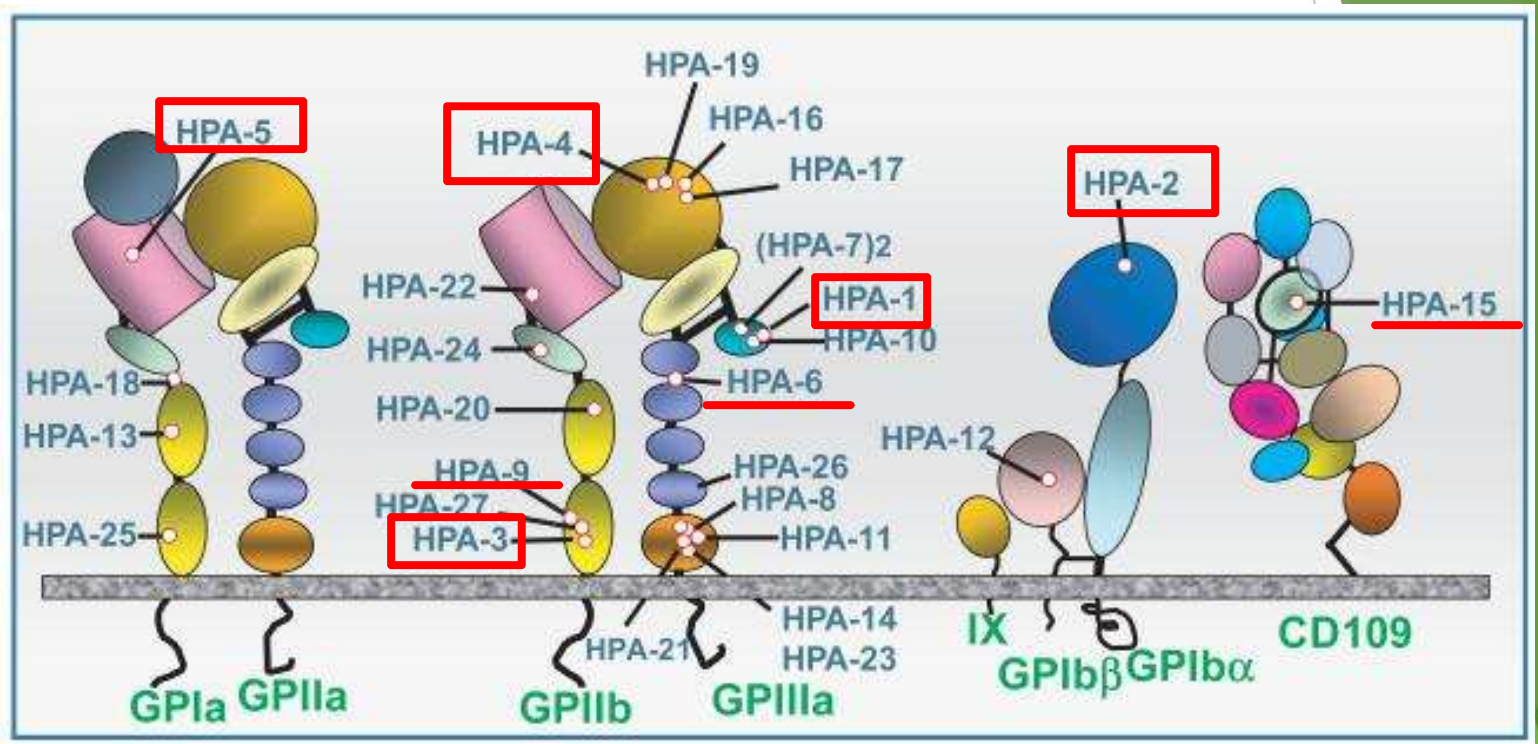
TROMBOCITNI ANTIGENI (HPA)



- za razliku od HLA antigena, **HPA antigeni nisu samostalni produkti gena**
- označavaju manifestaciju SNP* polimorfizama na određenom malom broju glikoproteina

** SNP = single nucleotid polymorphysm*

TROMBOCITNI ANTIGENI (HPA)



- tipizacija HPA davatelja i pacijenata – nadopuna testu za detekciju anti-HPA
- tipizacija davatelja u svrhu selekcije antigen-negativnih trombocita
- pomoć u selekciji podudarnih davatelja trombocita za refraktorne ili aloimunizirane pacijente
- dodatak kliničkoj slici aloimunih poremećaja trombocita (FNAIT, refrakternost transfuzije trombocita, post-transfuzijska purpura i sl.)

Tabela 13. **Humani trombocitni antigeni (Human Platelet Antigens – HPA).**
Preuzeto i adaptirano: *Metcalfe P. Nomenclature of human platelet antigens. Vox Sang 2003; 85: 240-5.i https://www.ebi.ac.uk/ipd/hpa/table1.html (Immuno Polymorphism Database)*

ANTIGEN	GLIKOPROTEIN	HGNC naziv*	Originalni naziv	Hromo-zom	Učestalost (%)
HPA-1	GPIIIa (CD61)	**ITGB3**	Zw^a, Pl^{A1}	17	HPA-1a: 98
			Zw^b, Pl^{A2}		HPA-1b: 28
HPA-2	GPIba (CD42b)	**GP1BA**	Ko^b	17	HPA-2a: 99
			Ko^a, Sib^a		HPA-2b: 16
HPA-3	GPIIb (CD41)	**ITGA2B**	Bak^a, Lek^a	17	HPA-3a: 84
			Bak^b		HPA-3b: 63
HPA-4	GPIIIa (CD61)	**ITGB3**	Yuk^b, Pen^a	17	HPA-4a:100
			Yuk^a, Pen^b		HPA-4b: <1
HPA-5	GPIa (CD49b)	**ITGA2**	Br^b, Zav^b	5	HPA-5a: 99
			Br^a, Zav^a, He^a		HPA-5b: 15
HPA-6w	GPIIIa (CD61)	**ITGB3**	Ca^a, Tu^a	17	<1
HPA-7w	GPIIIa (CD61)	**ITGB3**	Mo^a	17	<1
HPA-8w	GPIIIa (CD61)	**ITGB3**	Sr^a	17	<1
HPA-9w	GPIIb (CD41)	**ITGA2B**	Max^a	17	<1
HPA-10w	GPIIIa (CD61)	**ITGB3**	La^a	17	<1
HPA-11w	GPIIIa (CD61)	**ITGB3**	Gro^a	17	<1
HPA-12w	GPIbb (CD42b)	**GP1BB**	Iy^a	22	<1
HPA-13w	GPIa (CD49b)	**ITGA2**	Sit^a	5	<1
HPA-14w	GPIIIa (CD61)	**ITGB3**	Oe^a	17	<1
HPA-15	CD109	**CD109**	Gov^b	6	HPA-15a:74
			Gov^a		HPA-15b:76
HPA-16w	GPIIIa (CD61)	**ITGB3**	Duv^a	17	<1
HPA-17w	GPIIIa (CD61)	**ITGB3**	Va^a	17	<1
HPA-18w	GPIa (CD49b)	**ITGA2**	Cab^a	5	<1
HPA-19w	GPIIIa (CD61)	**ITGB3**	Sta	17	<1
HPA-20w	GPIIb (CD41)	**ITGA2B**	Kno	17	<1
HPA-21w	GPIIIa (CD61)	**ITGB3**	Nos	17	<1
HPA-22bw	GPIIb (CD41)	**ITGA2B**	Sey	17	<1
HPA-23bw	GPIIIa (CD61)	**ITGB3**	Hug	17	<1
HPA-24bw	GPIIb (CD41)	**ITGA2B**	$Cab2^{a+}$	17	<1
HPA-25bw	GPIa (CD49b)	**ITGA2**	Swi^a	5	<1
HPA-26bw	GPIIIa (CD61)	**ITGB3**	Sec^a	17	<1
HPA-27bw	GPIIb (CD41)	**ITGA2B**	Cab^{3a+}	17	<1
HPA-28bw	GPIIb (CD41)	**ITGA2B**	War	17	<1
HPA-29bw	GPIIIa (CD61)	**ITGB3**	Kha^b	17	<1

