

# Blood Typing

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The increased availability of blood components such as packed red blood cells (pRBCs) and platelet-rich plasma has improved treatment for some patients in emergency and critical care settings. Veterinary blood banks provide blood components, and most also perform blood typing and crossmatching. These procedures can also be performed in-house in veterinary practice laboratories. Veterinary technicians must understand the concepts of blood component transfusion and the procedures to help ensure that transfusion therapy is safe.

## Blood Groups and Immunity

Red blood cell (RBC) antigens are structures on RBC surfaces in an animal that may react with antibodies in the plasma of another animal. The specific surface markers in an individual animal are genetically determined and are referred to as *blood group antigens*. The number of blood groups varies among species. Antigen-antibody reactions can occur with blood transfusions due to variation in blood group antigens between the recipient and the donor. These reactions usually result in clumping or agglutination of RBCs or may manifest clinically as RBC lysis.

The erythrocytes of some domestic animals (i.e., cats, cattle, sheep, and pigs) have naturally occurring antibodies (alloantibodies). Once a transfusion has been given to an animal, antibodies against the RBC antigen (immune antibodies) of the transfused blood form. Breeding females should always be given properly matched blood to minimize the potential for production of antibodies that can result in destruction of a neonate's RBCs.<sup>1</sup>

## Blood Types

### Dogs

More than a dozen different canine blood groups have been described. Nomenclature for the blood group systems is designated by *DEA* (dog erythrocyte antigen) followed by a number. For *DEA* systems other than *DEA* 1, the erythrocytes are designated as positive or negative for the specific antigen. The *DEA* 1 group comprises subgroups *DEA* 1.1, *DEA* 1.2, and *DEA* 1.3. Canine erythrocytes may be positive or negative for each of the *DEA* 1 subgroups. *DEA* 3, *DEA* 4, *DEA* 5, and *DEA* 7 designate other major blood groups.<sup>2</sup> The blood groups considered to be clinically significant are *DEA* 1.1 and *DEA* 7. *DEA* 1.1 elicits the greatest antigen response and causes the most serious transfusion reactions.

Approximately 50% of dogs are positive for *DEA* 1.1. Transfusion reactions to the other blood groups are less likely to cause clinical signs. *Dal*, another canine antigen, has also been described.<sup>3</sup> Because naturally occurring anti-*DEA* 1.1 antibodies are not known to exist, the first transfusion of *DEA* 1.1-positive blood into a *DEA* 1.1-negative recipient may not result in an immediate reaction. However, antibodies can develop, resulting in a delayed transfusion reaction in as little as a week after the original mismatched transfusion. If a *DEA* 1.1-negative dog previously received *DEA* 1.1-positive blood, a severe reaction can occur in less than 1 hour if the dog is subsequently transfused with *DEA* 1.1-positive blood.<sup>2</sup>

### Cats

One blood group system, designated the *AB* system, has been identified in cats. The blood groups of cats are *A*, *B*, and *AB*. Few cats have group *AB* blood. The vast majority of cats in the United States have group *A* blood, which probably accounts for the low incidence of transfusion reactions in cats. Type *B* blood is found in certain breeds (e.g., Devon rex, British shorthair) and certain geographic areas (e.g., Australia). Unlike dogs, cats have naturally occurring antibodies to the erythrocyte antigen they lack. Type *B* cats have strong anti-*A* antibodies, while type *A* cats have weak anti-*B* antibodies. Transfusing type *B* cats with type *A* blood may result in a serious transfusion reaction and death. Thus, blood for transfusion of purebred cats should be selected by typing or crossmatching. *Mik*, another blood cell antigen, has also been described in cats.<sup>4</sup>

Neonatal isoerythrolysis has been documented in type *A* and type *AB* kittens of type *B* queens with naturally occurring anti-*A* antibodies.<sup>2</sup>

## Blood Typing

Methods of identifying some canine and feline blood groups are available for use in veterinary practice. These methods include an immunochromatography assay and a card/slide agglutination assay.<sup>4</sup> The tube method is the gold standard for blood typing but is primarily used in reference laboratories.

### The Tube Method

The tube method for determining blood type requires the use of antisera, which consist of antibodies specific for each possible

**Box 1. Canine and Feline Blood Types<sup>a</sup>**

Dogs	Cats
• DEA 1.1, 1.2	• Type A
• DEA 3	• Type B
• DEA 4	• Type AB
• DEA 5	
• DEA 7	

<sup>a</sup>Typing antisera are available for these blood types.

blood type of a given species. Commercial antisera for canine and feline group testing are available for a few canine and feline blood groups (**BOX 1**). The tube method requires collection of a whole blood sample using EDTA, heparin, or acid-citrate-dextrose anticoagulant. The blood is centrifuged at 1000g for 10 minutes. After removal of the plasma and buffy coat, the erythrocytes are washed three times in a saline solution, centrifuged, and resuspended. The RBC suspension is distributed among as many tubes as required for the number of blood type antisera being tested. A small amount (usually 0.1 mL) of the antisera is added to the appropriately labeled tube. The tubes are incubated for 15 minutes at room temperature and then recentrifuged for 15 seconds at 1000g. Each tube is examined macroscopically and microscopically for evidence of hemolysis or agglutination. Weak positive results may require repeat testing.<sup>2</sup>

