Cleveland Clinic

Antibody Identification

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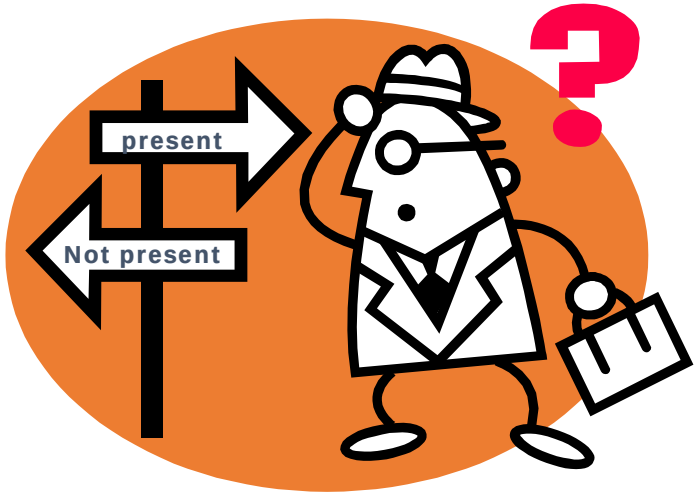
The Basics…..

- Antibody Screens use 2 or 3 Screening Cells to “detect” if antibodies are present in the serum
- If antibodies are detected, they must be identified…



Why do we need to identify?

- Locate/transfuse compatible blood
- Incompatible blood can cause adverse reactions

Key Concepts

- We test “knowns” with “unknowns”
- Antibody ID: we test patient serum (unknown) with reagent RBCs (known)

Known:		**Unknown**:
Reagent RBCs	+	patient serum
Reagent antisera	+	patient RBCs

Reagent RBCs

- Screening and Panel cells: Group O
- Eliminate ABO antibody interaction
- Negative DAT
- Each screen must have at least 1 cell positive for the following antigens (per FDA):
  - D, C, E, c, e, K, k, Fya, M, N, S, s
  - Most also include: P1, Le(a), Le(b), Fy(b), Jk(a), Jk(b)

Screening Cells	Panel Cells
• Antibody Detection • Set of 2 or 3 vials	• Antibody Identification • At least 10 vials per set

Antibody Panel vs. Screen

- An antibody panel is just an extended version of an antibody screen
- The screen only uses 2-3 cells:

	Rh							MNSs				P_1	Lewis		Lutheran		Kell		Duffy		Kidd					
Cell	D	C	E	c	e	f	C^w	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b				
I R1R1 (56)	+	+	0	0	+	0	0	+	+	0	+	0	+	0	0	+	+	+	+	0	+	+				
II R2R2 (89)	+	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0				

Antibody Panel

- An antibody panel usually includes at least 10 panel cells:

Cell Number	D	C	E	c	e	f	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	IS	37	AHG
1	0	+	0	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	0			
2	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0			
3	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+			
4	+	0	+	+	0	+	+	+	0	+	+	+	0	0	+	0	+	+	+			
5	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+			
6	0	0	0	+	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+			
7	0	0	0	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+			
8	0	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	+	0	+	+	0	+	0			
11	0	0	0	+	+	+	0	+	0	+	0	0	+	0	+	+	+	+	+			
Patient Typing																						
INTERPRETATION:																						

Panel

- Each of the panel cells has been antigen typed (shown on antigram)
  - + refers to the presence of the antigen
  - 0 refers to the absence of the antigen

Cell Number	D	C	E	c	e	f	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	IS	37	AHG
1	0	+	0	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	0			
2	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0			
3	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+			
4	+	0	+	+	0	+	+	+	0	+	+	+	0	0	+	0	+	+	+			
5	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+			
6	0	0	0	+	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+			
7	0	0	0	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+			
8	0	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	+	0	+	+	0	+	0			
11	0	0	0	+	+	+	0	+	0	+	0	0	+	0	+	+	+	+	+			
Patient Typing																						
INTERPRETATION:																						

Example: Panel Cell #10 has 9 antigens present: c, e, f, M, s, Le^b, k, Fy^a, and Jk^a

