POSSIBILITY OF WEAK D ANTIGEN DETECTION USING THE "SOLID PHASE" TECHNIQUE ON THE NEO IRIS AUTOMATED SYSTEM

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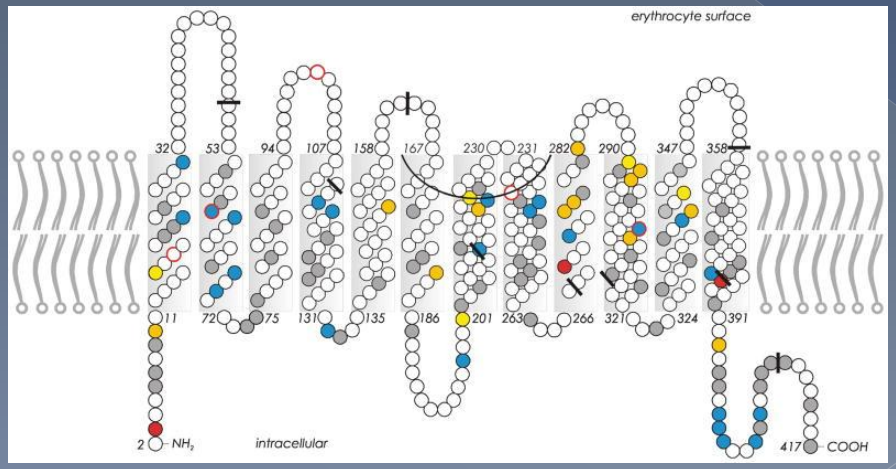
The importance of the Rh D antigen

- The Rhesus (Rh) blood group system **with variable expression of D antigen** is one of the most complex systems in immunohematology
- Antigen D is the **strongest immunogen**, not only among the antigens of the Rhesus system, but also among antigens of all other blood group systems
- Due to it **great clinical importance**, RhD antigen determination is a mandatory immunohematological test for all blood donors and recipients representing a constant challenge in transfusion daily practice



Why are D weak variants important?

- Individuals with weak Rh D antigen variants may be misclassified as Rh D negative
- Misclasification can lead to transfusion reactions and immunization
- Detection of weak variants enables safer blood selection and optimal patient care

Types of weak D variants

Serologically weak D antigen is consequence

- - quantitative/qualitative structural changes

D antigen variants can be grouped:

- - weak D- reduced expression of D antigen
- - partial D- certain epitopes are missing
  possible appearance of anti-D antibodies
- - DEL type- extremely low expression, detectable only by molecular methods

About 0.2-1% of routine blood tests for RhD results in a "serological weak D phenotype"

Serological methods of detection

- Direct agglutination -used in routine testing
- Indirect antiglobulin test (IAT) – enables the detection of Weak D variants

- Limitations:

- Can not distinguish Weak D from Partial D
- May give false negative results

Molecular methods

- PCR and genotyping of the RHD gene - precisely distinguish between types of weak variants
- Allow safe classification in cases with unclear serological findings
- Increasingly used in transfusion centers and prenatal diagnostics

Problems and challenges in practice

The possibility of misinterpretation of serological findings

Lack of standardization between laboratories
The use of different anti-D reagents (different clones), different methods (gel, tubes, automated systems) and interpretation of results

Need for staff education and protocol standardization

Lack of access to molecular diagnostics in all laboratories

Blood Transfusion Institute of Serbia (BTIS)

- The main and the biggest transfusion establishment in Serbia
- About 35 % of all donations collected in Serbia
- 75 000 – 80 000 donations per year

Donor Testing Department

- Determination of ABO/RhD blood group of voluntary blood donors at the BTIS is routinely performed using microplate technique on the automatic platforms

Qwalys 3

NEO Iris

COMPOSITION

ABD-Lys (DIAGAST Ref.: **79967**) is a kit consisting of:

- 10 microplates ABD-Lys for 10 x 16 ABO-RH1 groupings. Each microplate contains 16 wells of anti-A (ABO1), anti-B (ABO2), anti-D (RH1) and negative control, and 32 empty wells.
- 1 vial of 40-mL of MagneLys.

Reagents	Clones	Type	Origin
Anti-A (ABO1)	9113D10	IgM	Murine
Anti-B (ABO2)	9621A8	IgM	
Anti-D (RH1)	P3X61	IgM	Human

Groupa DVI Lys (DIAGAST Ref.: **79969**) is a kit consisting of:

- 12 microplates Groupa DVI Lys microplates for 12 x 8 groupings. Each microplate contains 8 wells of anti-A, anti-B, anti-A,B, anti-D1, anti-D3, negative control, and 8 x 6 empty wells.
- 1 vial of 40 mL of MagneLys.

