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## A Newly Recognized Blood Group in Domestic Shorthair Cats: The *Mik* Red Cell Antigen

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### Abstract

**Background**—Naturally occurring alloantibodies produced against A and B red cell antigens in cats can cause acute hemolytic transfusion reactions. Blood incompatibilities, unrelated to the AB blood group system, have also been suspected after blood transfusions through routine crossmatch testing or as a result of hemolytic transfusion reactions.

**Hypothesis**—Incompatible crossmatch results among AB compatible cats signify the presence of a naturally occurring alloantibody against a newly identified blood antigen in a group of previously never transfused blood donor cats. The associated alloantibody is clinically important based upon a hemolytic transfusion reaction after inadvertent transfusion of red cells expressing this red cell antigen in a feline renal transplant recipient that lacks this red cell antigen.

**Methods**—Blood donor and nonblood donor cats were evaluated for the presence of auto- and alloantibodies using direct antiglobulin and crossmatch tests, respectively, and were blood typed for AB blood group status. Both standard tube and novel gel column techniques were used.

**Results**—Plasma from 3 of 65 cats and 1 feline renal transplant recipient caused incompatible crossmatch test results with AB compatible erythrocytes indicating these cats formed an alloantibody against a red cell antigen they lack, termed *Mik*. The 3 donors and the renal transplant recipient were crossmatch-compatible with one another. Tube and gel column crossmatch test results were similar.

**Conclusions and Clinical Importance**—The absence of this novel *Mik* red cell antigen can be associated with naturally occurring anti-*Mik* alloantibodies and can elicit an acute hemolytic transfusion reaction after an AB-matched blood transfusion.

### Keywords

Blood type; Cat; Gel column crossmatch; Hemolytic transfusion reaction; Renal transplant

The AB blood group system, the only currently recognized system in cats, includes blood types A, B, and AB. The incidence of these blood types varies among cat breeds and geographic locations.<sup>1-5</sup> Naturally occurring alloantibodies formed against the red cell antigen the cat is lacking have been reported and identified in both type-A and type-B cats but not in type-AB cats.<sup>6-8</sup> Anti-A alloantibodies present in type-B cats can result in severe acute hemolytic transfusion reactions as well as neonatal isoerythrolysis, whereas anti-B alloantibodies formed by type-A cats can also cause hemolytic transfusion reactions, but apparently not neonatal isoerythrolysis.<sup>3,8-14</sup>

Naturally occurring alloantibodies and induced alloantibodies formed in humans after blood transfusion are well recognized, and the presence of these alloanti-bodies often requires extensive testing before transfusion to assure compatible and safe blood product administration. References to feline AB blood group compatible but otherwise incompatible crossmatch results have been reported in cats and suggest that additional blood type(s) exist.<sup>a,15</sup> We describe here a distinct clinically relevant alloantibody formed against a common antigen, named *Mik*, identified in 3 cats with no history of transfusion, as well as in a cat renal transplant recipient, using both a standard tube and a novel gel column crossmatch technique.

## Materials and Methods

### Animals

Sixty-five type-A blood donor cats and 1 type-A cat renal transplant recipient were included in blood compatibility testing at the Matthew J. Ryan Hospital of the University of Pennsylvania (VHUP). Blood donor cats of the Penn Animal Blood Bank (PABB), came from 2 groups: specific pathogen-free, castrated male, domestic shorthair (DSH) cats (n = 22) that are housed within VHUP, and a 2nd group of PABB community donors (n = 43), which includes DSH cats from students and staff at VHUP. PABB in-house specific pathogen free cats were obtained at approximately 1 year of age from either Harlan Sprague-Dawley<sup>b</sup> (n = 11), or Liberty Research Inc<sup>c</sup> (n = 11). Also included were 3 type-B cats and 2 type-AB community donor cats. Entry criteria for PABB donor cats require that they were housed strictly indoors and had not received a transfusion. All blood donor cats had an annual physical examination, CBC, and serum chemistry panel and were screened for FeLV, FIV, *Mycoplasma haemofelis* and *Candidatus Mycoplasma haemominutum*. The cat blood donor program and this clinical study were approved by the Institutional Animal Care and Use Committee.