Panel

- An autocontrol should also be run with ALL panels

Cell Number	D	C	E	c	e	f	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	IS	37	AHG
1	0	+	0	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	0			
2	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0			
3	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+			
4	+	0	+	+	0	+	+	+	0	+	+	+	0	0	+	0	+	+	+			
5	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+			
6	0	0	0	+	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+			
7	0	0	0	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+			
8	0	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	+	0	+	+	0	+	0			
11	0	0	0	+	+	+	0	+	0	+	0	0	+	0	+	+	+	+	+			
Patient Typing																						
INTERPRETATION:																						

Autocontrol

Patient RBCs
+
Patient serum

Panel

- The same phases used in an antibody screen are used in a panel

Cell Number	D	C	E	c	e	f	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	IS	37	AHG
1	0	+	0	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	0			
2	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0			
3	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+			
4	+	0	+	+	0	+	+	+	0	+	+	+	0	0	+	0	+	+	+			
5	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+			
6	0	0	0	+	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+			
7	0	0	0	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+			
8	0	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	+	0	+	+	0	+	0			
11	0	0	0	+	+	+	0	+	0	+	0	0	+	0	+	+	+	+	+			
Patient Typing																						
INTERPRETATION:																						

- **IS**
- **37°**
- **AHG**

You have agglutination…now what?

Cell Number	D	C	E	c	e	f	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	IS	37	AHG	CC
1	0	+	0	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	0	2^+	0	0	✓
2	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0	0	✓
3	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+	0	0	0	✓
4	+	0	+	+	0	+	+	+	0	+	+	+	0	0	+	0	+	+	+	2^+	0	0	✓
5	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	0	0	✓
6	0	0	0	+	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	0	0	✓
7	0	0	0	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+	2^+	0	0	✓
8	0	0	0	+	+	+	+	+	0	+	+	0	0	+	+	0	0	+	0	0	0	0	✓
9	0	0	0	+	+	+	+	0	+	0	0	+	0	0	+	0	+	+	+	2^+	0	0	✓
10	0	0	0	+	+	+	+	0	0	+	0	0	+	0	+	+	0	+	0	0	0	0	✓
11	0	0	0	+	+	+	0	+	0	+	0	0	+	0	+	+	+	+	+	0	0	0	✓
Patient Typing																				0	0	0	✓
INTERPRETATION:																							

??

Interpreting Antibody Panels

- There are a few basic steps to follow when interpreting panels:
  1. “Ruling out” means crossing out antigens that did not react
  2. Circle the antigens that are not crossed out
  3. Consider antibody’s usual reactivity
  4. Look for a matching pattern

Always remember:

An antibody will only **react** with cells that *have* the corresponding antigen; antibodies will **not react** with cells that *do not have* the antigen

Here’s an example:

1. Ruling Out

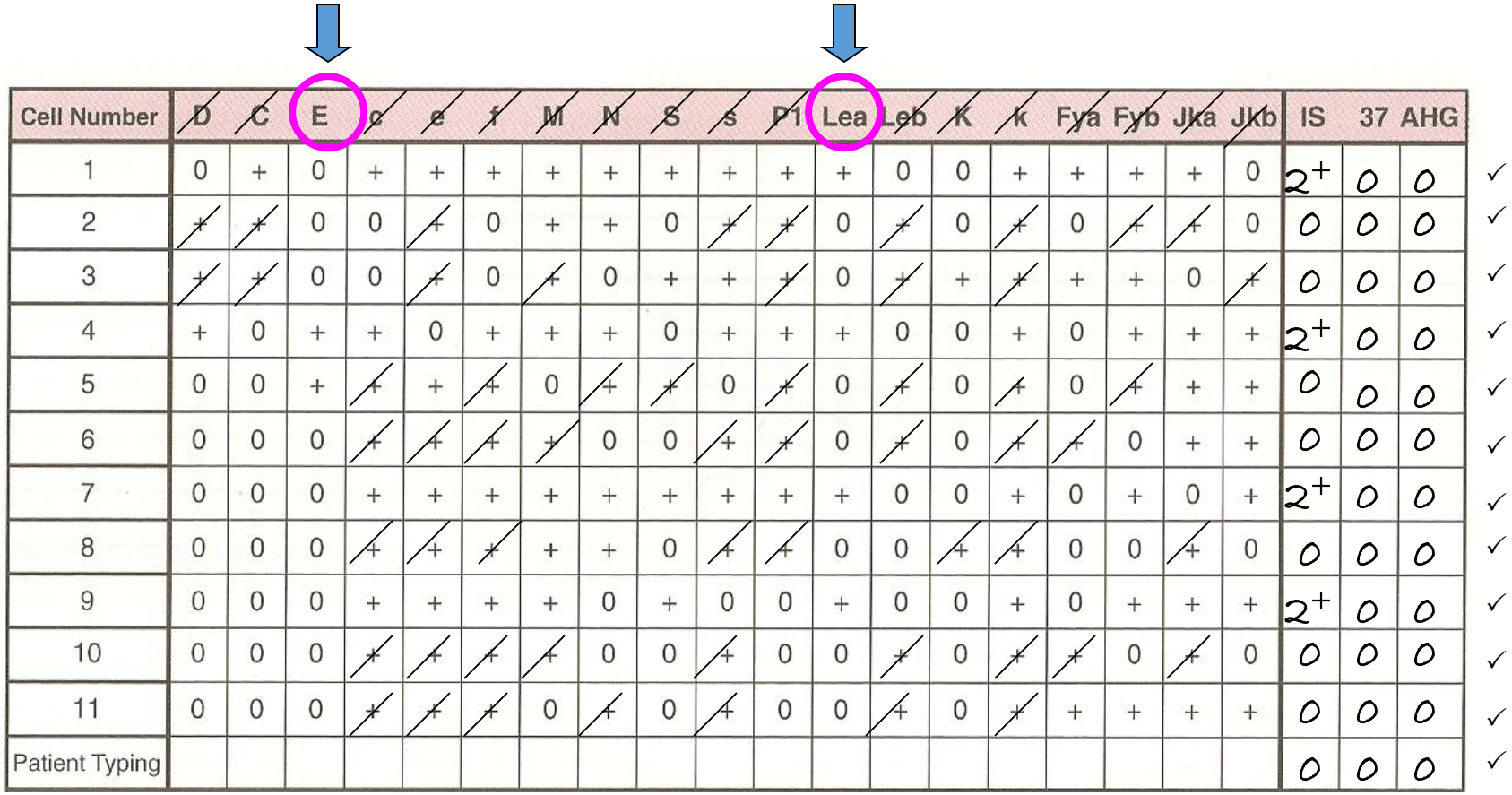
Cross out antigens that show NO REACTION in any phase; do **NOT** cross out heterozygous antigens that show dosage. Rule out each antibody twice homozygously.

Cell Number	D	C	E	c	e	f	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	IS	37	AHG	
1	0	+	0	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	0	2+	0	0	✓
2	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0	0	✓
3	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+	0	0	0	✓
4	+	0	+	+	0	+	+	+	0	+	+	+	0	0	+	0	+	+	+	2+	0	0	✓
5	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	0	0	✓
6	0	0	0	+	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	0	0	✓
7	0	0	0	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+	2+	0	0	✓
8	0	0	0	+	+	+	+	+	0	+	+	0	0	+	+	0	0	+	0	0	0	0	✓
9	0	0	0	+	+	+	+	0	+	0	0	+	0	0	+	0	+	+	+	2+	0	0	✓
10	0	0	0	+	+	+	+	0	0	+	0	0	+	0	+	+	0	+	0	0	0	0	✓
11	0	0	0	+	+	+	0	+	0	+	0	0	+	0	+	+	+	+	+	0	0	0	✓
Patient Typing																				0		0	✓
INTERPRETATION:																							

Exceptions to Homozygous Exclusion

- Ruling out D and P1
  - Do not have antithetical alleles
  - Ruled out with negative reaction on a positive cell
- Ruling out K
  - Little k = high frequency
  - Finding homozygous K antigen difficult (K+k-)
  - May rule out big K on heterozygous cells (K+k+)
- Ruling out when anti-D is suspected
  - dCe/dCe or and dcE/dcE cells are extremely rare
  - If anti-D is suspected, rule out C and E using heterozygous cells