Reagent	Clones	Type	Clones	Type	Origine
Anti-A (ABO1)	9113D10	IgM			Murine
Anti-B (ABO2)	9621A8				
Anti-A,B (ABO3)	9113D10 + 152D12				
Anti-D or D1 (RH1)	P3X61				Human
Anti-D2 (RH1)	HM10				
Anti-D3 (RH1)	P3X21223B10				
Anti-CDE (RH 2,1,3)	P3X25513G8 + P3X61 + P3X234				
1st determination			**2nd determination**		
Anti-C (RH2)	P3X25513G8 + MS24		DGC02 + MS24	IgM	
Anti-E (RH3)	906		P3X234		
Anti-c (RH4)	MS33		951		
Anti-e (RH5)	P3GD512 + MS63		BS260 + MS63		
Anti-K (KEL1)	MS56		601	IgG	
Negative control					

NEO Iris/Echo Lumena
ABO Grouping

Transfusion

REVERSE GROUPING

PRODUCT
Referencells – 4 (A_1, A_2, B and O) A_1, A_2, B and O reagent red blood cells for reverse grouping
Referencells – 2 (A_1 and B) A_1 and B reagent red blood cells for reverse grouping

SERUM

PRODUCT	CLONE
immuClone Anti-A IgM (ABO1) murine, monoclonal	Birma I
NOVACLONE Anti-A Murine Monoclonal (Galileo) (ABO1) IgM	F98 7C6
immuClone Anti-B IgM (ABO2) murine, monoclonal	LB2
NOVACLONE Anti-B Murine Monoclonal (Galileo) (ABO2) IgM	F84 3D6 + F97 2D6
immuClone Anti-A,B IgM (ABO3) murine, monoclonal	Birma I + ES4 + ES15
NOVACLONE Anti-A,B Murine Monoclonal (Galileo) (ABO3) IgM	F98 7C6 + F84 3D6 +F97 2D6 + F125 7B6

NEO Iris/Echo Lumena
RH1 Typing

Transfusion

PRODUCT	CLONE
immuClone Anti-D rapid Galileo IgM (RH1) human, monoclonal does not detect Category VI	RUM-1
NOVACLONE Anti-D IgM + IgG Monoclonal Blend (Galileo) (RH1) human, monoclonal agglutinates Category VI in IAT only	D175 (IgM) + D415 (IgG)
immuClone Anti-CDE Galileo IgM+IgG (RH2,1,3) human, monoclonal agglutinates Category VI in IAT only ***N**	Anti-C: MS24 (IgM) + Anti-D: MS201 (IgM) + Anti-D: MS26 (IgG) + Anti-E: MS80 (IgM)
immuClone Rh-Hr Control Galileo control serum for monoclonal RH-sera	
NOVACLONE Diluent Control Galileo control serum for monoclonal RH-sera	

All Rh D negative donors with **C** or **E** antigene are additionally checked for the presence of RhD antigen using gel technique

- Ccddee
- CcddEe
- CCddee
- ccddEe...

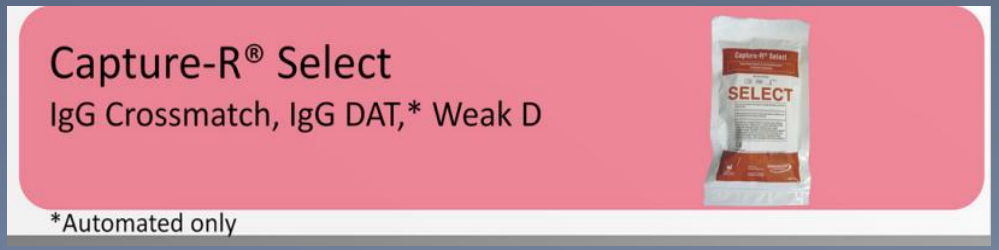
- CcDwee
- ccDwEe…

Reagent containing monoclonal IgG anti-D for confirmation of D^{weak} by IAT; (Cell Line: ESD1)

- In order to improve RhD testing, during 2020 the Blood Transfusion Institute of Serbia conducted a pilot study

