Blood Typing and Cross Matching
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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 the in-house 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.

Erythrocytes (RBCs) of some domestic animals have naturally occurring antibodies (alloantibodies). Once a transfusion has been given to an animal, antibodies against the RBC antigen (immune antibodies) form. Breeding females should always be given properly matched blood to avoid sensitization that results in destruction of the neonate’s RBCs.

Blood types

**Dogs**

More than a dozen different canine blood groups have been described. Nomenclature for the blood group systems is designated with the letters DEA (for Dog Erythrocyte Antigen) followed by a number. For DEA systems, the erythrocytes are designated as positive or negative for that specific antigen. The DEA 1 group was once considered to have three subgroups but recent research has documented that these reflected varying degrees of expression of the same gene.

DEA-3, DEA-4, DEA-5, and DEA-7 also designate major blood groups. The blood groups considered to be clinically significant are DEA-1 and DEA-7. The DEA1 sub-group elicits the greatest antigen response and causes the most serious transfusion reactions. Approximately 50% of all dogs are positive for the DEA 1 antigen. Transfusion reactions to the other blood groups are less likely to cause clinical signs. An additional canine antigen, designated Dal, has also been described. Because naturally occurring anti-DEA1 antibodies are not known to exist, the first transfusion of DEA1-positive blood into an DEA1-negative recipient may not result in an immediate reaction. However, antibodies can develop and result in a delayed transfusion reaction in as little as a week following the original mismatched transfusion. If a previously immunized DEA1-negative dog receives DEA1-positive blood, severe reactions occur in less than 1 hour.

**Cats**

One blood group system has been identified in the cat, designated the AB system. Blood groups of cats include A, B, and AB. Few cats have group AB. The vast majority of cats have group A, which probably accounts for the low incidence of transfusion reactions in cats. Type B occurs in certain purebred breeds and certain geographic areas. Unlike dogs, cats do possess naturally occurring antibodies to the erythrocyte antigen they are lacking. 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 serious transfusion reactions and death. Thus blood for transfusion of purebred cats should be selected by typing or crossmatching. An additional blood cell antigen, the Mik antigen, has also been described in cats. Neonatal isoerythrolysis has been documented in Type A or Type AB kittens born of Type B queens with naturally occurring anti-A antibodies.

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. 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 blood type of a given species. Commercial antisera for canine and feline group testing are available for canine and feline blood group. 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 additional testing.

**The card agglutination test**

Blood samples used to perform the card-based assay must not already show evidence of autoagglutination, 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 (DEA 1; DMS Laboratories) is a blood-typing test card used to classify dogs as positive or negative for DEA 1. Each typing card contains a monoclonal antibody specific to DEA 1. Each card has three visually defined wells labeled “DEA 1–positive control,” “Auto-agglutination Saline Screen” and “patient test.” One drop of EDTA-anticoagulated whole blood and 1 drop of phosphate-buffered saline are mixed onto the lyophilized reagents within each well. In the patient test well, the monoclonal antibody forms an antiserum and is then mixed with whole blood from the patient. DEA 1 positive erythrocytes react with the antiserum, causing agglutination. The antiserum in the patient test well does not react with DEA–1 negative erythrocytes.

RapidVet®-H (Feline) is a similar blood typing test card is available to classify cats 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 component consisting of a lectin. Erythrocytes from type A cats will agglutinate with anti-A monoclonal antibodies (the well labeled A on card) and erythrocytes from type B cats will agglutinate with anti-B solution (the well labeled B on card). Erythrocytes from type AB cats will agglutinate with both anti-A and anti-B reagents. The third well on the card serves as an auto-agglutination saline screen and must be negative for results to be valid. Samples are first screened for auto-agglutination. Should auto-agglutination be present, the red blood cells may be washed with phosphate buffered saline and the auto-agglutination screen repeated. If a negative auto-agglutination result is obtained, the typing test may be performed.

**Immunochromatographic assay**

Two commercial test kits use the immunochromatographic test principle rather than agglutination. The control band detects a separate antigen on the red blood cells. The canine test uses a monoclonal anti–DEA 1 antibody strip impregnated onto a paper strip 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, 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.

**Crossmatching**

In the absence of commercial antisera, crossmatching a blood donor and a recipient reduces the possibility of a transfusion reaction. The two-part procedure (major and minor crossmatches) requires a serum sample and a whole blood sample. RBC suspensions, collected as for blood typing, are prepared. In the manual major crossmatch, a few drops of serum from the recipient are added to a few drops of washed packed RBCs from the donor. The mixture is incubated and then centrifuged. Macroscopic or microscopic presence of hemolysis or agglutination indicates a blood-type mismatch. The 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.

Agglutination reactions are sometimes graded. Several classification schemes are used for this purpose. 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.

References available from the author.