MEDLAB BASICS · STUDY SHEET

ABO, H and Rh Typing

ABO and H antigens, forward and reverse grouping, ABO discrepancies and how to resolve them, D, weak D, partial D, Rh nomenclature and Rh null

26 sources + more detail
medlabbasics.com/sheets/lab-abo-rh.html
checked Nov 2026

1 ABO and H antigens

- ABH antigens are sugars added in steps to a precursor chain. The H gene (FUT1) adds fucose to make H on red cells; FUT2 (the secretor gene) makes H in saliva and other secretions.
- The A transferase adds N-acetylgalactosamine to H; the B transferase adds galactose. A and B transferases differ at four amino acids (176, 235, 266, 268); 266 and 268 set the sugar.
- The common O allele has a single-base deletion (261delG) that shifts the reading frame: no working enzyme, so H stays unconverted. Group O cells carry the most H; A1 and A1B cells the least.
- A2 differs from A1 in quantity and quality of A antigen: fewer sites and missing A determinants, which is why some A2 and A2B people make anti-A1.
- Anti-A and anti-B are 'naturally occurring', mostly IgM, made in response to gut flora and food antigens. They appear between 3 and 6 months of age.

2 Forward and reverse grouping

Forward grouping (cells) detects A and B antigens; reverse grouping (plasma or serum) detects the expected antibodies. Both must agree before a group is reported. Frequencies: 2026 US blood donor data.

GROUP	ANTI-A	ANTI-B	A1 CELLS	B CELLS	ANTIBODIES IN PLASMA	US DONORS
O	0	0	4+	4+	Anti-A, anti-B	48.8%
A	4+	0	0	4+	Anti-B	36.0%
B	0	4+	4+	0	Anti-A	11.3%
AB	4+	4+	0	0	None	3.9%
Bombay (Oh)	0	0	4+	4+	Anti-A, anti-B, anti-H (O cells also react)	Rare

EXAM TIP The two halves check each other: after infancy a person has the antibody to whichever A or B antigen they lack. A missing, weak, mixed-field or extra reaction is a discrepancy, and the ABO group is not reported until it is resolved.

3 ABO discrepancies: cause, clue and fix

First rule out clerical and technical error: check the labels and the history, repeat the test on the same sample (a new sample if mix-up is possible). Only then look for a problem with the cells or the plasma.

TYPE	CAUSE	CLUE	HOW IT IS RESOLVED
I: weak or missing antibodies	Newborns and infants under about 4-6 months, elderly patients, hypogammaglobulinemia and immunodeficiency, after stem cell transplant	Reverse reactions weak or absent; forward is clear	Incubate the reverse group at room temperature longer or at 4 C (with an autocontrol and O cells); review age, diagnosis and drugs
II: weak or missing antigens	A or B subgroups (A3 gives mixed field), leukemia, infants (antigens 25-50% of adult)	Forward reaction weak, mixed field or missing; the expected antibody is absent	Longer incubation, anti-A,B, anti-A1 and anti-H lectins, saliva inhibition in secretors, adsorption-elution, ABO genotyping
III: plasma protein problems	Rouleaux from high globulin (myeloma, Waldenstrom), plasma expanders such as dextran, Wharton jelly in cord samples	Reverse cells (and often all cells) look agglutinated; stacked-coin rouleaux under the microscope	Saline replacement for the reverse group; wash the patient's cells for the forward group; wash cord cells
IV: extra reactions	Cold autoantibody, cold alloantibody (anti-M and anti-Leb in donor studies), anti-A1 in A2 or A2B	O cells or the autocontrol react at room temperature; A1 cells react in an A or AB person	Warm-washed cells and prewarmed 37 C reverse testing, autoadsorption; antibody identification panel; anti-A1 lectin
Extra or unusual antigens	Acquired B, B(A), cis-AB, polyagglutination, chimerism or recent transfusion	Weak extra antigen in forward grouping; mixed field	See the next section

SAFETY Bombay phenotype types as group O on forward grouping, but the plasma agglutinates A1, B and group O cells because of anti-H. Group O blood is incompatible and has caused hemolytic transfusion reactions. Only Bombay red cells can be given.

4 Special ABO problems

PROBLEM	WHAT HAPPENS	TYPING PATTERN	RESOLUTION
A2 with anti-A1	A2 or A2B cells lack part of the A antigen; some make anti-A1 (1-8% of A2, 22-35% of A2B)	Forward A (or AB); reverse reacts with A1 cells, not A2 cells	Dolichos biflorus anti-A1 lectin: A1 cells agglutinate, A2 do not. Anti-A1 is usually cold IgM; if it reacts at 37 C give A2 or O red cells
Acquired B	Bacterial deacetylase turns A's N-acetylgalactosamine into a B-like sugar; elderly patients with colorectal cancer or gut infection; transient	Group A patient types as AB with some anti-B clones; plasma still contains anti-B	Different monoclonal anti-B, or anti-B acidified to pH 6.0; secretor saliva shows A and H, not B; transfuse group A or O red cells
B(A)	Inherited B allele with an A-type substitution: B transferase adds a little A	Group B with a weak anti-A reaction (2+ to 3+ in one series) while anti-B is 4+; the reverse group fits group B	ABO genotyping; until resolved, give red cells that suit the reverse group (group B or O)
cis-AB	One allele makes a hybrid enzyme carrying both A and B	AB in forward with one antigen (often B) weaker; may have anti-B; unexpected family results	ABO genotyping, family study
Bombay (Oh)	No H on cells or in secretions: both FUT1 and FUT2 null, so A and B cannot be made	Forward O; reverse reacts with A1, B and O cells; anti-H lectin (Ulex europaeus) negative	Confirm with Ulex, saliva and adsorption-elution; Bombay blood only, from rare donor registries and siblings

For education and exam preparation only. Not medical advice; follow current guidelines and your institution's protocols. MedLab Basics is an independent study aid not affiliated with any certifying body.

