

Elution and Eluates


Objectives:

1. List several reasons why an individual might have a positive direct antiglobulin test (DAT).
2. Explain the process of preparing an acid eluate.
3. Discuss how the reactivity of an eluate can explain the cause of a positive DAT.

Quick Lesson:

The following are reasons an individual might have a positive DAT:

- Transfusion reaction (donor cells are DAT+)
- Hemolytic Disease of the Fetus/Newborn (HDFN)
- Drugs (might be antibody to drug, or antibody to combination of drug and RBC membrane)
- Warm autoantibody (may or may not be causing hemolysis, can't differentiate serologically)
- Unknown reasons: some healthy donors have positive DATs



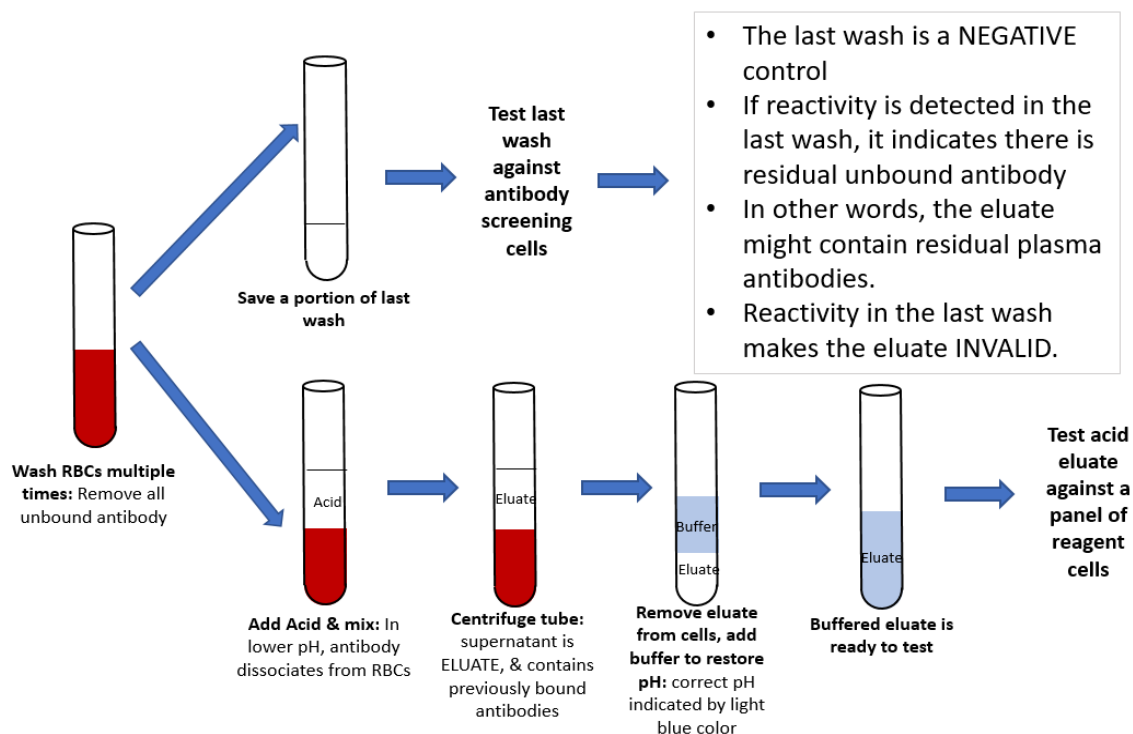
Reminder: a positive DAT means the RBCs are coated with antibody and/or complement

The most common method for preparing eluate:

- Acid Elution: it's quick & easy and a commercial kit is available

An eluate is prepared by removing antibody bound to RBCs. Then, those antibodies are tested. Results of eluate testing may help to determine the cause of the positive DAT.

Acid Elution: Method



Washing the Red Cells:

The eluate procedure begins with **WASHING** the RBCs. The purpose of washing is...

To wash away unbound antibody. Assures when you are testing eluate, it actually represents antibodies that once were attached to RBC instead of residual plasma antibody.

Last wash:

Supernatant from the “last wash” is tested as a control, and results of testing the “last wash” testing should be negative.

If the “last wash” is positive, the eluate is invalid. That means there is residual unbound antibody. More washes of the RBCs would be recommended.

The reactivity of the eluate helps to determine the cause of the positive DAT.

Reactivity of the Eluate	Possible cause of the positive DAT
Nonreactive	<ul style="list-style-type: none"> • Drugs (common reactions for drug induced hemolytic anemia: strong + DAT, negative eluate) • Alloantibody to low prevalence antigen
Panreactive, reactive with autocontrol	<ul style="list-style-type: none"> • Autoantibody
Contains alloantibody	<ul style="list-style-type: none"> • HDFN • Transfusion reaction

Eluate Case Studies

Study the following dry cases and determine the most likely cause of the positive DAT.