**The Card Agglutination Test**

Blood samples used to perform the card-based assay must not already show evidence of autoagglutination, which is usually visible as clumps in the blood sample. Washing the RBCs with phosphate-buffered saline may help salvage a sample that is showing evidence of agglutination. The RapidVet-H Canine DEA 1.1 (DMS Laboratories) is a blood-typing test card used to classify dogs as positive or negative for DEA 1.1 (**FIGURE 1**). The typing card contains a monoclonal antibody specific to DEA 1.1. Each card has three visually defined wells labeled “Autoagglutination patient screen,” “DEA 1.1–positive control,” and “Patient test.” One drop of EDTA-anticoagulated whole blood and 1 drop of phosphate-buffered saline are mixed on the lyophilized reagents within each well. In the DEA 1.1–positive test well, the monoclonal antibody forms an antiserum and is then mixed with whole blood from the patient. If present, DEA 1.1–positive erythrocytes react with the antiserum, causing agglutination. The antiserum in the patient test well does not react with DEA–1.1 negative erythrocytes.

RapidVet-H (feline) is a similar blood-typing test card for classifying feline blood as type A, B, or AB. The assay uses test wells that contain lyophilized reagent, representing an antibody to the A antigen, and an anti-B antigen, consisting of a lectin. Erythrocytes from type A cats agglutinate with anti-A monoclonal antibodies (the A well on the card), and erythrocytes from type B cats agglutinate with anti-B solution (the B well on the card).

Erythrocytes from type AB cats agglutinate with both anti-A and anti-B reagents. The third well on the card serves as an autoagglutination saline screen and must be negative for results to be valid. Samples are first screened for autoagglutination. If autoagglutination is present, the RBCs may be washed with phosphate-buffered saline and the autoagglutination screen repeated. If the autoagglutination result is negative, the typing test may be performed.<sup>5</sup>

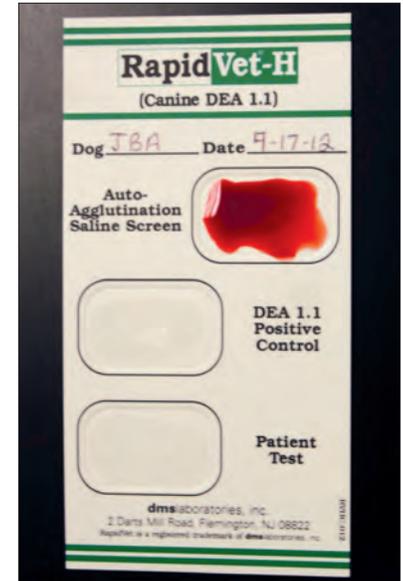


Figure 1. Card agglutination blood-typing test.

**Immunochromatographic Assay**

Two commercial test kits use the immunochromatographic test principle rather than agglutination. The control band detects a separate antigen on the RBCs. The canine test uses a paper strip impregnated with monoclonal anti-DEA 1.1 antibody and a second antibody to a universal RBC antigen as a control. An RBC solution diffuses up the strip, and if the cells express DEA 1.1, they concentrate in the area of antibody impregnation. The cells also concentrate in the area of the control antigen, demonstrating that cells have successfully diffused up the length of the strip. The feline test works the same way; however, it has an area containing an anti-A monoclonal antibody, an area containing an anti-B monoclonal antibody, and a control antibody for a common feline RBC antigen, allowing identification of blood type A, B, or AB<sup>6</sup> (**FIGURE 2**).

**Crossmatching**

In the absence of commercial antisera, crossmatching a blood donor and a recipient reduces the possibility of a transfusion reaction.<sup>7</sup>

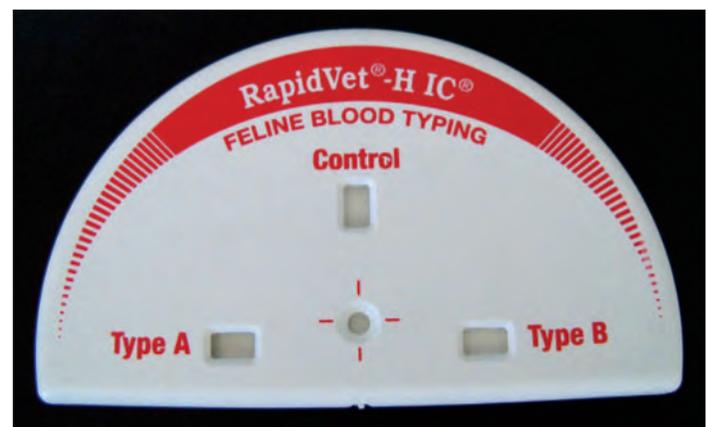


Figure 2. An immunochromatographic test for feline blood typing.