- In a three months period, in addition to routine testing, all serologycal weak D, as well as RhD negative donors, regardless of CE status, were tested on the NEO Iris device, with the "weak D" test using:

- **Capture R select**
- **Anti-D Novaclone**



Automated methods: Capture-R Select

Test principle:

- Based on a solid phase (solid phase red cell adherence assay)
- RBC are incubated with anti D antibodies adsorbed on a MTP
- The reaction is detected through indirect adhesion

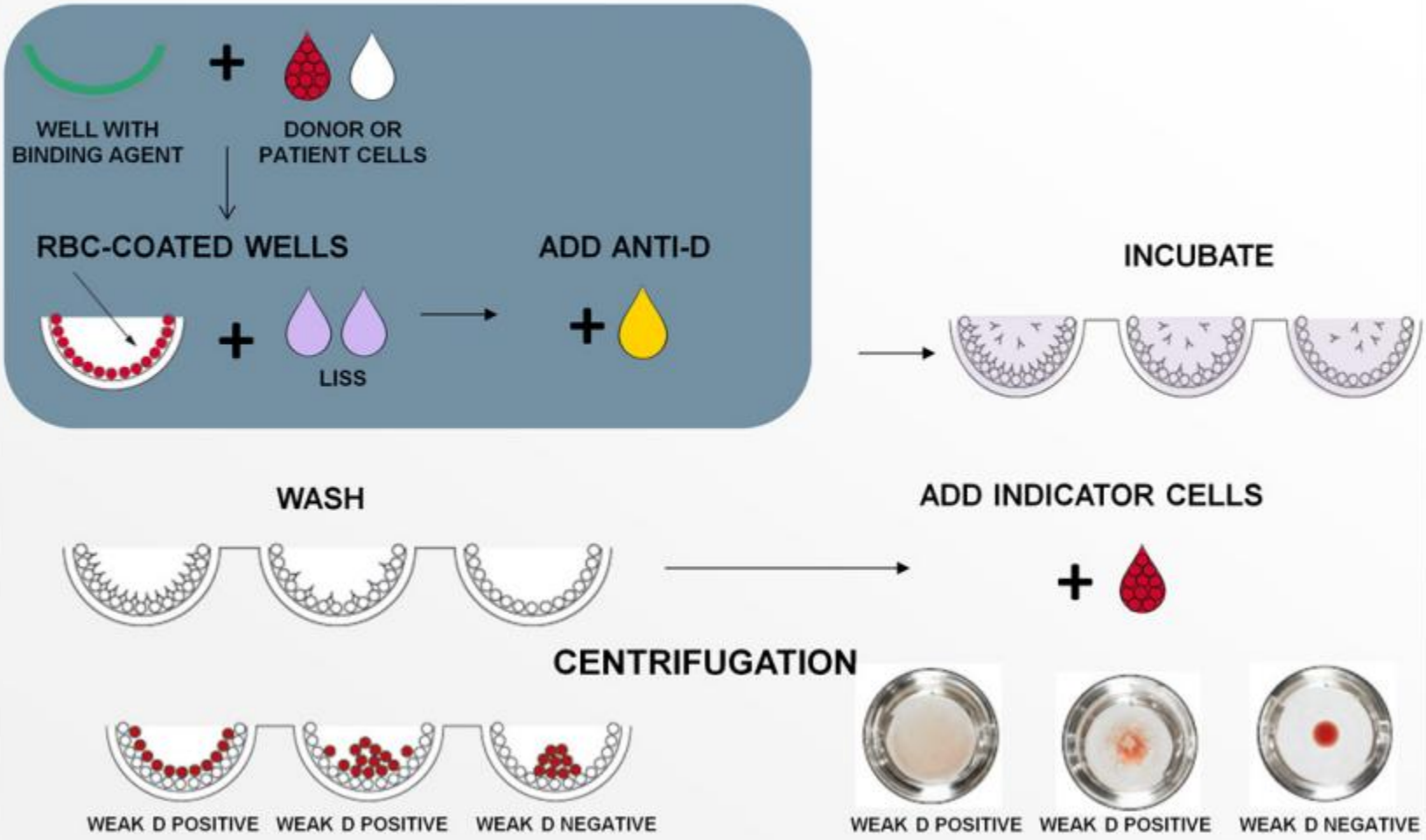
Advantages:

- High sensitivity – can detect even weakly expressed D antigens
- Automated process – reduces human error
- Suitable for high-throughput transfusion laboratories
- Compatible with LIS systems

Restrictions:

