



Resident Rotation: Immunohematology Reference Lab (IRL) Module 6: DATs & Eluates, Rh nomenclature



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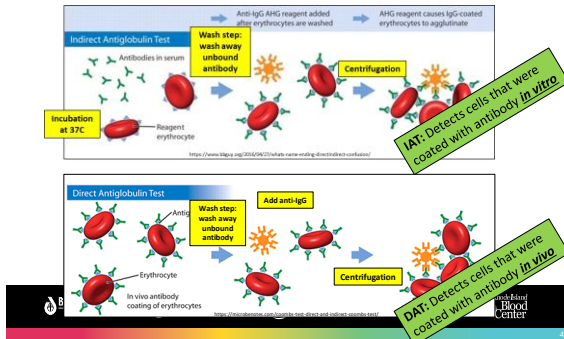


Objectives

- Describe the direct antiglobulin test (DAT), and list reasons why an individual might have a positive DAT.
- Discuss how an eluate is prepared, and how the results of eluate testing can help determine what's causing a patient's positive DAT.
- Review Rh haplotypes and nomenclature.



IAT vs DAT



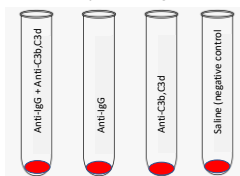
DAT testing

- Are the patient's cells coated with antibody (or complement components)?
- Complement components?
 - C3b/C3d
 - Some antibodies can fix complement
 - Complement components coating cells may indicate hemolytic process



DAT testing

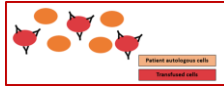
- 4 tubes:
 - "Polyspecific" reagent contains both anti-IgG and anti-complement components
 - Screening reagent
 - If polyspecific reagent is positive, test monospecific reagents
 - Anti-IgG
 - Anti-C3b/C3d
 - 4th tube is saline control
 - Negative control



What causes a positive DAT?

Positive DAT $\neq$ hemolysis

- HDFN – positive DAT on newborn RBCs
- Transfusion reaction – positive DAT on transfused cells
- Warm autoantibody
- Drug antibodies
 - Warm autoantibodies
 - Antibodies to drug-coated RBCs
 - Antibodies that react in the presence of drug
- Unknown cause



Objectives

- Describe DAT testing, and list reasons why an individual might have a positive DAT.
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Investigating positive DATs

- Elution: Harvest antibody bound to RBCs



Eluate

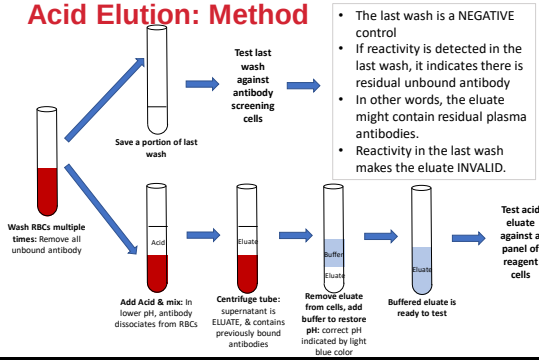


Investigating positive DATs

Cause of positive DAT	Reactivity of the Eluate
Transfusion Reaction	Alloantibody
Hemolytic disease of the fetus/newborn	Alloantibody
Warm autoantibody	Panreactive, reactive with autologous cells
Drug-induced antibody	Usually negative



Acid Elution: Method



More information: what does a warm autoantibody look like?

Pre-transfusion testing:

ABO/Rh						
Front type				Back type		Interpretation
Anti-A	Anti-B	Anti-D	Rh control	A ₁ cells	B cells	
4+	0	4+	0	0	4+	A+

Antibody Screen													
	Rh			Kell	Duffy		Kidd		MNS				Results
	D	C	E		Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	
SCI	+	+	0	0	+	0	+	+	+	+	+	+	5' RT
SCII	+	+	0	0	+	0	+	0	+	0	+	0	LISS 37C
SCIII	+	0	+	0	0	+	+	+	0	+	+	0	LISS IAT



Warm autoantibody workup

Antibody Panel																
	Rh					Kell		Duffy		Kidd		MNS				Results
	D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	Plasma UES IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	3+
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	3+
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	3+
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	3+
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	3+
6	0	0	+	+	+	0	+	0	+	+	0	+	0	+	+	3+
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	3+
8	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	3+
9	0	0	0	+	+	0	+	+	0	+	+	+	0	0	+	3+
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	3+
11	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	3+
Auto																3+

DAT			
Polyspecific	Anti-IgG	Anti-C3b,C3d	Saline Control
3+	3+	1+	0

Warm autoantibody workup

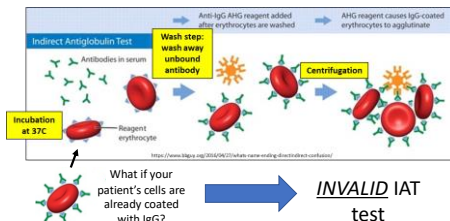
Antibody Panel																	
	Rh					Kell		Duffy		Kidd		MNS				Results	
	D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	Plasma LISS IAT	Etiator PEG IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	3+	3+
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	3+	3+
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	3+	3+
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	3+	3+
5	0	+	0	+	+	0	+	+	0	+	+	0	+	0	0	3+	3+
6	0	0	+	+	0	0	+	0	+	+	+	0	+	0	+	3+	3+
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	3+	3+
8	0	0	0	+	0	0	+	+	+	0	+	0	+	+	+	3+	3+
9	0	0	0	+	+	0	+	+	0	+	+	0	0	+	+	3+	3+
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	3+	3+
11	+	0	0	+	+	+	+	0	0	+	+	+	0	+	+	3+	3+
Auto																3+	