**Box 2. Crossmatching**

**Equipment**

- Normal saline
- Plastic, conical bottom, 12-mL tubes
- Centrifuge
- Microscope
- Slide/cover slip

**Procedure**

1. Obtain whole blood samples (in EDTA anticoagulant) from the donor and the recipient.
  - Samples may also be obtained from stored whole blood or pRBCs.
2. Centrifuge the EDTA tubes at 1000g for 10 min. Remove the plasma and place it in labeled tubes.
3. Place 3 to 5 drops of the pRBCs from each EDTA tube into the labeled conical centrifuge tubes.
4. Add 5 to 10 mL of saline to the pRBCs.
5. Centrifuge the tubes with RBCs for 2 to 5 min.
6. Pour off the supernatant and discard it.
7. Resuspend the pRBCs in saline and centrifuge them.
  - Repeat steps 6 and 7 one to three times until the supernatant is clear.
8. Add a few drops of saline to resuspend the pRBCs.
9. **Major crossmatch:** Label a plain tube with the donor name and “major.”
  - Add 2 drops of the recipient plasma and 2 drops of donor cell suspension.
10. **Minor crossmatch:** Label a tube with the donor number and “minor.”
  - Add 2 drops of the donor plasma and 2 drops of the recipient cell suspension.
11. **Controls:** Label two control tubes.
  - Add 2 drops of donor plasma and 2 drops of donor RBCs to the first tube.
  - Add 2 drops of recipient plasma and 2 drops of recipient RBCs to the second tube.
12. Incubate all four tubes at 37°C (98.6°F) for 15–30 min.
  - Room-temperature incubation is sometimes performed and generally yields accurate results.
13. Centrifuge all four tubes for 5 min.
14. Examine all four tubes macroscopically for evidence of hemolysis or agglutination.
15. Grade any agglutination reactions and examine the samples microscopically.
16. Positive reactions in the donor control tubes indicate unsuitable donors.

The two-part procedure (major and minor crossmatches) requires a serum sample and a whole blood sample (**BOX 2**). RBC suspensions, collected as for blood typing, are prepared. In the major crossmatch, a few drops of serum from the recipient are added to a few drops of washed pRBCs from the donor. The mixture is incubated and then centrifuged. Macroscopic or microscopic presence of hemolysis or agglutination indicates a blood-type mismatch. The



**Figure 3.** RapidVet-H test kits are available for in-house canine and feline blood crossmatching.

**Table 1. Grading Crossmatch Reactions**

Grade	Description
0	No evidence of agglutination or hemolysis
1	Many small agglutinates and some free cells
2	Large agglutinates and smaller clumps of cells
3	Many large agglutinates
4	Solid aggregate of cells

minor crossmatch is similar except that donor serum and recipient RBCs are used. Both procedures should be performed on all animals that require transfusion but whose blood types are unknown. Two controls are used for the test, which consists of using donor cells with donor serum as well as recipient cells with recipient serum. A commercial test kit for crossmatching is also available (**FIGURE 3**).

Agglutination reactions are sometimes graded. Several classification schemes are used for this purpose. **TABLE 1** shows one type of grading scale. The clinician determines whether evidence of agglutination constitutes an unsuitable transfusion.

**Summary**

Proper blood typing and crossmatching can provide valuable information to clinicians and help minimize problems in critically ill patients. Ideally, all critically ill patients should undergo blood typing and crossmatching before a transfusion.

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1. The most serious transfusion reactions in canine patients occur in dogs positive for \_\_\_\_\_ that are given mismatched transfusions.
  - a. DEA 1.1
  - b. DEA 3
  - c. DEA
  - d. Mik
2. The vast majority of cats in the United States have type \_\_\_\_ blood.
  - a. AB
  - b. B
  - c. A
  - d. Dal
3. A major crossmatch involves mixing
  - a. donor serum and recipient cells.
  - b. donor cells and recipient cells.
  - c. recipient serum and donor serum.
  - d. recipient serum and donor cells.
4. An agglutination reaction with evidence of many small agglutinates and some free cells would be designated as grade
  - a. 1.
  - b. 2.
  - c. 3.
  - d. 4.
5. The primary method of blood typing in reference laboratories is
  - a. crossmatching.
  - b. immunochromatography.
  - c. the tube method.
  - d. agglutination.
6. Canine agglutination blood typing is used to determine whether dogs are positive for
  - a. DEA 1.1.
  - b. DEA 3.
  - c. DEA.
  - d. Mik.
7. Neonatal isoerythrolysis has been documented in kittens of type \_\_ queens.
  - a. A
  - b. B
  - c. AB
  - d. Mik
8. The card agglutination assay for blood typing requires a sample collected with
  - a. no anticoagulant.
  - b. acid-citrate-dextrose anticoagulant.
  - c. EDTA.
  - d. heparin.
9. A minor crossmatch involves mixing
  - a. donor serum and recipient cells.
  - b. donor cells and recipient cells.
  - c. recipient serum and donor serum.
  - d. recipient serum and donor cells.
10. The most serious transfusion reactions in feline patients occur in cats with type \_\_\_\_ blood given mismatched transfusions.
  - a. Mik
  - b. B
  - c. AB
  - d. A