2. Circle antigens not crossed out



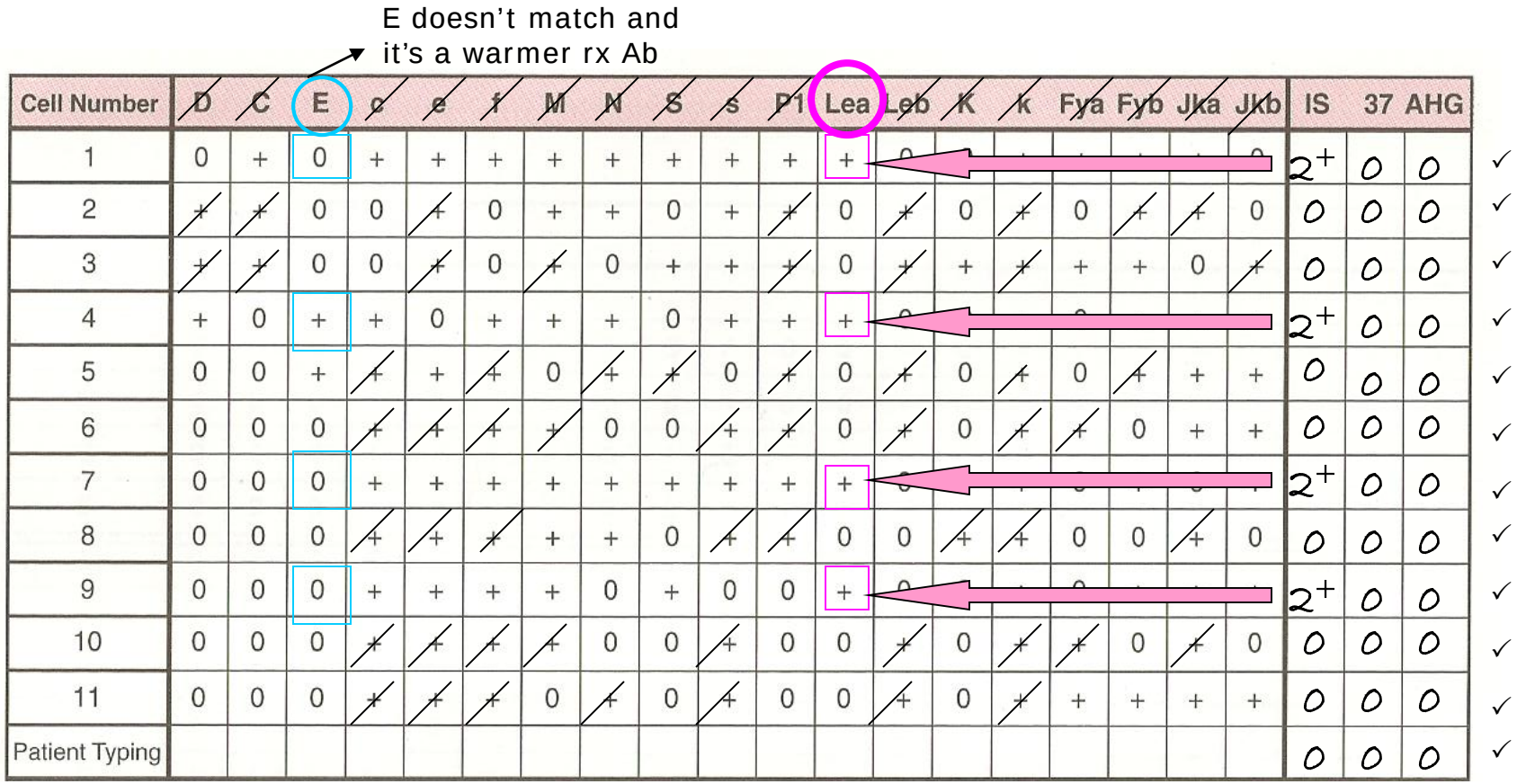
Cell Number	D	C	E	c	e	f	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	IS	37	AHG	
1	0	+	0	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	0	2^+	0	0	✓
2	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0	0	✓
3	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+	0	0	0	✓
4	+	0	+	+	0	+	+	+	0	+	+	+	0	0	+	0	+	+	+	2^+	0	0	✓
5	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	0	0	✓
6	0	0	0	+	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	0	0	✓
7	0	0	0	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+	2^+	0	0	✓
8	0	0	0	+	+	+	+	+	0	+	+	0	0	+	+	0	0	+	0	0	0	0	✓
9	0	0	0	+	+	+	+	0	+	0	0	+	0	0	+	0	+	+	+	2^+	0	0	✓
10	0	0	0	+	+	+	+	0	0	+	0	0	+	0	+	+	0	+	0	0	0	0	✓
11	0	0	0	+	+	+	0	+	0	+	0	0	+	0	+	+	+	+	+	0	0	0	✓
Patient Typing																				0	0	0	✓