* Naziv prema HUGO (Human Genome Organisation) komitetu za nomenklaturu gena

HPA – *human platelet antigen*

- do sada otkriveno 29 sustava
- 6 klinički značajnih (1,2,3,4,5,15)
- svi su bialelični – unutar svakog HPA sustava u ljudskoj populaciji postoji alel „a" (učestaliji) i alel „b" (manje učestao)
- pojedini čovjek ima sve HPA sustave, i za svaki HPA sustav fenotipski može biti:
  - homozigot aa ili
  - homozigot bb ili
  - heterozigot ab

TROMBOCITNI ANTIGENI (HPA)

Table 1: Phenotypic frequencies for the HPA systems shown are for the Caucasian population.

Alloantigen System	Other Published Names	Allelic Forms	Phenotypic Frequency	Glycoprotein Location
HPA-1	$P1^A$, Zw	HPA-1a ($P1^{A1}$) HPA-1b ($P1^{A2}$)	72% a/a 26% a/b 2% b/b	GPIIIa Leu = Pro_{33}
HPA-2	Ko, Sib	HPA-2a (Ko^b) HPA-2b (Ko^a)	85% a/a 14% a/b 1% b/b	GPIb Thr = Met_{145}
HPA-3	Bak, Lek	HPA-3a (Bak^a) HPA-3b (Bak^b)	37% a/a 48% a/b 15% b/b	GPIIb Ile = Ser_{843}
HPA-4	Pen, Yuk	HPA-4a (Pen^a) HPA-4b (Pen^b)	99% a/a <0.1% a/b <0.1% b/b	GPIIIa Arg = Gin_{143}
HPA-5	Br, Hc, Zav	HPA-5a (Br^b) HPA-5b (Br^a)	80% a/a 19% a/b 1% b/b	GPIa Glu = Lys_{505}
HPA-6	Ca, Tu	HPA-6a (Ca^b) HPA-6b (Ca^a)	99% a/a <1% a/b <1% b/b	GPIIIa Arg = Gin_{489}
HPA-7	Mo	HPA-7a (Mo^b) HPA-7b (Mo^a)	99% a/a <1% a/b <1% b/b	GPIIIa Pro = Ala_{407}
HPA-8	Sr^2	HPA-8a (Sr^b) HPA-8b (Sr^a)	99% a/a <1% a/b <1% b/b	GPIIIa Arg = Cys_{636}

Adapted from: McFarland J. In: Rossi E, Simon T, Moss G, Gould S (eds.) Principles of Transfusion Medicine 2nd ed. Williams & Wilkins; 1995

TROMBOCITNI ANTIGENI (HPA)

HPA-FluoGene KITS

ARTICLE NO.	ARTICLE	TESTS/PLATE	TESTS/KIT
003 010 048 / 10	**HPA-FluoGene** HPA-1 a/b, -2 a/b, -3 a/b, -4 a/b, -5 a/b, -6 a/b, -9 a/b, -15 a/b	6 / 1	48 / 10
003 011 048 / 96	**HPA-FluoGene HPA-1 a/b Screen**	12 / 96	48 / 96

TROMBOCITNI ANTIGENI (HPA)

ID HPA XT antigen list

Human platelet antigens	alleles assayed	phenotypes (antigens)
HPA-1	HPA1a, HPA1b	HPA-1a, HPA-1b
HPA-2	HPA2a, HPA2b	HPA-2a, HPA-2b
HPA-3	HPA3a, HPA3b	HPA-3a, HPA-3b
HPA-4	HPA4a, HPA4b	HPA-4a, HPA-4b
HPA-5	HPA5a, HPA5b	HPA-5a, HPA-5b
HPA-6	HPA6a, HPA6b	HPA-6bw
HPA-7	HPA7a, HPA7b	HPA-7bw
HPA-8	HPA8a, HPA8b	HPA-8bw
HPA-9	HPA9a, HPA9b	HPA-9bw
HPA-10	HPA10a, HPA10b	HPA-10bw
HPA-11	HPA11a, HPA11b	HPA-11bw
HPA-15	HPA15a, HPA15b	HPA-15a, HPA-15b

TROMBOCITNI ANTIGENI (HPA)

INDIKACIJE ZA TEST GENOTIPIZACIJE HPA

- testiranje fetusa / novorođenčeta
  - kod roditelja s ranijom trudnoćom gdje je utvrđen NAIT
  - utvrđeno neobjašnjivo intrakranijalno krvarenje

- testiranje majke i oca djeteta
  - kod fetusa / novorođenčeta gdje postoji sumnja na NAIT

- testiranje žena
  - koje planiraju trudnoću, a NAIT je dijagnosticiran kod bližih ženskih srodnika (pr. sestra ili sestra partnera)
  - s posttransfuzijskom purpurom

- testiranje može pomoći kod
  - procjene rizika za NAIT u budućim trudnoćama
  - procjene rizika za posttransfuzijsku purpuru i trombocitopeniju

SADRŽAJ

1. Metode molekularne tipizacije i detekcije protutijela
2. Eritrocitni antigeni (RBC)
3. Leukocitni antigeni (HLA)
4. Trombocitni antigeni (HPA)

4.a Antitrombocitna protutijela – klinički slučajevi i detekcija

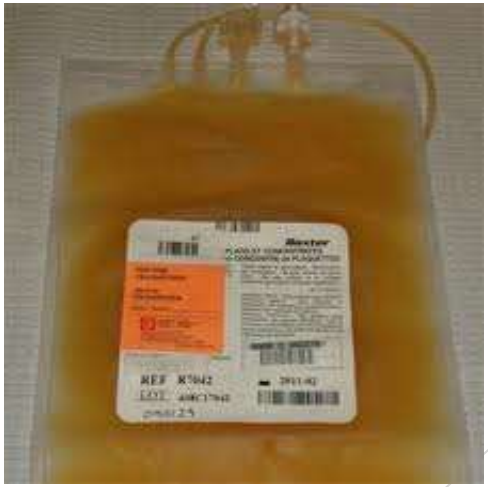
ANTI-HPA PROTUTIJELA

POTREBA ZA TRANSFUZIJOM TROMBOCITA

- za vrijeme nekih kirurških zahvata trombociti se gube ili troše zbog krvarenja (kardiokirurške, ortopedske, abdominalne ili procedure transplantacija organa)

- tijekom nekih bolesti, trombociti mogu biti uništeni ili se ne proizvode dovoljno (tumori, lekuemije)

- žrtve nesreća mogu gubiti trombocite zbog značajnog gubitka krvi

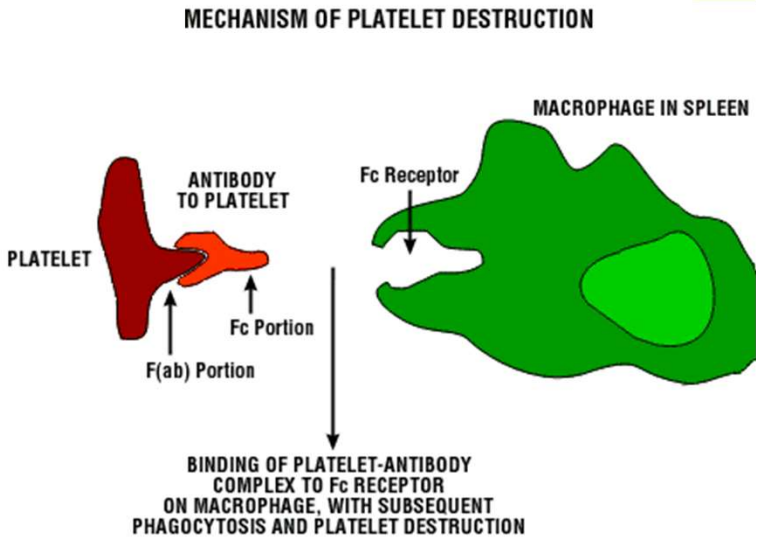


ANTI-HPA PROTUTIJELA

ZBOG ČEGA TESTIRATI SERUME NA ANTITROMBOCITNA (I ANTI-HLA) PROTUTIJELA?