Definition of warm auto in eluate:
eluate reacts with autologous cells

Blood Bank of Maryland	Community Blood Center	INNOVATIVE BLOOD RESOURCES	New York Blood Center	Rush-Kimble Blood Center
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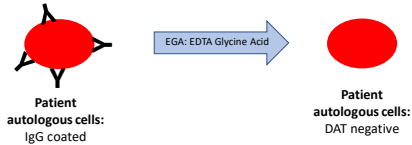
Reminder: you can't test DAT-positive cells using the IAT!



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Treatment to remove IgG from autologous cells

- EDTA-glycine acid (EGA) treatment:
 - Commercial kit
 - Treatment removes IgG from cells, preserves the cells for further testing
 - Some blood group antigens are destroyed by EGA (Kell)



Warm autoantibody workup

Antibody Panel																			
Rh						Kell		Duffy		Kidd		MNS				Results			
	D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	Plasma ULS IAT	Eluate PEG IAT		
1	+	+	0	0	+	0	+									3+	3+		
2	+	+	0	0	+	+	+									3+	3+		
3	+	0	+	+	0	0	+									3+	3+		
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	3+	3+		
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	3+	3+		
6	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	3+	3+		
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	3+	3+		
8	0	0	0	+	+										+	3+	3+		
9	0	0	0	+	+										0	+	3+		
10	+	+	0	0	+										+	0	3+		
11	+	0	0	+	+										+	+	3+		
Auto																		WT	
* EGA-treated, DAT-negative autologous RBCs																3+	3+		

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Rh phenotypes and genotypes

- Rh antigens inherited as **haplotypes** (example: DCe).
- One paternal haplotype and one maternal haplotype (example: DCe/DCe)



Rh phenotypes and genotypes

	Haplotype Fisher-Race nomenclature	Haplotype Weiner nomenclature
Rh (D) positive	DCe	R ₁
	DcE	R ₂
	Dce	R ₀
	DCE	R ₂
Rh (D) negative	dce	r
	dCe	r'
	dCe	r''
	dCE	r ^y

Weiner nomenclature: used
to discuss RBC units or
patient phenotypes

You
need
to
know
these!



Rh phenotypes and genotypes

	Haplotype Fisher-Race nomenclature	Haplotype Weiner nomenclature
Rh (D) positive	DCe	R ₁
	DcE	R ₂
	Dce	R ₀
	DCE	R ₂
Rh (D) negative	dce	r
	dCe	r'
	dCe	r''
	dCE	r ^y

The prevalence of
these haplotypes
varies according to
ethnicity.

What are the most
common
haplotypes?



Inheritance of haplotypes



So, if you inherit **DcE** from mom, and **DcE** from dad, your genotype (using Fisher-Race nomenclature) would be:

DcE/DcE

In Weiner nomenclature, your genotype would be

R₁R₁

The common Rh antigens expressed on your cells would be... **D, C, & e**

The common Rh antigens **NOT** expressed on your cells would be... **E & c**



"Probable" genotype

By knowing the reactivity of RBCs with common anti-Rh reagents, we can arrive at the probable genotype:

Anti-D	Anti-C	Anti-E	Anti-c	Anti-e	Probable genotype
+	+	0	+	+	DcE/dcE = R₁r

We say this is the "probable" genotype because R₁ and r haplotypes are more common than r' or R₀

You might notice that there are a number of haplotypes that could lead to these reactions. Example(s):

R₀r' = DcE/dcE = D+, C+, c+, e+ & E-

or

R₁R₀ = DcE/DcE = D+, C+, c+, e+ & E-



One example of how many genotypes can lead to the same antigens expressed on RBCs (phenotype)

Anti-D	Anti-C	Anti-E	Anti-c	Anti-e	Probable genotype
+	+	+	+	+	R₁R₂ = DcE/DcE = D+C+E+c+e+

Phenotype (what's expressed on RBCs)	Possible Genotype
D+C+E+c+e+	• R₁R₂ = DcE/DcE • R₁r' = DcE/dcE • R₂r' = DcE/dcE • R₂R₀ = DcE/DcE • R₁r = DcE/dce • R₀r' = DcE/dce

We say this is probable, because R₁ and R₂ haplotypes are very common. All the other possibilities include less common haplotypes



Moving forward

- Discuss adsorption studies of warm autoantibody samples
- Choose adsorbing cells based on antigen types
- Will need to have a basic understanding of Rh haplotypes:
 - R_1, R_2, r
 - What antigens are negative for each haplotype?
 - $R_1 = E-, C-$
 - $R_2 = C-, e-$
 - $rr = D-, C-, E-$



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