

If both the serum and eluate are nonreactive, there is evidence of immune hemolysis, and the patient has received a drug previously reported to have caused hemolysis, testing to demonstrate drug-related antibodies should be considered (see later section on Laboratory Investigation of Drug-Induced Immune Hemolysis).

AUTOIMMUNE HEMOLYTIC ANEMIA

Immune-mediated hemolysis is the shortening of red cell survival by the product(s) of an immune response. If the marrow is able to adequately compensate, the reduced red cell survival may not result in anemia. Immune-mediated hemolysis is only one cause of hemolytic anemia, and many causes of hemolysis are unrelated to immune reactions. The serologic investigations carried out in the blood bank do not determine whether a patient has a “hemolytic” anemia. The diagnosis of hemolytic anemia rests on clinical findings and such laboratory data as hemoglobin or hematocrit values; reticulocyte count; red cell morphology; bilirubin, haptoglobin, and LDH levels; and, sometimes, red cell survival studies. The serologic findings help determine whether the hemolysis has an immune basis and, if so, what type of immune hemolytic anemia is present. This is important because the treatment for each type is different.

In some cases, the destruction of red cells takes place in the intravascular space, with release of free hemoglobin into the plasma. The red cells are ruptured following activation of the classical complement cascade. The characteristic features of this rare type of hemolysis are hemoglobinemia and hemoglobinuria. Conversely, extravascular hemolysis is characterized by phagocytosis of red cells by macrophages of the mononuclear phagocyte system, with a subsequent increase in serum

bilirubin. Such a distinction is a simplification, however, because hemoglobin can also be released into the plasma following extravascular destruction.

Immune hemolytic anemias can be classified in various ways. One classification system is shown in Table 17-3. The AIHAs are subdivided into five major types: warm AIHA (WAIHA), cold agglutinin syndrome (CAS), mixed- or combined-type AIHA, paroxysmal cold hemoglobinuria (PCH), and DAT-negative AIHA. Not all cases fit neatly into these categories. Table 17-4 shows the typical serologic characteristics of the AIHAs. Drugs (discussed in a later section of this chapter) may also induce immune hemolysis; drug-induced *autoantibodies* are serologically indistinguishable from WAIHA.

Warm Autoimmune Hemolytic Anemia

The majority of AIHA cases are caused by warm-reactive autoantibodies, optimally reactive with red cells at 37 C. The autoantibody is usually IgG (but can be IgM or IgA).

TABLE 17-3. Classification of Immune Hemolytic Anemias

Autoimmune Hemolytic Anemia (AIHA)
Warm AIHA
Cold agglutinin syndrome
Mixed-type AIHA
Paroxysmal cold hemoglobinuria
DAT-negative AIHA
Alloimmune Hemolytic Anemia
Hemolytic transfusion reaction
Hemolytic disease of the fetus and newborn
Drug-Induced Immune Hemolytic Anemia
Drug-dependent
Drug-independent

TABLE 17-4. Typical Serologic Findings in Autoimmune Hemolytic Anemia

	WAIHA	CAS	Mixed-type AIHA	PCH
DAT (routine)	IgG IgG + C3 C3	C3 only	IgG + C3 C3	C3 only
Immunoglobulin type	IgG	IgM	IgG, IgM	IgG
Eluate	IgG antibody	Nonreactive	IgG antibody	Nonreactive
Serum	IAT; 35% agglutinate untreated red cells at 20 C	IgM agglutinating antibody; titer ≥ 1000 (60%) at 4 C; react at ≥ 30 C	IgG IAT-reactive antibody plus IgM agglutinating antibody react at ≥ 30 C	Routine IAT negative; IgG biphasic hemolysis in Donath-Landsteiner test
Specificity	Broadly reactive; multiple specificities reported	Usually anti-I	Usually unclear	Anti-P

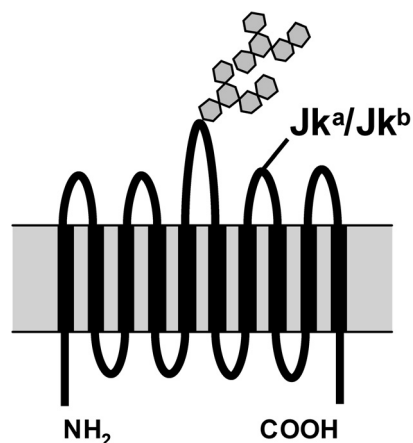


FIGURE 14-6. Diagram of the Kidd glycoprotein, a urea transporter, with cytoplasmic N- and C-terminal domains, 10 membrane-spanning domains, and an N-glycan on the third extracellular loop. The position of the Jk^a/Jk^b polymorphism is shown on the fourth external loop.