- pacijenti koji primaju transfuzije trombocita mogu se senzibilizirati na trombocitne glikoproteine i antigene HLA razreda I
  - davatelji trombocita mogu u membrani trombocita eksprimirati antigene koji su različiti od pacijenta (primatelja)
  - višestruke transfuzije dovode do aloimunizacije i potencijalnog razaranja transfundiranih trombocita u sljedećim transfuzijama
  - može se javiti refrakternost trombocita, kad naknadne transfuzije ABO podudarnih trombocita ne postižu kliničko poboljšanje

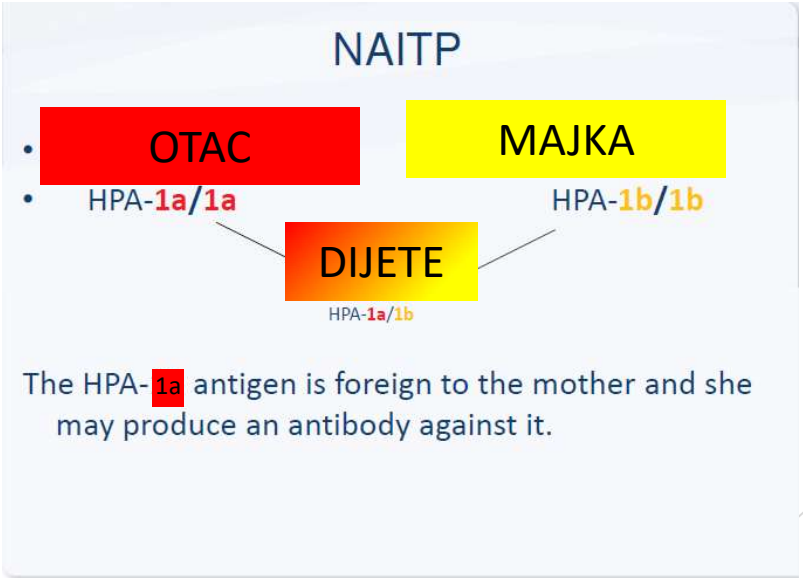
- neka patološka stanja dovode do stvaranja protutijela na vlastite trombocite pacijenta (autoprotutijela)

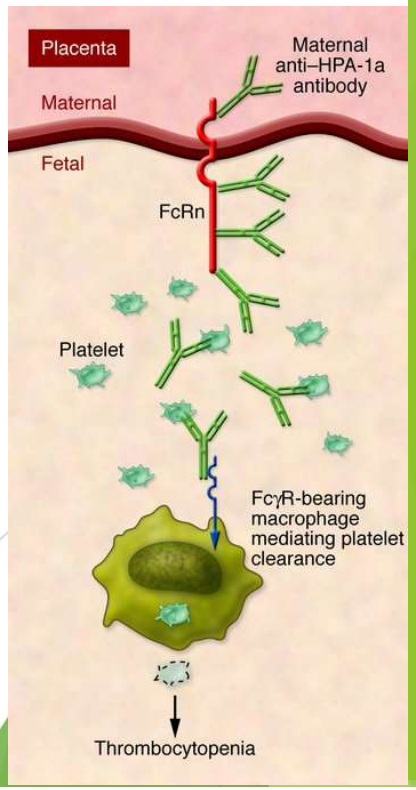


ANTI-HPA PROTUTIJELA

ZBOG ČEGA TESTIRATI SERUME NA ANTITROMBOCITNA (I ANTI-HLA) PROTUTIJELA?

- trudnice mogu stvoriti protutijela na fetalne IPA* antigene
  - ukoliko fetus naslijedi alel od oca kojeg majka nema, majka može stvoriti protutijela na taj antigen
  - protutijela mogu proći placentu i vezati se za fetalne trombocite *in utero* uzrokujući trombocitopeniju = NAITP





** IPA = inherited paternal antigens - antigeni nasljeđeni od oca*

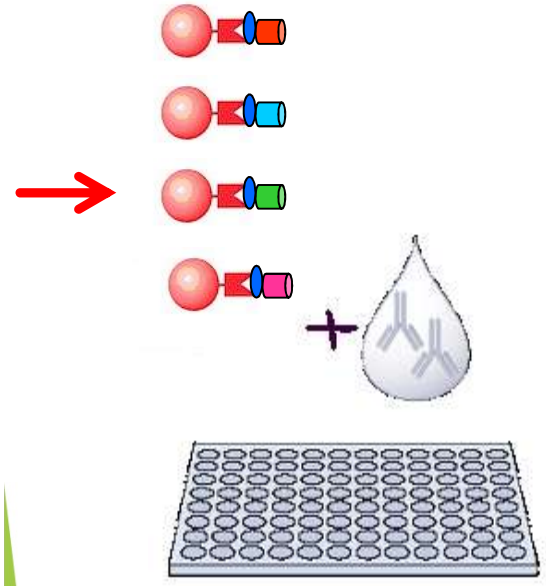
LIFECODES PAK LX™ - DETEKCIJA anti-HPA

LIFECODES Pak LX: kvalitativni imunoesej metodom mikrosfera koji detektira i diferencira IgG protutijela na HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, GPIV i anti-HLA protutijela razreda I u ljudskom serumu

Ključne karakteristike:

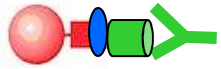
- koristi Luminexovu xMAP bead array tehnologiju, standardiziran CE-IVD protokol
- reakcija u pojedinačnoj jažici omogućuje detekciju višestrukih analita – jednostavna manipulacija
- rezultati unutar 2 h, manualni rad kraći od 20 minuta
- potreban mali volumen seruma (10 µl), što uz mogućnost multipleksiranja doprinosi efikasnoj analizi ukoliko su limitirane količine (pr. novorođenče)
- interpretacija: software MATCHIT! Platelet Ab