- Does not distinguish weak D from partial D

Weak D Testing



A total of **2529** blood donor samples, routinely determined as RhD negative and Dweak were tested with the **"weak D" test**

Rh phenotyp	N	%
ccddee	2333	92,25
Ccddee	137	5,42
CCddee	2	0,08
ccddEe	41	1,62
CcddEe	2	0,08
CcDwee ccDwEe	14	0,55

- For **3 (0,12%)** blood samples, we obtained results that did not match with the other immunohematological results
- In routine testing the blood group was typed as **RhD negative**
- Rh phenotype **Ccddee**
- **ID DiaClon anti D - (neg)**
- **Capture R select/Novaclone Anti-D "weak test" positive**
- The RhD antigen was serologically proven by the adsorption and elution technique

- **FluoQube device**
- **Fluogene PCR-SSP method**
- **RBC FluoGene D® weak/variant**
- RBC Fluogene D weak/variant weak D type 1, 2, 3, 4.1, 4.0/4.2/4.3, 4.2.1/4.2.2, 5, 11, 14, 15, 17, DEL1, DEL8

- Molecular testing of these samples showed the presence of a weak variant of the D antigen, **D weak type 11**

- as a donor RhD+
- as a patient RhD-

The obtained results during this study showed that the **Capture R select / Novaclone Anti-D "weak test"** is more sensitive than other routine methods in the detection of weaker variants of the RhD antigen

The introduction of the "weak D" test was justified and planned

From **2023** the **"weak D"** test is routinely performed in the laboratory for immunohematological testing of blood donors

In the 2 year period (01.07.2023. -30.06.2025.) a total of **154 225** voluntary blood donors samples were tested in BTIS

24 380 samples were determined as Rh D negative

Rh D negative **donors** were additionally checked for Rh D antigen with the "weak D" test

The total number of samples tested by this method was **13 835**

For **18 (0.13 %)** of the tested samples, we obtained results that did not match with the other immunohematological results

Molecular testing

In **15** blood donors, the following weak Rh D variants were identified:

Rh phenotype (serology)	*RHD* variants	Total
Ccddee	weak D type 11	11
	weak D type 1	1
CCddee	weak D type 1	1
	D-DNB	1
ccddee	D-DNB	1

- For 3 donors, results obtained by molecular testing indicate the existence of one of the partial forms of Rh D ag, which can not be classified by the current method and tests

  2 donors Rh phenotype (serology) Ccddee
  1 donor Rh phenotype (serology) CcddEe

- For 2 blood donors, the finding was detected during the first donation, the other donors had 1-14 previously donations

- The obtained test results are register into the BTIS donor database
- The blood donors are informed about the test results
- The results of **"weak D"** test are visible in the worksheets during the next blood donation, which enables the easier interpretation of the results

What to implement in practice

- A combination of serological and molecular methods is the optimal approach
- Molecular method should become the standard in all unclear cases
- In order to adopt the appropriate algorithm and optimal testing strategy, it is necessary to conduct a study of the frequency of RHD variants in the particular population
- National guidelines should incorporate clear algorithms for classification of weak variants

Studies related to the population of Serbia and the region

- In the last decade, studies were conducted in Serbia and the region, that investigated the presence and distribution of weak D variants in blood donors and patients

- Guzijan G, Jovanovic Srzentic S, Pavlovic Jankovic N, Djilas I, Lilić M. ***Implementation of Molecular RHD Typing at Two Blood Transfusion Institutes from Southeastern Europe (Belgrade and Banja Luka).*** *Transfus Med Hemother*. 2019;46(2):114–120. doi:10.1159/000496751

- serological weak D donors and serological RhD negative samples, C/E positive, were analyzed for the presence of RHD variants

Distribution of *RHD* variants after molecular testing of initially serologically RhD-negative donors from BTIS