Jk(a-b-) and Jk3

The null phenotype, Jk(a-b-) Jk:-3, usually results from homozygosity for a silent gene at the *JK* locus. Although very rare in most populations, the null phenotype is relatively common in Polynesians with a prevalence of around one in 400, but as high as 1.4% in Niueans. The Polynesian null allele contains a splice site mutation in intron 5 resulting in the loss of exon 6 from the mRNA. In Finns,

TABLE 14-5. Kidd Phenotypes in Three Populations

Phenotype	Prevalence (%)		
	Whites	Blacks	Asians
Jk(a+b-)	26	52	23
Jk(a+b+)	50	40	50
Jk(a-b+)	24	8	27

where Jk(a-b-) is less rare than in other populations of European ethnicity, the mutation responsible encodes a Ser291Pro substitution. Immunized individuals with the Jk(a-b-) phenotype may produce anti-Jk3. An extremely rare form of Jk(a-b-) phenotype found in Japanese results from heterozygosity for a dominant inhibitor gene, named *In(Jk)* in analogy with the *In(Lu)* dominant inhibitor of Lutheran and other antigens. Very weak expression of Jk^a and/or Jk^b can be detected on *In(Jk)* red cells by adsorption/elution tests.

Kidd Antibodies and Their Clinical Significance

Anti-Jk^a and -Jk^b are not common antibodies and are generally found in antibody mixtures. They are usually IgG1 and IgG3, but some are partly IgG2, IgG4, or IgM. About 50% of anti-Jk^a and -Jk^b bind complement.^{6(pp216-217)} Kidd antibodies are often difficult to detect. Some directly agglutinate antigen-positive cells, but the reactions are usually weak by this method. Generally, an antiglobulin test is required and use of enzyme-treated cells may be necessary to detect weaker antibodies.

Kidd antibodies are dangerous as they may cause severe acute HTRs. They are also a very common cause of delayed HTRs, probably because they are often not detected in pretransfusion testing because of their tendency to drop to low or undetectable levels in the plasma. Anti-Jk3 can also cause acute or delayed HTRs. Despite their hemolytic potential, Kidd antibodies only very rarely cause severe HDFN.

Kidd antibodies can behave as histocompatibility antigens in renal transplants and may have been responsible for an acute transplant rejection.³⁰

The Kidd Glycoprotein, a Urea Transporter

The Kidd antigens are located on a red cell urea transporter, also known as human urea transporter 11 (HUT11 or UT-B1).³¹ When

red cells approach the renal medulla, which contains a high concentration of urea, the urea transporter permits rapid uptake of urea and prevents the cells from shrinking in the hypertonic environment. As the red cells leave the renal medulla, urea is transported rapidly out of the cells, preventing the cells from swelling and carrying urea away from the kidney. HUT11 was detected on endothelial cells of the vasa recta, the vascular supply of the renal medulla, but it is not present in renal tubules.

Normal red cells are rapidly lysed by 2M urea because urea transported into the cells makes them hypertonic and they burst as a result of the osmotic influx of water. Because of the absence of the urea transporter, Jk(a-b-) cells are not hemolyzed by 2M urea and this can be used as a method for screening for Jk(a-b-) donors.³²

The Jk(a-b-) phenotype is not associated with any clinical defect, although two unrelated Jk(a-b-) individuals had a mild urine-concentrating defect.³³

THE DIEGO SYSTEM

Band 3, the Red Cell Anion Exchanger

The 21 antigens of the Diego system are located on Band 3, the common name for the red cell anion exchanger or solute carrier family 4 A1 (SLC4A1).³⁴ Band 3 is a major red cell membrane glycoprotein with approximately 10^6 copies per red cell. In addition to a transmembrane domain that traverses the membrane about 14 times, with an N-glycan on the fourth extracellular loop, Band 3 has a long cytoplasmic N-terminal domain that interacts with the membrane skeleton proteins ankyrin, 4.1R, and protein 4.2, and also functions as a binding site for hemoglobin (Fig 14-7). The short, cytoplasmic C-terminal domain binds carbonic anhydrase II. Band 3 in red cells has at least two major functions: the rapid exchange of HCO_3^- and Cl^- ions, important in