### Blood Typing

One or several EDTA-anticoagulated blood samples (<7 days old stored at 4°C) were available from all cats in the study and plasma (fresh or fresh frozen) or serum samples were available from most cats. Blood type was initially determined upon enrollment into the PABB donor program using both slide and tube agglutination methods.<sup>16</sup> In the feline renal transplant recipient, as well as PABB donors in which there was evidence of an alloantibody, AB blood type was confirmed with 2 additional methods: a commercially available feline blood-typing card<sup>d</sup> and a gel column method.<sup>e</sup> The gel column test was recently evaluated for blood typing in cats.<sup>f,16</sup>

<sup>a</sup>Henson MS, Kristensen AT, Armstrong PJ, et al. Feline blood component therapy: Retrospective study of 246 transfusions. American College of Veterinary Internal Medicine, 12th Congress, San Francisco, CA, 1994

<sup>b</sup>Harlan Sprague Dawley, Indianapolis, IN

<sup>c</sup>Liberty Research Inc. Waverly, NY

<sup>d</sup>RapidVet-H (Feline) DMS laboratories, Flemington, NJ

<sup>e</sup>DiaMed-Vet ID Card A + B, DiaMed AG, Switzerland

## Tube and Gel Column Crossmatch Test

Serologic evaluation of cats for the presence of possible non-AB blood group alloantibodies was accomplished by a tube method as well as by a gel column technique when sample size permitted. “Major” and “minor” crossmatch terminology refers to a blood recipient’s potential serologic compatibility with possible blood donors. A major crossmatch is performed to identify alloantibodies in the recipient’s serum or plasma against a potential donor’s red cells, whereas a minor crossmatch tests for alloantibodies in the donor’s serum or plasma.

**Tube Method**—A recently described standard tube crossmatch procedure<sup>g</sup> was followed: Briefly, 50  $\mu$ L of plasma plus 25  $\mu$ L of a 3–5% suspension of washed red blood cells in phosphate buffered saline (PBS) were incubated for 15 minutes at 37°C followed by a 15-second centrifugation (1,000  $\times$  g).<sup>h</sup> To assess for agglutination of erythrocytes and hemolysis after gentle resuspension of the red cell button, the following scale was used: 4+, single agglutinate of erythrocytes; 3+, few large agglutinates of erythrocytes; 2+, large agglutinates of erythrocytes amid many smaller erythrocyte clumps; 1+, many small red blood cell agglutinates amid a background of free cells; weak: weak granularity; and negative, no granularity. Hemolysis of erythrocytes was also recorded as positive or negative.

**Gel Column Method**—This technique was adopted from the human protocol and uses a saline or NaCl/enzyme test and cold agglutinins gel test,<sup>i</sup> which consists of 6 microtubes or columns filled with neutral dextran-acrylamide gel beads and no added reagent.<sup>f</sup> Red blood cells (10  $\mu$ L) are suspended in 1,000  $\mu$ L of low ionic strength saline (LISS)<sup>j</sup> to make a resultant 0.8% red cell suspension. In each incubation chamber, 25  $\mu$ L of serum or plasma plus 50  $\mu$ L of the 0.8% red cell suspension are pipetted and the gel cards are incubated<sup>k</sup> at 22°C for 10 minutes. A 10-minute centrifugation step follows in a special centrifuge.<sup>l</sup> A negative or compatible result is one where all red cells pass through the gel column forming a pellet at the bottom of the gel. A positive or incompatible reaction is graded from 1+ to 4+ and results when red cells fail to pass through the gel matrix, becoming entrapped above or within it.<sup>16</sup> Autocontrols, referring to red cells incubated with plasma or serum from the same animal, were also performed with each sample and were all found to be negative.

## Agglutinin Titer

Alloantibody characterization, both in magnitude and immunoglobulin type, was accomplished through agglutinin titers both with and without dithiothreitol treatment of plasma. The agglutinin titer is the highest dilution of serum or plasma at which hemagglutination is still appreciated (1+); this is determined by making serial 2-fold dilutions of an animal’s plasma or serum in PBS after which a standard tube crossmatch procedure is performed using the serial dilutions.<sup>7,17</sup> Treatment of plasma with dithiothreitol, which eliminates agglutination and complement-binding activities of IgM antibodies by cleaving disulfide bonds, was also used in similar agglutination titer studies as described previously.<sup>7,17</sup>

<sup>f</sup>Giger U, Blais MC, Weinstein NM. Assessment of a commercial gel column technique for feline AB blood typing and cross-matching. American Society Veterinary Clinical Pathology 40th Annual Meeting, Boston, MA, 2005

<sup>g</sup>Scott M et al. Standard crossmatching protocol. Association for Veterinary Hematology and Transfusion Medicine, American College of Veterinary Internal Medicine, 21st Congress, Charlotte, NC 2003