LIFECODES PAK Lx METODA

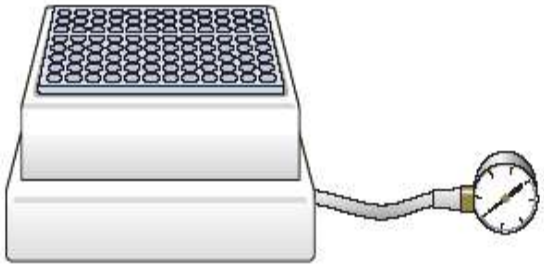


40ul mješavine mikrosfera s antigenima
+ **10ul seruma**

60 min
@ RT°



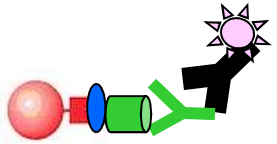
vacuum manifold

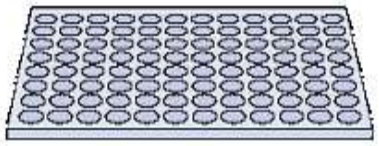


isprati 4X ostatke seruma

dodati 50ul anti-ljudskog IgG-PE

30 min
@ RT°





razrijediti uzorak i očitati rezultate u Luminex aparatu

LIFECODES PAK Lx MATCH IT! software

Bead	Glycoprotein	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	GPIV	HLA	Raw	Adj Val 1	Adj Val 2	Adj Val 3	Bead Assignment
18	Con1													43	-3.64	-2.70	-4.13	Negative
19	Con2													118	-3.37	-2.58	-3.82	Negative
21	Con3													50	-3.42	-2.69	-4.02	Negative
17	POS													20775	247.32	176.81	247.32	Positive
11	GPIV											+		119	-1.96	-1.56	-2.4	Negative
13	HLA Class I												+	163	-2.27	-1.60	-2.58	Negative
22	GPIIb-IIIa	+	-			+	-	+	-					19488	222.73	159.39	222.57	Positive
26	GPIIb-IIIa	+	-			-	+	+	-					18757	215.61	153.67	214.78	Positive
28	GPIIb-IIIa	-	+			+	-	+	-					291	-6.84	-5.37	-8.42	Negative
29	GPIIb-IIIa	-	+			-	+	+	-					276	-7.19	-5.43	-8.27	Negative
30	GPIIb-IIIa	+	+			+	+	+	-					14579	162.06	115.19	160.19	Positive
34	GPIIb-IIIa	+	-			+	+	-	+					19073	218.06	155.89	217.63	Positive
35	GPIb/IX			+	-									108	-3.65	-2.77	-4.21	Negative
37	GPIb/IX			+	-									92	-3.73	-3.05	-4.46	Negative
38	GPIb/IX			+	+									133	-3.65	-2.96	-4.07	Negative
39	GPIb/IX			-	+									100	-4.19	-3.15	-5.11	Negative
42	GPIb/IX			-	+									91	-3.63	-2.85	-4.52	Negative
44	GPIa-IIa									+	-			239	-10.49	-7.01	-10.9	Negative
47	GPIa-IIa									+	-			318	-9.08	-5.99	-7.29	Negative
49	GPIa-IIa									+	+			263	-8.61	-5.27	-8.7	Negative
50	GPIa-IIa									-	+			345	-6.10	-3.88	-5.98	Negative
51	GPIa-IIa									-	+			275	-7.05	-4.32	-6.24	Negative

THE LIFECODES PAK Lx Antigen Panel

Bead	Antigen	HPA-type	Expected sera reactivity												
			1a	1b	3a	3b	4a	4b	2a	2b	5a	5b	GPIV	HLA-CI I	aHu-IgG
1	GPIIb-IIIa	1a-3a-4a	++++	-	++++	-	++++	-	-	-	-	-	-	-	-
2	GPIIb-IIIa	1a-3b-4a	++++	-	-	++++	++++	-	-	-	-	-	-	-	-
3	GPIIb-IIIa	1b-3a-4a	-	++++	++++	-	++++	-	-	-	-	-	-	-	-
4	GPIIb-IIIa	1b-3b-4a	-	++++	-	++++	++++	-	-	-	-	-	-	-	-
5	GPIIb-IIIa	1ab-3ab-4a	++	++	++	++	++++	-	-	-	-	-	-	-	-
6	GPIIb-IIIa	1a-3ab-4b	++++	-	++	++	-	++++	-	-	-	-	-	-	-
7	GPIb/IX	2a	-	-	-	-	-	-	++++	-	-	-	-	-	-
8	GPIb/IX	2a	-	-	-	-	-	-	++++	-	-	-	-	-	-
9	GPIb/IX	2ab	-	-	-	-	-	-	++	++	-	-	-	-	-
10	GPIb/IX	2b	-	-	-	-	-	-	-	++++	-	-	-	-	-
11	GPIb/IX	2b	-	-	-	-	-	-	-	++++	-	-	-	-	-
12	GPIa-IIa	5a	-	-	-	-	-	-	-	-	++++	-	-	-	-
13	GPIa-IIa	5a	-	-	-	-	-	-	-	-	++++	-	-	-	-
14	GPIa-IIa	5ab	-	-	-	-	-	-	-	-	++	++	-	-	-
15	GPIa-IIa	5b	-	-	-	-	-	-	-	-	-	++++	-	-	-
16	GPIa-IIa	5b	-	-	-	-	-	-	-	-	-	++++	-	-	-
17	GPIV	-	-	-	-	-	-	-	-	-	-	-	++++	-	-
18	HLA-CI I	-	-	-	-	-	-	-	-	-	-	-	-	++++	-
19	Pos Con	-	-	-	-	-	-	-	-	-	-	-	-	-	++++
20	Con1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	Con2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	Con3	-	-	-	-	-	-	-	-	-	-	-	-	-	-

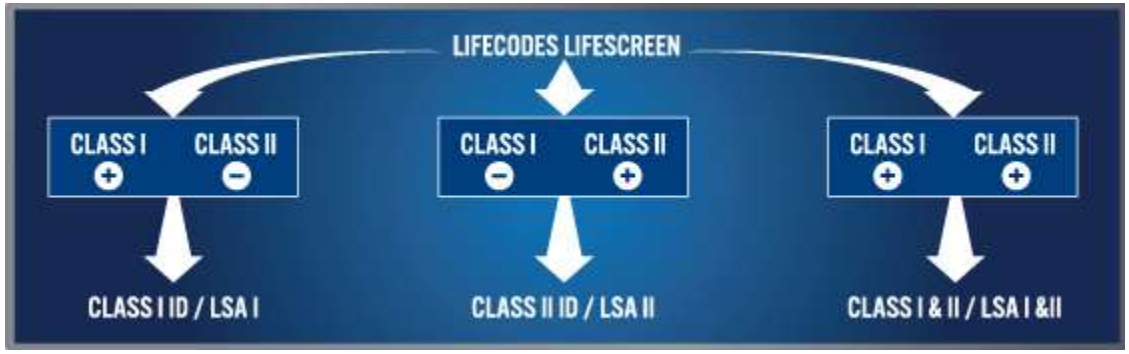
LIFECODES® HLA Antibody Kits

test za screening (probir) anti-HLA protutijela:

- **LifeScreen Deluxe** – Class I & II (Pos/Neg)

testovi za identifikaciju anti-HLA protutijela:

- **LSA Class I** – HLA Class I Lifecodes Single Antigen test
- **LSA Class II** – HLA Class II Lifecodes Single Antigen test



DIJAGNOSTIKA NAITP

KARAKTERISTIKE

- anti-HLA protutijela su najčešća aloprotutijela
- anti-HPA-1a je implicirano u oko 80% NAITP slučajeva
- oko 1/3 višerotkinja (>= 3 trudnoće) razvijaju anti-HLA protutijela, no, ona rjeđe uzrokuju NAITP

- za razliku od Rh inkompatibilnosti, javlja se i kod prve trudnoće

- ispitati:
  - HPA genotip majke djeteta
  - HPA genotip oca djeteta
  - HPA genotip novorođenčeta
  - odrediti anti-HPA (anti-HLA?) protutijela u serumu majke