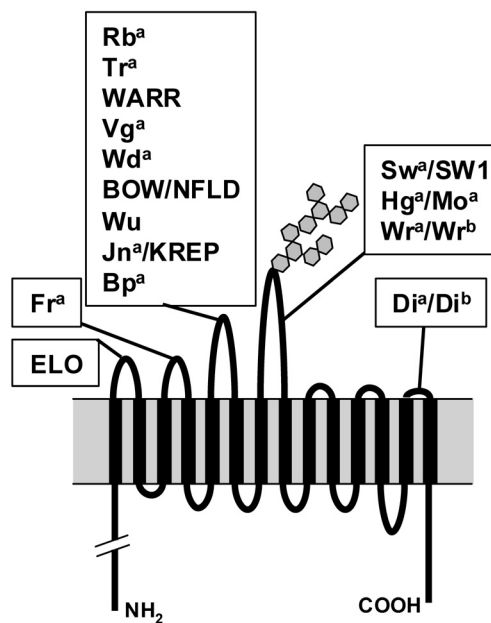


FIGURE 14-7. Diagram of Band 3, the Diego glycoprotein and anion exchanger, with cytoplasmic N- and C-terminal domains, 14 membrane-spanning domains, and an N-glycan on the fourth extra-cellular loop (although the precise conformation is still controversial). The locations of the 21 antigens of Diego system on the extracellular loops are shown.

CO_2 transport, and attachment of the red cell membrane to the cytoskeleton.³⁵ Tetramers of Band 3 form the core of the Band 3/Rh macrocomplex of red cell membrane proteins (Fig 14-1), which could function as a gas channel for O_2 and CO_2 .¹⁰ The Band 3 gene (*SLC4A1*) consists of 20 exons of coding sequence and is on chromosome 17.

Di^a (DI1) and Di^b (DI2); Anti-Di^a and -Di^b

Di^a, the original Diego antigen, is very rare in people of European and African ancestry, but has a prevalence of 5% in Chinese and Japanese and a higher prevalence in the native peoples of North and South America, reaching

Antibody Detection, Identification, and Compatibility Testing



METHOD 3-1. USING IMMEDIATE-SPIN COMPATIBILITY TESTING TO DEMONSTRATE ABO INCOMPATIBILITY

Principle

See Chapter 15 for a discussion of the principles of compatibility testing.

Specimen

Patient's serum or plasma may be used. The age of the specimen must comply with the pretransfusion specimen requirements in *AABB Standards for Blood Banks and Transfusion Services*.¹

Reagents

1. Normal saline.
2. Donor red cells.

Procedure

1. Prepare a 2% to 5% suspension of donor red cells in normal saline or EDTA saline. Some serologists using serum for testing prefer to suspend the donor red cells in EDTA saline because high-titer anti-A or anti-B can initiate complement coating, which can cause steric hindrance of agglutination.² The use of a patient's sample col-

lected in EDTA is an alternative approach to prevent this phenomenon.

2. Label a tube for each donor red cell suspension being tested with the patient's serum.
3. Add 2 drops of the patient's serum or plasma to each tube.
4. Add 1 drop of the suspension of donor red cells to the appropriate test tube.
5. Mix the contents of the tube(s) and centrifuge according to the calibration of the centrifuge.
6. Examine the tube(s) for hemolysis, gently resuspend the red cell button(s), and examine for agglutination.
7. Read, interpret, and record test results.

Interpretation

1. Agglutination or hemolysis constitutes a positive (incompatible) test result.
2. A smooth suspension of red cells after resuspension of the red cell button constitutes a negative result and indicates a compatible immediate-spin crossmatch.

References

1. Price TH, ed. *Standards for blood banks and transfusion services*. 25th ed. Bethesda, MD: AABB, 2008:44.
2. Judd WJ, Steiner EA, O'Donnell DB, Oberman HA. Discrepancies in ABO typing due to prozone: How safe is the immediate-spin crossmatch? *Transfusion* 1988;28:334-8.