<sup>h</sup>Clay Adams Sero-Fuge 2002

<sup>i</sup>DiaMed ID card “NaCl, enzyme test and cold agglutinins,” Switzerland

<sup>j</sup>LISS; ID-Diluent “Vet 2,” DiaMed AG, Switzerland

<sup>k</sup>ID-incubator 37 SI, DiaMed Microtyping System, Switzerland

<sup>l</sup>ID-centrifuge 12 S II, DiaMed Microtyping System, Switzerland

## Direct Antiglobulin Test

A direct antiglobulin test (direct Coombs' test), which assesses for the presence of autoantibodies on the surface of erythrocytes, was performed on donors 1, 2, and 3, the renal transplant recipient, as well as 4 control donor cats. Serial dilutions using goat anti-cat IgG, IgM, and C3 reagent<sup>m</sup> in PBS from 1 : 1 to 1 : 1,024 in addition to a control containing only PBS were incubated with a 3–5% suspension of washed erythrocytes; the protocol provided by the manufacturer was followed for incubation and scoring.<sup>m</sup>

## Results

Incompatible crossmatch reactions with plasma from donor 1 (cat's name Mike) resulted in a detailed survey of blood compatibilities in PABB blood donors as a measure of quality control. Three PABB donors (donors 1, 2, and 3) and a cat renal transplant recipient were extensively studied after identification of a novel alloantibody. Plasma from donors 1, 2, and 3 caused agglutination (1+ to 3+) of erythrocytes from blood type-A cats in tube crossmatch assays at 22°C and 37°C (Table 1). Using a single cat's type-A red blood cells in crossmatch tests with plasma or serum from donors 1, 2, and 3 yielded comparable incompatible reactions suggesting donors 1, 2, and 3 produced an alloantibody against the same red cell antigen expressed on the other type-A cats' red blood cells. Hemagglutination reactions were also seen using plasma from donors 1, 2, and 3 with erythrocytes from type-AB and B cats (Table 1). Plasma and erythrocytes from donors 1, 2, and 3 were compatible with each other in major and minor cross-match tests. Autocontrol tests were consistently negative for all donor cats. Furthermore, tube and gel crossmatch tests using plasma from additional type-A cats yielded compatible results with both *Mik* positive and *Mik* negative, type-A red cells (Table 1). Both tube and gel column methods consistently identified the same incompatible (positive for hemagglutination) as well as compatible (negative or no agglutination) samples. The magnitude of the hemagglutination reaction between the 2 techniques varied slightly and likely was owing to the different methodology. Harlan Sprague Dawley<sup>b</sup> provided blood samples from 3 additional type-A cats related to donor 1. Plasma from 2 of these cats caused agglutination (2+ and 1+) of *Mik* negative red cells in tube crossmatch tests, whereas plasma from the 3rd did not cause hemagglutination.

Donor 1 had a reproducibly positive direct antiglobulin test result up to a plasma dilution of 1 : 32 indicating the presence of a red cell autoantibody, whereas the direct antiglobulin test results for donors 2 and 3 as well as the 4 control cats were negative. Despite the positive direct antiglobulin test for donor 1, anemia was never documented and review of peripheral blood smears did not reveal abnormalities.

The agglutinin titer of plasma samples from donors 1, 2, and 3 varied among cats as well as among testing dates from 1 : 16 to 1 : 64, 1 : 16 to 1 : 32 and  $\leq 1 : 1$ , respectively. Hemolysin activity was not identified in any of the donor plasma or serum samples. Dithiothreitol treatment of plasma from either donor 1 or 2 noticeably weakened the hemagglutination titer to  $\leq 1 : 8$ . Because some degree of hemagglutination persisted after dithiothreitol treatment of plasma both IgG and IgM alloantibodies are likely present. Dithiothreitol-treated plasma from donor 3 did not cause hemagglutination of any cat's erythrocytes as expected.

## Case Study

A 4.5-year-old castrated male DSH cat (index cat) was evaluated for renal transplantation because of chronic renal failure. Ten days before evaluation, this cat received 20 mL of type A packed RBCs because of anemia. There was no history of any previous transfusions. The

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<sup>m</sup>Feline Coombs Reagent Catalog number 592-2 VMRD, Inc. Pullman, WA