3. Consider antibody's usual reactivity

Cell Number	D	C	E	c	e	f	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	IS	37	AHG	
1	0	+	0	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	0	2^+	0	0	✓
2	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0	0	✓
3	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+	0	0	0	✓
4	+	0	+	+	0	+	+	+	0	+	+	+	0	0	+	0	+	+	+	2^+	0	0	✓
5	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	0	0	✓
6	0	0	0	+	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	0	0	✓
7	0	0	0	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+	2^+	0	0	✓
8	0	0	0	+	+	+	+	+	0	+	+	0	0	+	+	0	0	+	0	0	0	0	✓
9	0	0	0	+	+	+	+	0	+	0	0	+	0	0	+	0	+	+	+	2^+	0	0	✓
10	0	0	0	+	+	+	+	0	0	+	0	0	+	0	+	+	0	+	0	0	0	0	✓
11	0	0	0	+	+	+	0	+	0	+	0	0	+	0	+	+	+	+	+	0	0	0	✓
Patient Typing																				0	0	0	✓

Le^a is normally a Cold-Reacting antibody (IgM), so it makes sense that we see the reaction in the IS phase of testing; The E antigen will usually react at warmer temperatures

4. Look for a matching pattern



Cell Number	D	C	E	c	e	f	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	IS	37	AHG
1	0	+	0	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	0	2^+	0	0
2	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0	0
3	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	+	+	0	+	0	0	0
4	+	0	+	+	0	+	+	+	0	+	+	+	0	+	+	+	+	+	+	2^+	0	0
5	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	0	0
6	0	0	0	+	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	0	0
7	0	0	0	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	2^+	0	0
8	0	0	0	+	+	+	+	+	0	+	+	0	0	+	+	0	0	+	0	0	0	0
9	0	0	0	+	+	+	+	0	+	0	0	+	0	+	+	+	+	+	+	2^+	0	0
10	0	0	0	+	+	+	+	0	0	+	0	0	+	0	+	+	0	+	0	0	0	0
11	0	0	0	+	+	+	0	+	0	+	0	0	+	0	+	+	+	+	+	0	0	0
Patient Typing																				0	0	0

…Yes, there is a matching pattern!

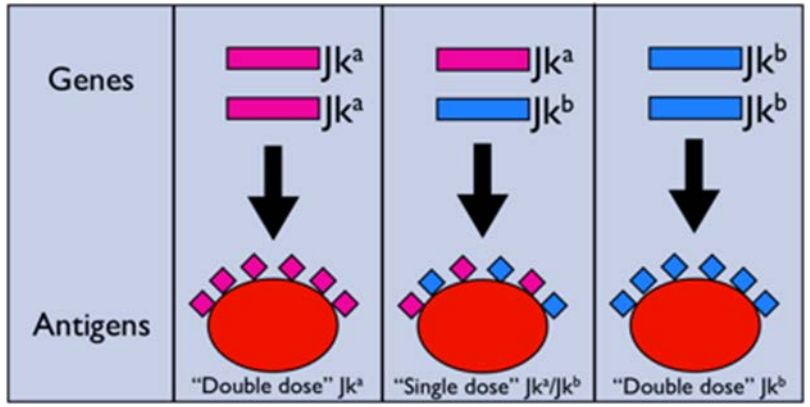
Interpretation

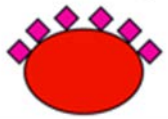
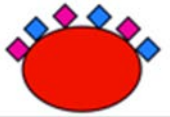
Guidelines