4 Special ABO problems CONTINUED

PROBLEM	WHAT HAPPENS	TYPING PATTERN	RESOLUTION
Para-Bombay	FUT1 weak or absent; secretors still make H (and A or B) in saliva	Forward O, but the reverse group fits A or B; weak or no H on cells; anti-H may react only in the cold	Adsorption-elution, saliva testing, FUT1/FUT2 genotyping
Chimerism or transfusion	Two red cell populations: twin chimera, recent transfusion, stem cell transplant	Mixed-field forward reaction; in a twin chimera the plasma lacks the antibody to the twin's antigen	Transfusion and transplant history; STR or ABO genotyping; flow cytometry
Polyagglutination	Hidden red cell antigens (T, Tk, Tn and others) exposed by microbial enzymes or by inherited defects	Cells agglutinated by most adult human sera	Rare; a lectin panel (Dolichos and others such as Salvia and Vicia lectins) identifies the antigen

5 Rh: D, weak D, partial D

- Two neighboring genes on chromosome 1: RHD (D antigen) and RHCE (C or c with E or e). Both need RhAG (RHAG gene, chromosome 6) to reach the membrane.
- D-negative usually means the whole RHD gene is deleted. About 17% of US donors are D-negative.
- D is the most immunogenic antigen after A and B: more than 80% of D-negative people given D-positive red cells are expected to make anti-D.
- Weak D: fewer D sites because amino acid changes in or under the membrane hinder the protein's insertion. Reacts 2+ or weaker (or not at all) with anti-D at immediate spin but moderately or strongly at the antiglobulin phase. No anti-D has been reported in weak D types 1, 2 and 3.
- Partial D: changes on the outside of the cell remove some D epitopes (for example DVI). The person can make anti-D against the missing parts.
- DEL: D so weak it is found only by adsorption-elution; types as D-negative; common in East Asian populations.

WHO	WEAK D TEST (ANTI-D THEN AHG)	REPORTED AS
Donor	Required by 21 CFR 640.5 when the direct anti-D test is negative	Rh positive if either test is positive; Rh negative only if both are negative. DVI donors type Rh positive this way
Patient (transfusion recipient)	Not required (AABB Standards: unnecessary); licensed anti-D reagents are chosen not to detect DVI at immediate spin	Negative direct test: D-negative, gets D-negative blood. Weak or discordant reaction: refer for RHD genotyping
Newborn of a D-negative mother	Required, to decide the mother's RhIG candidacy	Weak D positive baby: mother is a RhIG candidate
Pregnant patient with serologic weak D	RHD genotyping recommended (AABB/CAP)	Weak D type 1, 2 or 3: manage as D-positive, no RhIG. Other types: D-negative, give RhIG

EXAM TIP Exams test the logic of weak D: a donor is called Rh positive so a weak D unit never goes to a D-negative patient; a patient is called D-negative so a partial D patient never gets D-positive blood. The safe call differs because the risk differs.

6 Rh nomenclature

Wiener: capital R = D present, small r = D absent; 1 = C, 2 = E, 0 = neither, z or y = both; prime = C, double prime = E. 'd' means no D antigen; there is no anti-d.

FISHER-RACE HAPLOTYPE	WIENER	ANTIGENS MADE
DCe	R1	D, C, e
DcE	R2	D, c, E
Dce	R0	D, c, e
DCE	Rz	D, C, E
dce	r	c, e (no D)
dCe	r'	C, e (no D)
dcE	r''	c, E (no D)
dCE	ry	C, E (no D)

ISBT numbers: first three digits = system (ABO 001, RH 004, LW 016, H 018, RHAG 030), last three = antigen.

ANTIGEN	ISBT SYMBOL	ISBT NUMBER
D	RH1	004001
C	RH2	004002
E	RH3	004003
c	RH4	004004
e	RH5	004005
G	RH12	004012
Total Rh (absent on Rh null)	RH29	004029

7 Rh null

- No Rh antigens at all (D, C, c, E, e and the rest); LW is also missing.
- Regulator type (more common): both RHAG alleles mutated, so no Rh complex reaches the membrane, even though RHD and RHCE are normal and can be passed on.
- Amorph type (considerably rarer): normal RHAG, the common RHD deletion and a mutation in each RHCE allele.
- Rh deficiency syndrome: chronic mild to moderate hemolytic anemia with stomatocytes and spherocytes.
- After transfusion or pregnancy the patient can make anti-Rh29, which reacts with every cell except Rh null. Blood comes from family members, frozen rare units and rare donor registries.

MORE Infants and the under-4-month rule

- At birth A and B antigens are about 25-50% of adult strength and reach full strength by 2 to 4 years.
- The infant's own anti-A and anti-B appear between 3 and 6 months and reach adult titers by 5 to 10 years. Maternal IgG crosses the placenta, can be detected in the infant and can suppress the infant's own antibody production.
- So under 4 months of age only the forward ABO group is done; a reverse group would show maternal antibody, not the infant's. As described from the AABB Standards, repeat ABO typing is unnecessary for infants under 4 months.
- If non-group-O red cells are to be given to an infant under 4 months, the infant's plasma is tested for maternal anti-A or anti-B with an antiglobulin method.

For education and exam preparation only. Not medical advice; follow current guidelines and your institution's protocols. MedLab Basics is an independent study aid not affiliated with any certifying body.