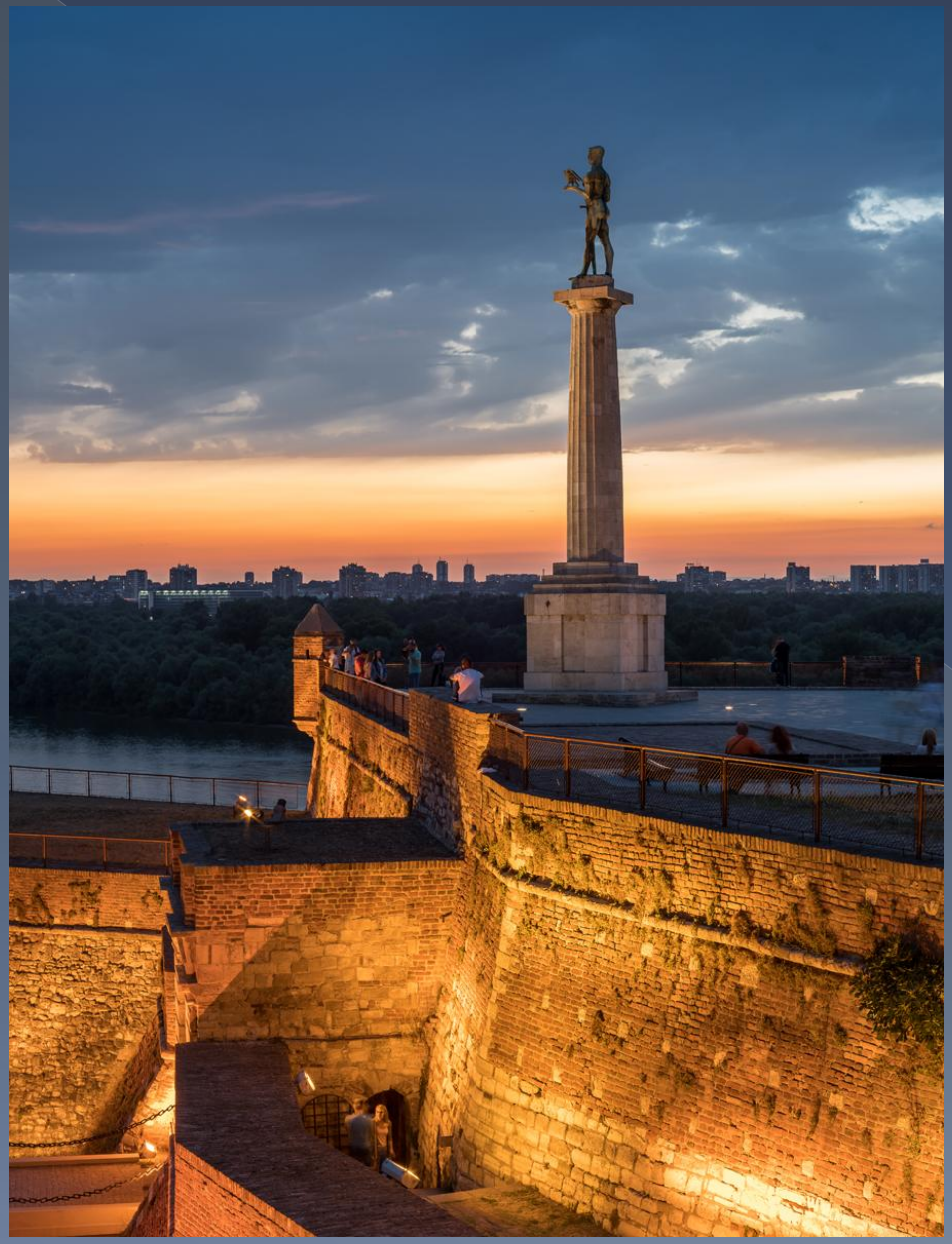
Rh phenotype (serology)	No presence of *RHD* confirmed, *n* (%)	*RHD* variants			Total, *n* (%)
		total quantity, *n* (%)	Specifi-cation	quantity, *n*	
Ccddee	39 (63.9)	1 (1.6)	weak D type 11	1	40 (65.6)
CcddEe	2 (3.3)	1 (1.6)	weak D type 11	1	3 (4.9)
ccddEe	11 (18.0)	3 (4.9)	weak D type 2	1	14 (23.0)
			weak D type 15	2	
CCddee	2 (3.3)	1 (1.6)	D-CE(2–9)-D	1	3 (4.9)
ccddEE	1 (1.6)	0 (0.0)	–	–	1 (1.6)
Total	55 (90.2)	6 (9.8)			61 (100.0)

BTIS results

- Detected weak D variants in BTIS

- type 1 (41.9%)
- type 3 (30.7%)
- type 14 (6.5%)
- type 15 (1.6%)
- DNB with anti-D (1.6%)

- In 17.7% of serologically weak D samples the molecular typing result was standard D



The new testing method brings more accurate data, greater transfusion safety and, most importantly, the safety and well-being of patients – because they are at the center of all our efforts