DIJAGNOSTIKA NAITP

Test protutijela

- probir (screening) seruma majke za antitrombocitna protutijela

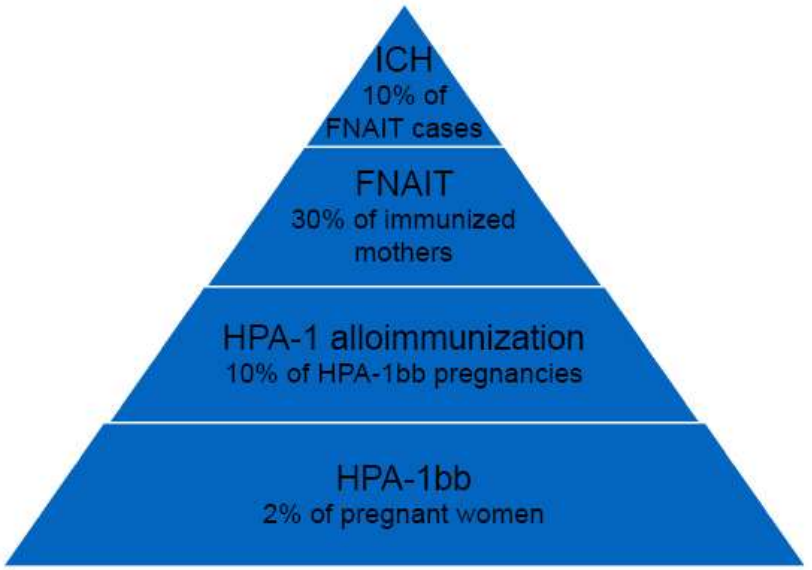
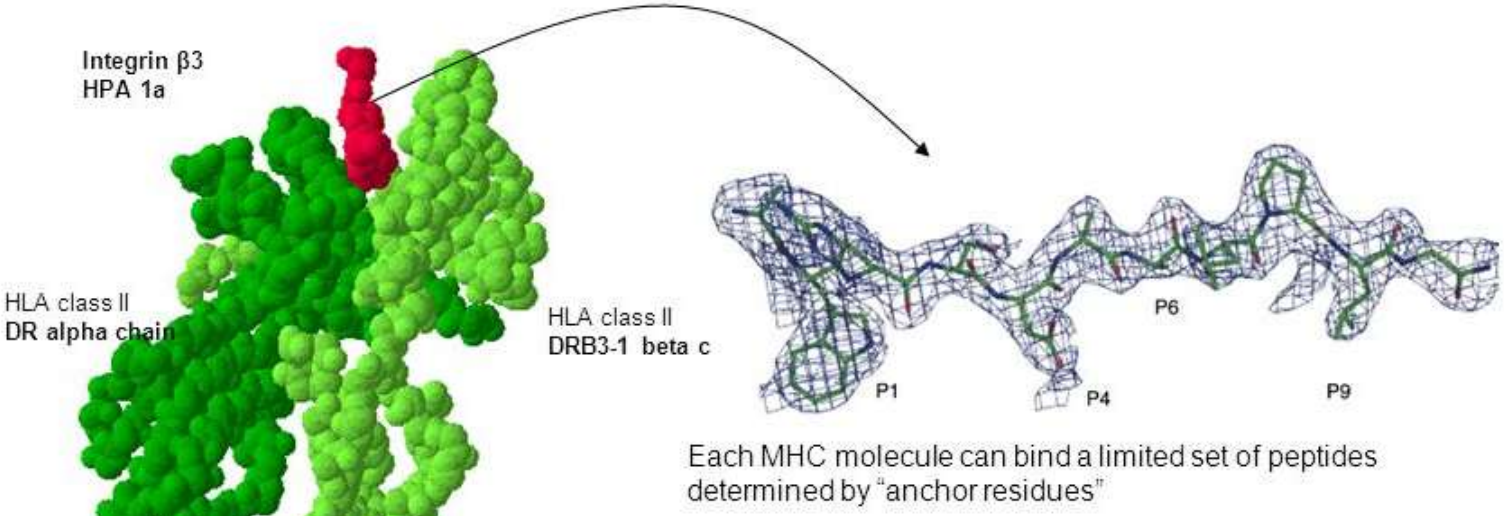


Figure 2 Baseline characteristics of HPA-1-induced fetal and neonatal alloimmune thrombocytopenia.
Abbreviations: ICH, intracranial hemorrhage; FNAIT, fetal and neonatal alloimmune thrombocytopenia; HPA, human platelet antigen.

Test DNA (molekularni test)

- genotipizacija HPA majke, fetusa i / ili oca djeteta

HPA 1a peptide presented by HLA DR B3 0101 positive APC's



Parry, C.S. *et al.*, *J. Mol. Biol.* (2007), 371(2):435-46

- Svaka HLA molekula veže određeni broj peptida ovisno o aminokiselinama koje tvore utor za vezanje. Taj utor kodiraju egzoni 2 i 3 alela HLA razreda I i egzoni 2 alela HLA razreda II.
- Utvrđena vrlo jaka poveznost alela HLA-DRB3*01:01 i alogenih razlika između majčinskih i fetalnih HPA-1 antigena majke i fetusa s razvojem NAIT-a, sugerira da vrlo jak imunosni odgovor anti-HPA-1a protutijelima ovisi o T limfocitima koji prepoznaju peptid nastao od HPA-1a, a koji im prezentira molekula HLA-DRB3*01:01.
- Da taj antigen HLA uistinu i veže taj peptid dokazano je kristalografijom.

PRIKAZ SLUČAJA - Sarajevo

PAK Lx SAMPLE ANALYSIS AND RESULTS | Batch Name: 20181206 PLX 3006971-1 | Assay Date: 12.06.18

PAK Lx Kit Lot #: 3006971-PLX | Assay Tech: | Analysis Date: 12/6/2018

SAMPLE ID: Unknown11

Minimum Cutoff (MC). If the MFI of the Con beads is < MC, the Adjusted Ratios are calculated using MC. 130

Antibody Target	GPIV	HLA	GPIIbIIIa (HPA-1,-3,-4)	GPIbIX (HPA-2)	GPIaIIa (HPA-5)
Result	Neg	Pos	Neg	Neg	Neg

Bead Region	Glycoprotein Group	Antigen	MFI	Bead Reactivity	Adjusted Ratio 1	Adjusted Ratio 2	Adjusted Ratio 3
13	Con1	Con1	516				
14	Con2	Con2	192				
18	Con3	Con3	528				
11	POS	POS	23221				
6	GPIV	GPIV	375	Negative	-1.84	-2.76	-2.11
10	HLA Class I	HLA Class I	23708	Positive	43.52	118.59	42.06
21	GPIIb-IIIa	HPA - 1a-3a-4a	443	Negative	-4.46	-8.01	-5.01
22	GPIIb-IIIa	HPA - 1a-3b-4a	441	Negative	-4.73	-7.56	-4.88
23	GPIIb-IIIa	HPA - 1b-3a-4a	454	Negative	-3.4	-4.53	-3.22
24	GPIIb-IIIa	HPA - 1b-3b-4a	395	Negative	-3.39	-5.2	-3.69
25	GPIIb-IIIa	HPA - 1ab-3ab-4a	626	Negative	-4.23	-6.54	-4.4
26	GPIIb-IIIa	HPA - 1a-3ab-4b	345	Negative	-3.77	-5.83	-3.93
27	GPIb/IX	HPA - 2a	478	Negative	-2.45	-3.26	-2.51
28	GPIb/IX	HPA - 2a	565	Negative	-2.29	-2.49	-2.22
29	GPIb/IX	HPA - 2ab	582	Negative	-1.84	-2.22	-2.1
30	GPIb/IX	HPA - 2b	810	Negative	-1.52	-1.3	-1.59
32	GPIb/IX	HPA - 2b	751	Negative	-1.43	-1.18	-1.59
33	GPIa-IIa	HPA - 5a	788	Negative	-2.43	-2.95	-2.55
42	GPIa-IIa	HPA - 5a	644	Negative	-2.1	-2.95	-2.29
48	GPIa-IIa	HPA - 5ab	786	Negative	-2.12	-2.73	-2.16
51	GPIa-IIa	HPA - 5b	810	Negative	-2.17	-2.99	-2.37
54	GPIa-IIa	HPA - 5b	872	Negative	-1.54	-1.85	-1.97

