MEDLAB BASICS · STUDY SHEET

Antibody Screen and Identification

Screen and panel workflow, phases, rule-outs and dosage, the 3+3 rule, autocontrol and DAT, enzymes, DTT, neutralization, adsorption, elution, the blood group systems and a worked panel

24 sources + more detail
medlabbasics.com/sheets/lab-antibody-id.html
checked Nov 2026

1 Workflow

1. Antibody screen: the patient's plasma against 2 or 3 group O screening cells by indirect antiglobulin test (IAT). The cells together carry the antigens of the common clinically significant antibodies.
2. Screen positive: test a panel (often 10 to 12 cells plus an autocontrol) with the same method, reading every phase the method uses.
3. Rule out: on each nonreactive cell, cross out the antigens it carries (homozygous cells where dosage applies).
4. Match the pattern: the antigens left should fit the reactive cells. Add selected cells to exclude what remains.
5. Confirm: at least 3 antigen-positive cells react and 3 antigen-negative cells do not (p = 0.05), and the patient types negative for the antigen.
6. Give antigen-negative units that are compatible by AHG crossmatch, and record the antibody so it is honored on every later sample.

EXAM TIP A patient makes an antibody only to an antigen the patient lacks. A patient with anti-Jka must type Jk(a-). If the patient types positive, think autoantibody, partial antigen, recently transfused cells in the sample, or a wrong answer.

2 What reacts where

Gel and solid phase read only the antiglobulin end point, so cold IgM antibodies show less often.

PHASE	USUALLY DETECTS	EXAMPLES
Immediate spin or room temperature	IgM, often naturally occurring, usually not clinically significant	Anti-M, anti-N, anti-Lea, anti-Leb, anti-P1, anti-I
37 °C (tube reading before AHG)	Some IgG in tube; cold IgM with a wide thermal range; hemolysis by complement-binding antibodies may show here	Strong anti-D; hemolysis with anti-Lea, anti-Jka or anti-Vel
AHG (IAT)	IgG warm antibodies: the clinically significant ones	Rh, Kell, Duffy, Kidd, anti-S, anti-s, anti-U

EXAM TIP An antibody is usually clinically significant if it reacts at 37 °C or AHG (IgG): Rh, Kell, Kidd, Duffy, S, s, U. Antibodies reacting only at room temperature or below (M, N, Lewis, P1, I) usually are not.

3 Rule-out and dosage

- Cross out only on nonreactive cells. Reactive cells are for the pattern, not for exclusions.
- Dosage: a homozygous cell (for example Jk(a+b-)) carries more antigen and reacts more strongly than a heterozygous Jk(a+b+) cell. A weak antibody may miss a heterozygous cell, so exclude on a homozygous cell.
- Dosage is shown by Rh (C, c, E, e), Kidd, Duffy and MNS antibodies.
- No dosage is listed for K, Lewis or P1 (Bio-Rad). Anti-K is excluded on one K+k- cell or two K+k+ cells (K+k- cells are scarce). Antigrams give no zygosity for Lewis and P1, so any antigen-positive cell is used, but P1 strength varies widely between cells (partly with zygosity), so a weak P1+ cell can miss a weak anti-P1.
- Published protocols (Manduzio 2024) exclude on two homozygous nonreactive cells; some labs accept one. Follow the local procedure.

4 Confirming: the 3+3 rule

p is the one-sided Fisher exact probability that the pattern fits the antigen by chance when every cell agrees: 1 divided by the number of ways to choose the positive cells from all cells tested. Some authors (Harris and Hochman; Kanter, Poole and Garratty) argue p values should not be applied mechanically; the rule of three is the working standard.

POSITIVE CELLS REACTING	NEGATIVE CELLS NOT REACTING	P (FISHER EXACT)	MEETS P ≤ 0.05?
3	3	1/20 = 0.05	Yes (the standard)
2	3	1/10 = 0.10	No (accepted by some labs as a modified rule)
3	5	1/56 = 0.018	Yes
4	4	1/70 = 0.014	Yes

EXAM TIP With two antibodies, a cell carrying both antigens counts for neither. Each specificity needs its own 3 positive cells that lack the other antigen.

5 Autocontrol and DAT

PANEL	AUTOCONTROL OR DAT	THINK OF
Some cells react, a pattern	Negative	One or more alloantibodies
All cells react, same strength	Negative	Antibody to a high-prevalence antigen (or several alloantibodies)
All cells react, same strength	Positive	Warm autoantibody (DAT IgG, sometimes C3); possible underlying alloantibodies
A pattern	Positive, recently transfused	Alloantibody coating transfused cells: delayed hemolytic or serologic reaction; send an eluate
All cells react, usually weak, by IAT only	May be positive	Anti-CD38 drug (daratumumab, isatuximab): ABO/RhD unaffected

SAFETY A new antibody with a positive DAT in a patient transfused in the last weeks may be a delayed hemolytic transfusion reaction. Tell the medical director and the patient's team; do not just report the antibody.