- Again, it's important to look at:
  - **Autocontrol**
    - Negative - alloantibody
    - Positive – autoantibody or DTR (i.e.,alloantibodies)
  - **Phases**
    - IS – cold (IgM)
    - 37° - cold (some have higher thermal range) or warm reacting
    - AHG – warm (IgG)…significant!!
  - **Reaction strength**
    - 1 consistent strength – one antibody
    - Different strengths – multiple antibodies or **dosage**

About reaction strengths……

- Strength of reaction may be due to "dosage"
  - If panel cells are homozygous, a strong reaction may be seen
  - If panel cells are heterozygous, reaction may be weak or even non-reactive
- Panel cells that are heterozygous should not be crossed out because antibody may be too weak to react (see first example)



Antibody	RBCs	Reaction
Anti-Jk^a	Jk(a+b–) 	3+
Anti-Jk^a	Jk(a+b+) 	1+

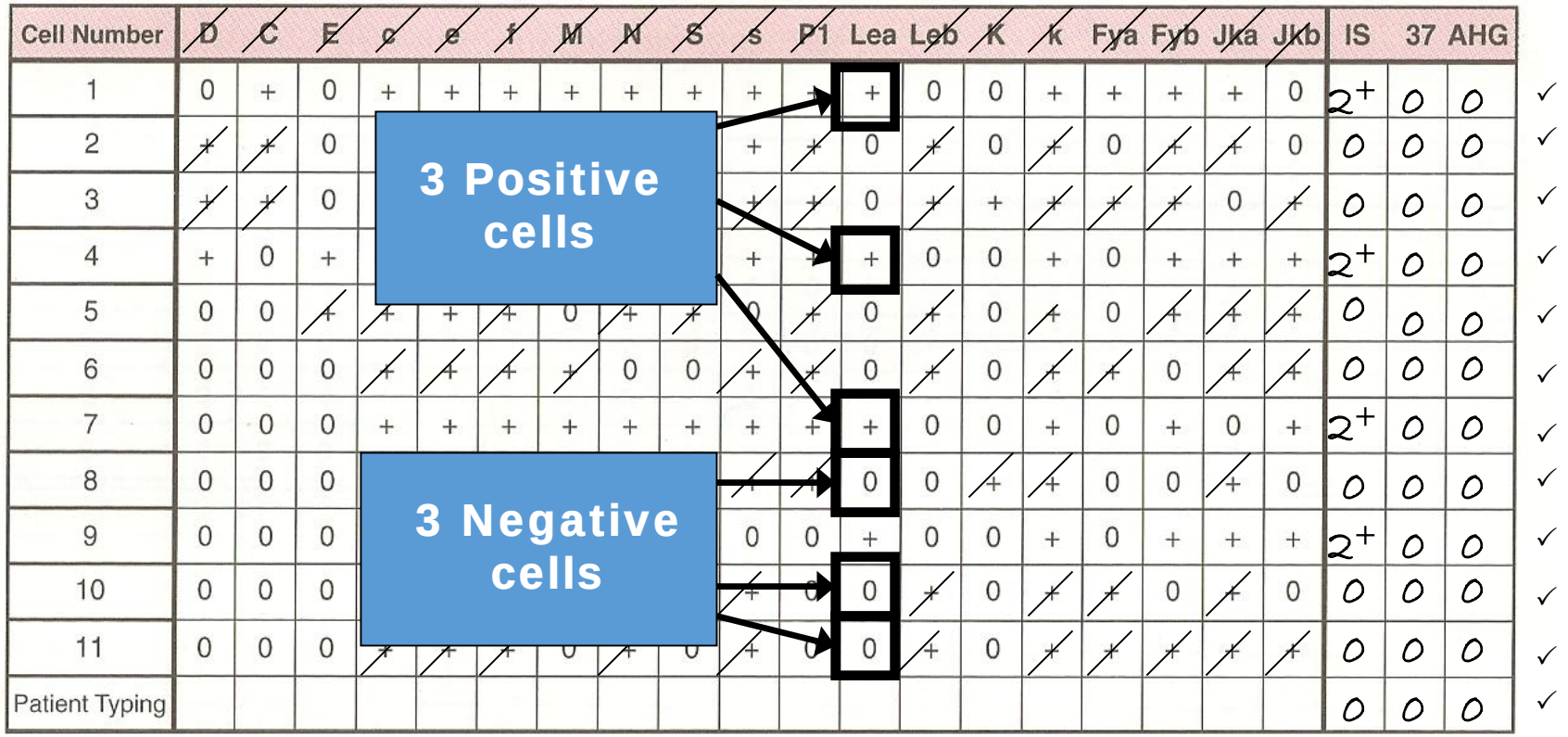
Guidelines (continued)