PRIKAZ SLUČAJA - Sarajevo

PAK Lx SAMPLE ANALYSIS AND RESULTS | Batch Name: 20181206 PLX 3006971-1 | Assay Date: 12.06.18

PAK Lx Kit Lot #: 3006971-PLX | Assay Tech: | Analysis Date: 12/6/2018

SAMPLE ID: Unknown4

Minimum Cutoff (MC). If the MFI of the Con beads is < MC, the Adjusted Ratios are calculated using MC. 130

Antibody Target	GPIV	HLA	GPIIbIIIa (HPA-1,-3,-4)	GPIbIX (HPA-2)	GPIaIIa (HPA-5)
Result	Neg	Neg	Reactive	Neg	Neg

Bead Region	Glycoprotein Group	Antigen	MFI	Bead Reactivity	Adjusted Ratio 1	Adjusted Ratio 2	Adjusted Ratio 3
13	Con1	Con1	663				
14	Con2	Con2	408				
18	Con3	Con3	593				
11	POS	POS	25459				
6	GPIV	GPIV	611	Negative	-1.65	-3.21	-1.79
10	HLA Class I	HLA Class I	587	Negative	-1.58	-3.45	-1.89
21	GPIIb-IIIa	HPA - 1a-3a-4a	10486	Positive	10.5	15.41	11.83
22	GPIIb-IIIa	HPA - 1a-3b-4a	10360	Positive	10.05	15.57	11.75
23	GPIIb-IIIa	HPA - 1b-3a-4a	8988	Positive	9.28	15.17	11.08
24	GPIIb-IIIa	HPA - 1b-3b-4a	9248	Positive	9.79	15.44	11.15
25	GPIIb-IIIa	HPA - 1ab-3ab-4a	11817	Positive	12.38	19.2	14.34
26	GPIIb-IIIa	HPA - 1a-3ab-4b	9270	Positive	9.54	15.13	11.05
27	GPIb/IX	HPA - 2a	458	Negative	-2.69	-4.63	-2.65
28	GPIb/IX	HPA - 2a	565	Negative	-2.54	-4.04	-2.34
29	GPIb/IX	HPA - 2ab	580	Negative	-2.1	-3.83	-2.22
30	GPIb/IX	HPA - 2b	509	Negative	-2.32	-4.27	-2.27
32	GPIb/IX	HPA - 2b	395	Negative	-2.29	-4.12	-2.34
33	GPIa-IIa	HPA - 5a	1629	Negative	-1.5	-3.05	-1.29
42	GPIa-IIa	HPA - 5a	1938	Negative	-0.43	-1.55	-0.24
48	GPIa-IIa	HPA - 5ab	1577	Negative	-1.26	-2.95	-0.99
51	GPIa-IIa	HPA - 5b	1830	Negative	-0.98	-2.72	-0.81
54	GPIa-IIa	HPA - 5b	1224	Negative	-1.38	-3.39	-1.56

PRIKAZ SLUČAJA - Sarajevo

PAK Lx SAMPLE ANALYSIS AND RESULTS | Batch Name: 20181206 PLX 3006971-1 | Assay Date: 12.06.18

PAK Lx Kit Lot #: 3006971-PLX | Assay Tech: | Analysis Date: 12/6/2018

SAMPLE ID: Unknown8

Minimum Cutoff (MC). If the MFI of the Con beads is < MC, the Adjusted Ratios are calculated using MC. 130

Antibody Target	GPIV	HLA	GPIIbIIIa (HPA-1,-3,-4)	GPIbIX (HPA-2)	GPIaIIa (HPA-5)
Result	Neg	Neg	Neg	Neg	Pos

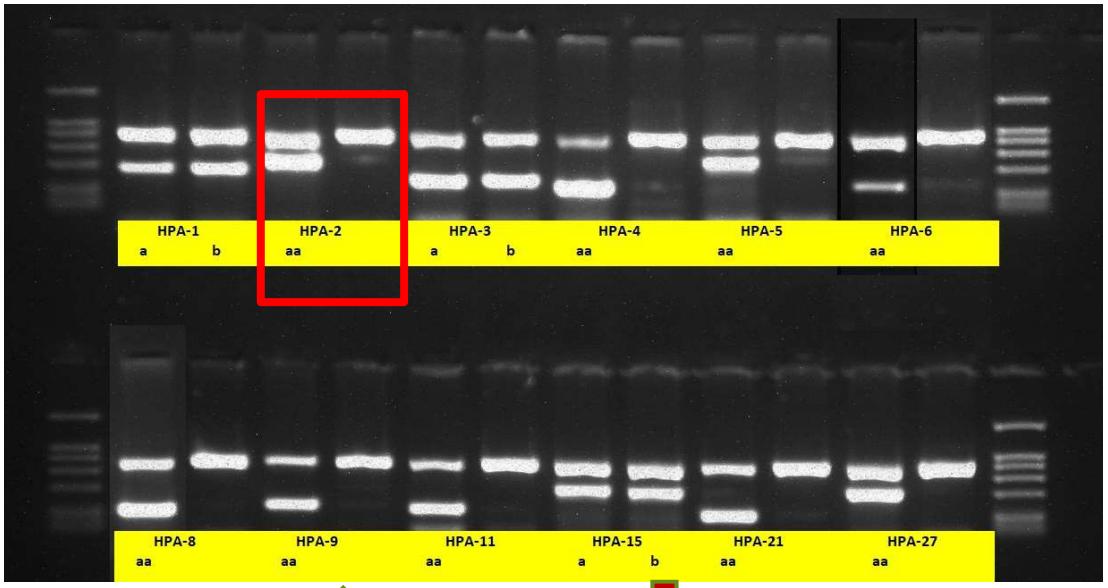
Bead Region	Glycoprotein Group	Antigen	MFI	Bead Reactivity	Adjusted Ratio 1	Adjusted Ratio 2	Adjusted Ratio 3
13	Con1	Con1	253				
14	Con2	Con2	251				
18	Con3	Con3	185				
11	POS	POS	22981				
6	GPIV	GPIV	263	Negative	-1.53	-3.66	-1.39
10	HLA Class I	HLA Class I	202	Negative	-1.67	-4.09	-1.79
21	GPIIb-IIIa	HPA - 1a-3a-4a	933	Negative	-1.63	-6.6	-0.8
22	GPIIb-IIIa	HPA - 1a-3b-4a	992	Negative	-1.65	-5.9	-0.35
23	GPIIb-IIIa	HPA - 1b-3a-4a	579	Negative	-1.99	-4.58	-0.94
24	GPIIb-IIIa	HPA - 1b-3b-4a	564	Negative	-1.93	-5	-1.38
25	GPIIb-IIIa	HPA - 1ab-3ab-4a	1030	Negative	-1.36	-5.7	-0.01
26	GPIIb-IIIa	HPA - 1a-3ab-4b	656	Negative	-1.84	-5.01	-1.02
27	GPIb/IX	HPA - 2a	341	Negative	-2.03	-4.39	-1.57
28	GPIb/IX	HPA - 2a	352	Negative	-2	-4.03	-1.38
29	GPIb/IX	HPA - 2ab	373	Negative	-1.49	-3.76	-1.18
30	GPIb/IX	HPA - 2b	296	Negative	-1.92	-4.34	-1.53
32	GPIb/IX	HPA - 2b	321	Negative	-1.62	-3.81	-1.27
33	GPIa-IIa	HPA - 5a	892	Negative	-0.43	-3.5	0.79
42	GPIa-IIa	HPA - 5a	652	Negative	-0.77	-3.7	0.02
48	GPIa-IIa	HPA - 5ab	9108	Positive	32.43	29.47	45.72
51	GPIa-IIa	HPA - 5b	15694	Positive	58.41	55.31	81.16
54	GPIa-IIa	HPA - 5b	16953	Positive	63.91	61.15	88.27