6 Tools for a hard panel

TOOL	WHAT IT DOES	USE IT TO
Selected cells	Cells chosen for one antigen combination	Exclude what is left, or separate two antibodies
Enzyme-treated cells (ficin, papain)	Remove Fy, M, N, S; enhance Rh, Kidd	Unmask an Rh or Kidd antibody hidden under anti-Fya or anti-M

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Antibody Screen and Identification

6 Tools for a hard panel CONTINUED

TOOL	WHAT IT DOES	USE IT TO
DTT-treated cells	Break disulfide bonds: Kell, Do, Yt and CD38 lost; Lu weakened	Remove anti-CD38 drug interference; point to a Kell antibody
DTT or 2-ME on plasma	Break IgM pentamers	Tell IgM from IgG (prenatal titers)
Neutralization (MLS)	Soluble antigen blocks the antibody	Saliva: Lewis, ABH. Hydatid cyst fluid or pigeon egg: P1. Urine: Sda. Pooled plasma: Ch/Rg
Adsorption	Cells take antibody out of plasma	Remove a warm or cold autoantibody to see alloantibodies under it
Elution	Frees antibody from the patient's coated cells	Identify what is on the cells when the DAT is positive
Prewarm	Cells and plasma kept at 37 °C, no spin, warm washes	Avoid cold autoantibody interference; it can weaken or lose real IgG antibodies

⚠ **SAFETY** Prewarming can weaken or lose clinically significant IgG antibodies. Use it only for a known cold problem, under a written procedure, and exclude warm antibodies by another method.

7 Blood group systems

HTR: hemolytic transfusion reaction. HDFN: hemolytic disease of the fetus and newborn. Sources: Bio-Rad bulletin H005165; AABB Technical Manual table via De Santis 2019.

SYSTEM (ANTIGENS)	ANTIBODY	PHASE	FICIN/PAPAIN	DTT	HTR	HDFN
Kell (K, k, Kpa, Kpb, Jsa, Jsb)	IgG; immune	AHG	Not affected	Destroyed	Mild to severe, delayed	Mild to severe; anti-K suppresses fetal red cell production
Kidd (Jka, Jkb, Jk3)	IgG, often with IgM; binds complement	AHG	Enhanced	Not affected	No to severe, immediate or delayed; the classic delayed HTR	Rare; mild to moderate at most
Duffy (Fya, Fyb, Fy3)	IgG	AHG	Fya, Fyb destroyed (Fy3 not)	Not affected	Mild to severe (rare)	Anti-Fya mild to severe (rare); anti-Fyb mild (rare)
MNS (M, N, S, s, U)	Anti-M, anti-N: IgM (some IgG), natural. Anti-S, -s, -U: IgG	M, N: RT. S, s, U: AHG	M, N destroyed; S, s variable	Not affected	M, N: almost never. S: rare, up to moderate. s: rare, mild. U: mild to severe	M, N: almost never. S, s: rare but can be severe. U: mild to severe
Lewis (Lea, Leb)	IgM, natural, in Le(a-b-) people	RT; some 37 °C or AHG	Enhanced	Not affected	Rare (anti-Lea)	No
P1PK (P1)	IgM, natural	RT	Enhanced	Not in the AABB table (carbohydrate; expected unaffected)	Rare	No
Ii (I, i)	IgM cold autoantibody	RT or 4 °C	Enhanced	Not affected	No (except cold agglutinin disease)	No
Lutheran (Lua, Lub)	IgM and IgG	Lua: RT. Lub: AHG	Not affected or weakened	Weakened or destroyed	Lua: no. Lub: mild to moderate	Lua: no to mild (rare). Lub: mild

💡 **EXAM TIP** Enzymes destroy Duffy and MNS (Fya, Fyb, M, N, S variably) and enhance Rh, Kidd, Lewis, P1 and I. DTT destroys Kell. Kell resists enzymes; Duffy resists DTT.

8 Worked panel

Invented teaching panel, tube IAT. Screen was positive at AHG only.

CELL	RH	D	C	E	C	E	K	FYA	FYB	JKA	JKB	M	N	S	S	LEA	P1	IS / 37 / AHG
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0 / 0 / 2+
2	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0 / 0 / 0
3	r'r	0	+	0	+	+	+	+	+	0	+	+	+	+	+	0	0	0 / 0 / 0
4	rr	0	0	0	+	+	0	0	+	+	+	+	+	0	+	0	+	0 / 0 / 1+
5	R1R1	+	+	0	0	+	0	+	0	0	+	+	+	+	+	0	+	0 / 0 / 0
6	R0r	+	0	0	+	+	+	0	+	+	0	0	+	+	+	+	0	0 / 0 / 2+
7	rr	0	0	0	+	+	0	+	+	0	+	+	0	+	0	0	+	0 / 0 / 0
8	r''r	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0 / 0 / 0
Auto																		0 / 0 / 0

1. Reactions only at AHG and a negative autocontrol: an IgG alloantibody.
2. Cross out on nonreactive cells: cell 2 excludes D, E, c, Fyb, Jkb, N, s, Lea, P1; cell 3 excludes K; cell 5 excludes C, e, Fya; cell 7 excludes M, S. This uses one nonreactive cell per antigen (homozygous where dosage applies); a lab that requires two would add selected cells for E, C, Fya, M, S, Lea and K.
3. Left standing: Jka only. Cells 1, 4 and 6 are Jka+ and react; cells 2, 3, 5, 7 and 8 are Jka- and do not (3 positive, 5 negative: p = 0.018).
4. Dosage fits: Jk(a+b-) cells 1 and 6 give 2+, Jk(a+b+) cell 4 gives only 1+.
5. Finish: type the patient Jk(a-) (genotype if recently transfused), give Jk(a-) AHG-compatible units, and flag the record: Kidd antibodies fade and come back.

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