CASE 1																	
	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	Eluate PEG IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	2+	3+
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	2+	3+
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	2+	3+
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	2+	3+
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	2+	3+
6	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	2+	3+
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	2+	3+
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	2+	3+
9	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	2+	3+
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	2+	3+
11	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	2+	3+
Auto																2+	3+*

*DAT-negative, autologous cells

1. Case 1 Interpretation:

CASE 2																	
	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 PEG IAT	Eluate PEG IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	0	0
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	0	0
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	0	0
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	0	0
6	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	0	0
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	0	0
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	0	0
9	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	0	0
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	0	0
11	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	0	0
Auto																4+	NT

NT= not tested

2. Case 2 Interpretation:

CASE 3																	
	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 PEG IAT	Eluate PEG IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	0	0
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	2+
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	0	0
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	0	0
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	0	0
6	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	0	0
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	0	2+
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	0	0
9	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	0	0
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	0	0
11	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	0	2+
Auto																2+mf	NT

mf = mixed field reactivity

3. Case 3 Interpretation:

CASE 4																	
	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 PEG IAT	Eluate PEG IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	2+	3+
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	2+	0
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	0	3+
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	0	0
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	1+	3+
6	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	0	0
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	0	0
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	0	3+
9	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	0	3+
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	2+	3+
11	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	0	0
Auto																2+mf	NT

mf = mixed field reactivity

4. Case 4 Interpretation:

Eluate Case Studies: Answers

1. **Warm autoantibody** (eluate panreactive, reacts with autocontrol)
2. **Drug antibody** (strongly positive DAT/negative eluate) If antibody is to drug coated cells and/or is only reactive “in the presence of drug”, remember that you remove antibody from RBCs and then test them with untreated panel cells (and have removed the plasma which contains drug) – that’s why eluate reactions are negative. (When drug antibodies react “in the presence of drug,” plasma may be panreactive)
3. **No allos in the plasma/ anti-K in eluate = transfusion reaction** Patient recently received K+ cells, and is newly forming anti-K. Autocontrol is mixed field because donor cells have positive DAT, autologous cells don’t. Alloantibodies often can be detected in eluate before they “spill over” to the plasma. These results provide evidence of a transfusion reaction due to anti-K.
4. **Anti-C in plasma/ anti-Fy^a in eluate = transfusion reaction** Patient had anti-C in plasma, and was transfused C-negative, Fy(a+) unit. Newly forming anti-Fy^a in eluate, not yet detected in plasma. These results provide evidence of a transfusion reaction due to anti-Fy^a.

Assessing Understanding:

- A patient has a 2+ positive DAT with polyspecific reagent and anti-C3b,-C3d. A tech prepares an acid eluate from the patient's red cells and tests it against a panel of reagent red cells. The eluate is weakly reactive with all cells tested. The last wash is also weakly reactive with all cells tested. What is the best way to interpret these test results?**

 - The patient has a warm reactive autoantibody.
 - The testing results are invalid.
 - The results are consistent with drug induced hemolytic anemia.
 - The patient is having a delayed transfusion reaction.

- Matching: Fill in the chart with the most probable cause of the positive DAT:**

 - Drug antibody
 - Transfusion reaction
 - Hemolytic disease of the fetus/newborn
 - Warm autoantibody

Patient/diagnosis	Last transfusion	Eluate reactivity	Interpretation
79 year old female, pneumonia	Never	Nonreactive	
Newborn female	Never	Anti-K	
35 year old male, pre- elective surgery	Never	Panreactive	
25 year old male, serious car accident	7 days ago	Anti-K	

- What is the most appropriate interpretation of the following results?**

	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 PEG IAT	Eluate PEG IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	0	0
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	4+	0
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	0	3+
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	0	0
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	0	0
6	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	0	3+
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	4+	0
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	0	0
Auto																2+mf	NT

mf = mixed field reactivity

- Transfusion reaction due to anti-K
- Transfusion reaction due to anti-E
- Transfusion reaction due to both anti-K and anti-E
- Warm autoantibody

Assessing Understanding: Answers

1. **b**

2.

Patient/diagnosis	Last transfusion	Eluate reactivity	Interpretation
79 year old female, pneumonia	Never	Nonreactive	a Drug antibody
Newborn female	Never	Anti-K	c HDFN
35 year old male, pre- elective surgery	Never	<u>Panreactive</u>	d warm autoantibody
25 year old male, serious car accident	7 days ago	Anti-K	b transfusion reaction

3. **b**