PRIKAZ SLUČAJA - Sarajevo

- pacijentica
  - trombocitopenija; 50000 trombocita
  - dvije prethodne trudnoće (dvoje djece)
  - planira 3. trudnoću

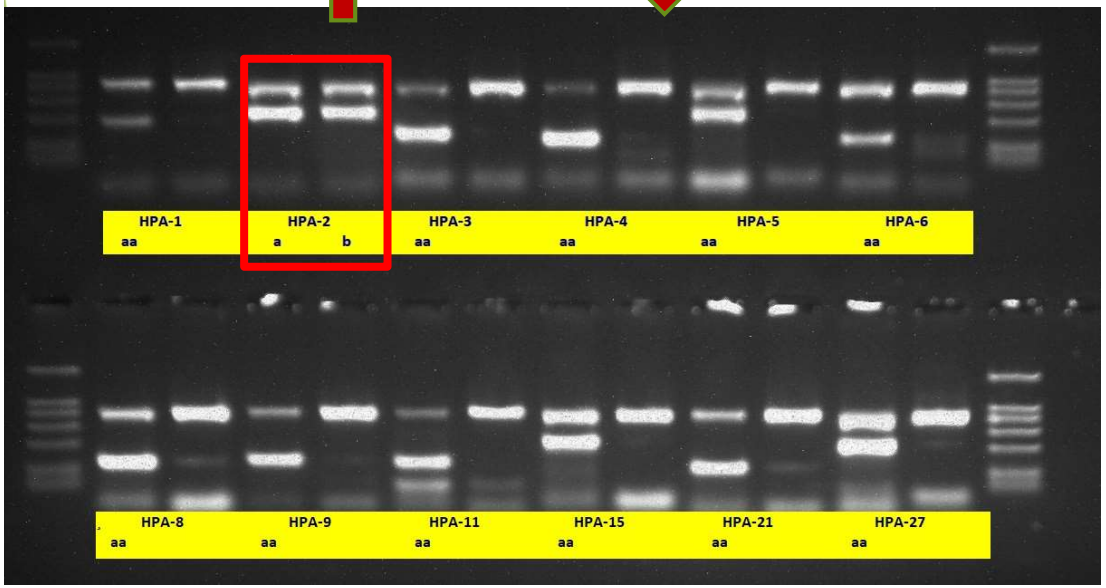
- ispitati:
  - HPA genotip majke djeteta
  - HPA genotip oca djeteta
  - HPA genotip novorođenčeta
  - odrediti anti-HPA (anti-HLA?) protutijela u serumu majke

PRIKAZ SLUČAJA - Sarajevo



PACIJENTICA

PARTNER



PACIJENTICA			PARTNER		
HPA-1	a	b	HPA-1	aa	
HPA-2	aa		HPA-2	a	b
HPA-3	a	b	HPA-3	aa	
HPA-4	aa		HPA-4	aa	
HPA-5	aa		HPA-5	aa	
HPA-6	aa		HPA-6	aa	
HPA-8	aa		HPA-8	aa	
HPA-9	aa		HPA-9	aa	
HPA-11	aa		HPA-11	aa	
HPA-15	a	b	HPA-15	aa	
HPA-21	aa		HPA-21	aa	
HPA-27	aa		HPA-27	aa	

HPA tipizacija djece?

PRIKAZ SLUČAJA - Sarajevo

PAK Lx SAMPLE ANALYSIS AND RESULTS | Batch Name: 20181206 PLX 3006971-1 | Assay Date: 12.06.18

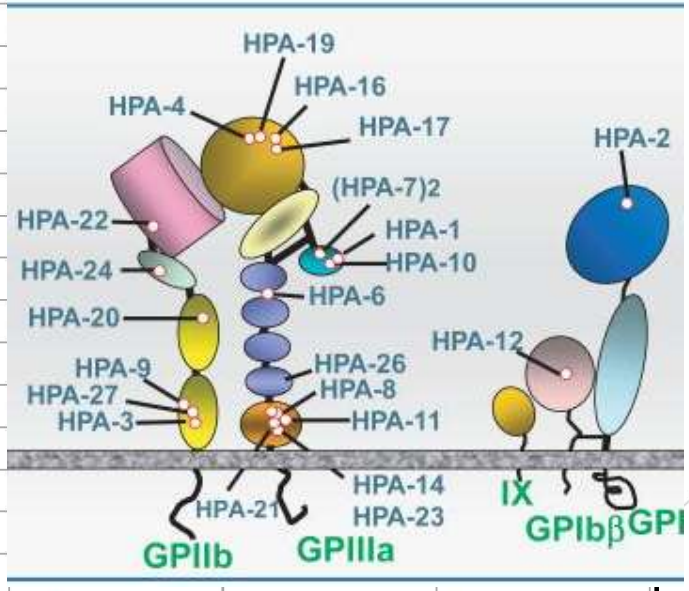
PAK Lx Kit Lot #: 3006971-PLX | Assay Tech: | Analysis Date: 12/6/2018

SAMPLE ID: Unknown12

Minimum Cutoff (MC). If the MFI of the Con beads is < MC, the Adjusted Ratios are calculated using MC. 130

Antibody Target	GPIV	HLA	GPIIbIIIa (HPA-1,-3,-4)	GPIbIX (HPA-2)	GPIaIIa (HPA-5)
Result	Neg	Neg	Neg	Neg	Neg

Bead Region	Glycoprotein Group	Antigen	MFI	Bead Reactivity	Adjusted Ratio 1	Adjusted Ratio 2	Adjusted Ratio 3
13	Con1	Con1	1054				
14	Con2	Con2	273				
18	Con3	Con3	1218				
11	POS	POS	23060				
6	GPIV	GPIV	476	Negative			
10	HLA Class I	HLA Class I	609	Negative			
21	GPIIb-IIIa	HPA - 1a-3a-4a	2305	Negative			
22	GPIIb-IIIa	HPA - 1a-3b-4a	2117	Negative			
23	GPIIb-IIIa	HPA - 1b-3a-4a	1087	Negative			
24	GPIIb-IIIa	HPA - 1b-3b-4a	1014	Negative			
25	GPIIb-IIIa	HPA - 1ab-3ab-4a	2494	Negative			
26	GPIIb-IIIa	HPA - 1a-3ab-4b	1314	Negative			
27	GPIb/IX	HPA - 2a	740	Negative			
28	GPIb/IX	HPA - 2a	450	Negative			
29	GPIb/IX	HPA - 2ab	332	Negative			
30	GPIb/IX	HPA - 2b	334	Negative			
32	GPIb/IX	HPA - 2b	352	Negative			
33	GPIa-IIa	HPA - 5a	596	Negative			
42	GPIa-IIa	HPA - 5a	672	Negative	-2.71	-3.83	-2.96
48	GPIa-IIa	HPA - 5ab	887	Negative	-2.8	-3.57	-2.92
51	GPIa-IIa	HPA - 5b	881	Negative	-2.9	-3.98	-3.18
54	GPIa-IIa	HPA - 5b	697	Negative	-2.57	-3.83	-3.05



PACIJENTICA		
HPA-1	a	b
HPA-3	a	b
HPA-4	aa	
HPA-2	**aa**	
PARTNER		
HPA-1	aa	
HPA-3	aa	
HPA-4	aa	
HPA-2	**a**	**b**

- negativna na IgG alo-anti HPA – i na anti-gpIb/IX (-> HPA2) – novi serum??
- lijekovi ??
- potencijalna auto-protutijela na gpIIb/IIIa ???
  HPA-1a ???
  (alo-protutijela na gpIIb/IIIa nisu moguća zbog HPA genotipa)
- transfuzije ??