- **Matching the pattern**
  - Single antibodies usually shows a pattern that matches one of the antigens (see previous panel example)
  - Multiple antibodies are more difficult to match because they often show mixed reaction strengths

Rule of three

- The **rule of three** must be met to *confirm* the presence of the antibody
- A *p*-value ≤ 0.05 must be observed
- This gives a 95% confidence interval
- How is it *demonstrated*?
  - **Patient serum MUST be:**
    - **Positive with 3 cells with the antigen**
    - **Negative with 3 cells without the antigen**

Our previous example fulfills the "rule of three"



Cell Number	D	C	E	c	e	f	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	IS	37	AHG
1	0	+	0	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	0	2+	0	0
2	+	+	0							+	+	0	+	0	+	0	+	+	0	0	0	0
3	+	+	0							+	+	0	+	+	+	+	+	0	+	0	0	0
4	+	0	+							+	+	+	0	0	+	0	+	+	+	2+	0	0
5	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	0	0
6	0	0	0	+	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	0	0
7	0	0	0	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+	2+	0	0
8	0	0	0							+	+	0	0	+	+	0	0	+	0	0	0	0
9	0	0	0							0	0	+	0	0	+	0	+	+	+	2+	0	0
10	0	0	0							+	0	0	+	0	+	+	0	+	0	0	0	0
11	0	0	0	+	+	+	0	+	0	+	0	0	+	0	+	+	+	+	+	0	0	0
Patient Typing																				0	0	0

Panel Cells 1, 4, and 7 are **positive** for the antigen and gave a reaction at immediate spin

Panel Cells 8, 10, and 11 are **negative** for the antigen and did not give a reaction at immediate spin

Ruling-In Antibodies

- Must rule-in 3 times
- If there are multiple antibodies (say Jka and E) they must be ruled in on separate cells.
  - 3 cells that are Jka+ and E negative
  - 3 cells that are E+ and Jka negative

What if the “rule of three” is not fulfilled?

- If there are not enough cells in the panel to fulfill the rule, then additional cells from another panel could be used
- Most labs carry different lot numbers of panel cells
- This is called a **select cell panel**

Select Cell Panels

- Chosen cells that will finish all necessary rule-outs and rule-ins
- You must run a positive control with each select cell panel
  - Positive for the suspected antibody
  - Can be homozygous or heterozygous
- If you are only running rule-ins, a negative control must then be run

Selected Cells

Selected cells	S	Jk^a	P_1	IS	LISS 37°	AHG
#1	+	0	0	0	0	2+
#5	0	+	0	0	0	3+
#8	0	0	+	0	0	0 ✓

These results show that instead of 3 antibodies, there are actually 2: anti-S and anti-Jk^a

Phenotyping

- In addition to the rule of three, **antigen typing** the patient red cells can also **confirm an antibody**
- How is this done?
  - If reagent antisera (of the suspected antibody) is added to the patient RBCs, a **negative** reaction should result…Why?

Individuals **DO NOT** make allo-antibodies against antigens they have

Tips for Difficult Panels

- Determine patient history (transfusion, pregnancy, etc.)
- Look at variation in reaction strength
- Test in different methods (gel, PEG, LISS, solid phase, etc.)
- Phenotype the patient
- Enzyme treated cells
- Phenotypically matched cells

Cleveland Clinic

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