UČINAK RUTINSKOG PROVOĐENJA HLA I HPA TESTOVA U TRANSFUZIJSKOJ MEDICINI

- smanjenje aloimunizacija zbog anti-HLA i anti-HPA protutijela u višestruko transfundiranim pacijentima – sigurnija transfuzija krvi

- smanjenje broja izdavanja krvnih pripravaka pacijentima (liječenje antigen podudarnim pripravcima)

- poboljšanje iskoristivosti krvnih pripravaka

EUROPEAN FEDERATION FOR IMMUNOGENETICS

STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING

Version 7.0

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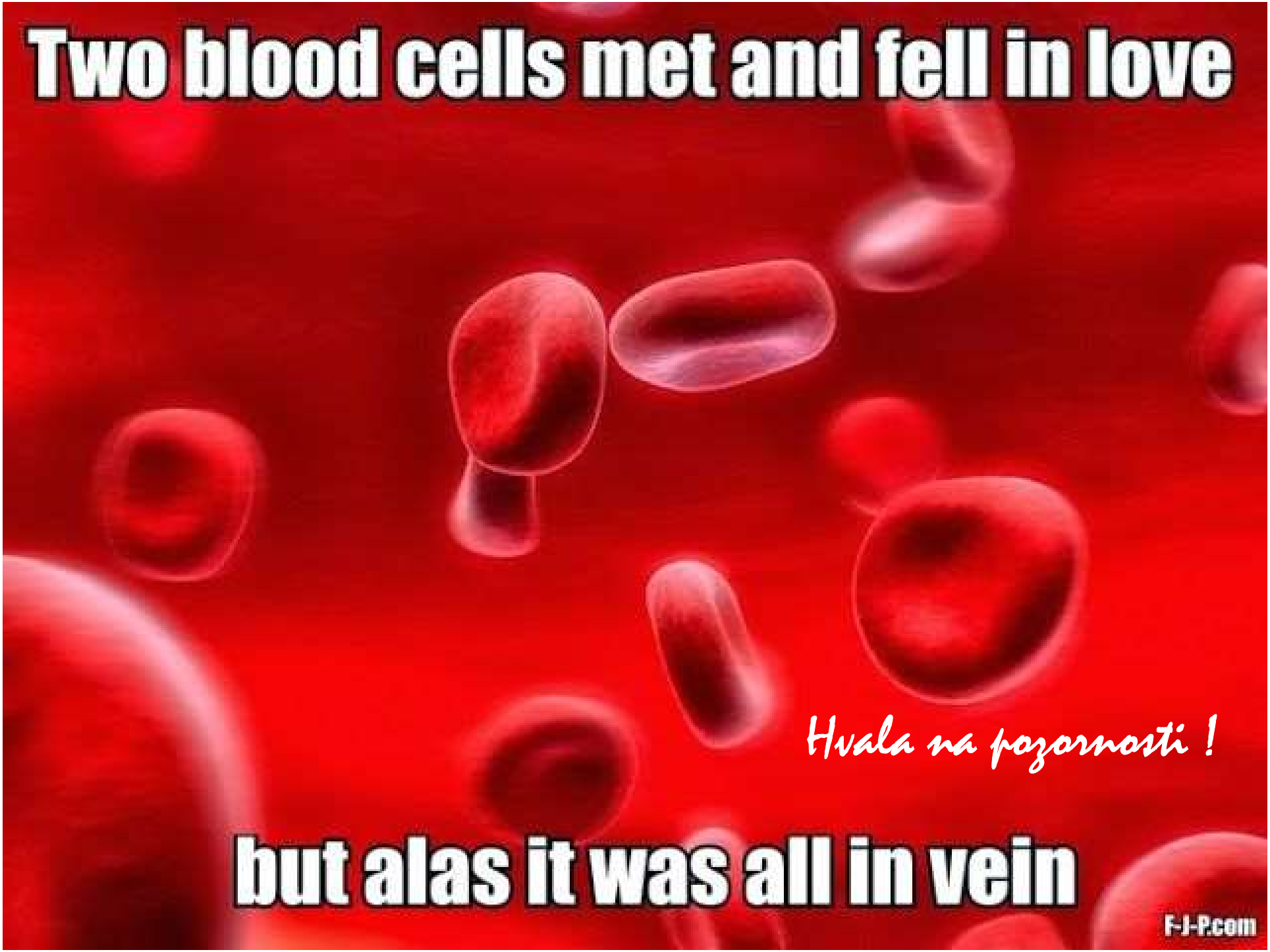
E5.4.1.1	Platelet refractoriness
E5.4.1.1.1	Platelet refractory patients who require HLA matched platelets
E5.4.1.1.1.1	Must be typed for HLA-A and HLA-B
E5.4.1.1.1.2	If alloimmune refractoriness is suspected the patient must be tested for HLA class I antibodies
E5.4.1.1.2	To provide compatible platelets, either:
E5.4.1.1.2.1	The specificity of detected HLA antibodies against HLA-A and HLA-B must be defined and recorded, or
E5.4.1.1.2.2	Crossmatching must be performed, or
E5.4.1.1.2.3	Platelets from donors with acceptable mismatches for HLA-A and -B must be provided.
E5.4.1.1.3	All selected plateletpheresis donors used for the provision of HLA matched platelets must be typed for HLA-A and HLA-B
E5.4.4	HPA and Transfusion
E5.4.4.1	Neonatal Alloimmune Thrombocytopenia (NAIT)
E5.4.4.1.1	The maternal serum must be investigated for the presence of HPA antibodies
E5.4.4.1.2	HPA typing of the mother, father and neonate should be performed
E5.4.4.2	Post Transfusion Purpura (PTP)
E5.4.4.2.1	The patient must be HPA typed
E5.4.4.2.2	The patient's serum must be investigated for HPA antibodies

ZAKLJUČCI
Primjena metoda ispitivanja DNA u suvremenim imunohematološkim laboratorijima

- Suvremeni imunohematološki laboratorij, koji svoj rad temelji isključivo na serološkim metodama, danas ima ograničene mogućnosti u rješavanju složenih slučajeva i nepodudarnosti vezanih uz transfuziju krvi i krvnih produkata.
- Ispitivanja davatelja i primatelja krvi temeljena na analizi DNA pružaju važne informacije koje serološka testiranja, sama po sebi, ne mogu dati, bez obzira na mogućnost nasljeđivanja nefunkcionalnog (utišanog) gena.
- Ovo DNA ispitivanje ne upotrebljava se za utvrđivanje ili dijagnostiku genetske bolesti.
- Stručnjaci u referentnim laboratorijima za imunohematologiju i molekularnu imunohematologiju moraju poznavati zakonske propise te propise u okviru dobre laboratorijske prakse i osiguravanja kontrole kvalitete, kao i imati znanja i sposobnosti u povezivanju i interpretaciji rezultata DNA i seroloških testiranja kod rješavanja kliničkih problema koji su im upućeni.
- Molekularno testiranje sve više postaje neophodna nadopuna testovima hemaglutinacije, pa time značajno povećava sigurnost transfuzije krvi.



Hvala na pozornosti !