PCV did not appreciably rise after transfusion, and, within 1–2 days of this initial RBC transfusion, marked hemoglobinemia and hyperbilirubinemia were noted. Serum from this cat before this transfusion was not available for testing. The cat was administered immunosuppressive agents in preparation for renal transplantation. Tube crossmatch tests were performed to identify a compatible kidney donor as well as potential blood donors before surgery. These tests revealed compatible major (index cat plasma) cross-match reactions with red cells from 3 type-A cats (kidney donor X, blood donor Y, and blood donor Z) and 1+ incompatible hemagglutination reactions with 6 other type-A cats tested. Renal transplantation was performed on the index cat on day 6 of hospitalization. A total of 85 mL blood were transfused the day of surgery because of anemia (PCV = 15%). This included 45 mL of whole blood from kidney donor X at the start of surgery, 20 mL of packed PRCs from blood donor Y during surgery, and 20 mL of packed RBCs from blood donor Z a few hours after surgery. One hour after completion of the surgery and 5 hours after the 1st transfusion, the PCV was 22% and plasma appeared hemolyzed in a spun microhematocrit tube. No comments were made regarding plasma appearance during the surgery itself, when blood was collected for assessment of PCV, total solids, and acid-base status. The PCV fell to 15% 10 hours postoperatively, and hemoglobinemia was observed for the next 3–4 days. Index cat and blood donor compatibility was reassessed using both tube and gel column AB blood typing and crossmatch assays in the Transfusion Medicine Laboratory. These crossmatch tests incorporated index cat plasma from both before and after the transplant surgery and VHUP blood transfusions. Further testing confirmed that the index cat and donors had type-A blood, but revealed index cat plasma from before and after surgery and the 3 blood transfusions was incompatible to erythrocytes from donors X, Y, and Z (Table 2). The index cat's plasma was compatible with red cells from donors 1, 2, and 3 (*Mik* negative) but was incompatible with all other type-A cats tested (n = 10) including a known sibling. Plasma from donors 1 and 2 was also compatible with red blood cells from the index cat; plasma from donor 3 was not available for testing (Table 2). A direct antiglobulin test for the index cat after the transfusions was negative. Serum creatinine concentration remained elevated after surgery (6.3 mg/ dL) and poor perfusion to the renal allograft was noted on ultrasound indicative of transplant rejection. Histopathologic evaluation of biopsy samples from the renal allograft taken 4 days after surgery and histopathology of the entire renal allograft removed 1 week after surgery revealed acute necrosis of endothelial walls and vascular fibrin thrombi deposition. Complications from the multiple surgeries resulted in the cat's further decline and death approximately 2 weeks after the transplant surgery.

## Discussion

Although red cell antigens outside of the AB blood group system in cats have been suspected based upon incompatible crossmatch results and presumed hemolytic transfusion reactions, this is, to the authors' knowledge, the first description of a blood group and corresponding clinically relevant, naturally occurring alloantibody distinct from the AB blood group system in cats. Crossmatch results reproducibly demonstrated that plasma from donors 1, 2, and 3 was incompatible with red cells from all type-A cats but not with each others' red cells or red cells from the renal transplant recipient. These findings suggest the presence of an alloantibody produced against a common red cell antigen, termed *Mik*, absent in all 3 donors. The clinical relevance of anti-*Mik* alloantibodies was documented after an acute hemolytic transfusion reaction after inadvertent transfusion of *Mik*-positive blood to the *Mik*-negative renal transplant recipient.

The lack of a prior transfusion history in donors 1, 2, and 3 indicates the anti-*Mik* alloantibody can be naturally occurring similar to anti-A and anti-B alloantibodies present in type-B and type-A cats, respectively. Anti-A and anti-B alloantibodies develop by 2–3

months of age without known sensitization but are believed to result from exposure to structural epitopes from a variety of organisms including plants, bacteria, and protozoa, which are similar or identical to blood group antigens.<sup>7,18</sup> Anti-*Mik* alloantibodies in the renal transplant recipient were also likely naturally occurring as the very 1st transfusion this cat received at the referral practice resulted in hemolysis. The timing of this hemolytic transfusion reaction indicates the presence at the time of transfusion of alloantibodies against a foreign red cell antigen.

The strength of the *Mik* alloantibody differed among blood donors and depended on which *Mik*-positive red cells were used. This might reflect varied expression of the *Mik* antigen on erythrocytes. *Mik* is also likely expressed on the type-B and AB red cells tested as plasma from donors 1 and 2 caused agglutination (3+) of these red cells similar to reactions with known *Mik*-positive, type-A red cells. As expected, plasma from type-A, *Mik*-positive cats caused only weak agglutination of type-B and AB red cells. Previous reports demonstrate type-A cats' anti-B alloantibodies are weak to moderate with a titer of  $\leq 1 : 32$  whereas type-B cats anti-A titers are stronger and range from 1 : 64 to 1 : 2,048.<sup>7</sup> Hemagglutinin activity of the anti-*Mik* allo-antibody was diminished by dithiothreitol treatment of plasma from *Mik*-negative cats indicating the presence of both IgG and IgM alloantibodies, similar to anti-B antibody type in type-A cats.<sup>7</sup> Although the lack of hemagglutinin activity seen with dithiothreitol-treated plasma from donor 3 suggests his anti-*Mik* alloantibody is primarily IgM in composition the more likely explanation is that remaining IgG concentrations are of insufficient quantity or strength to cause detectable hemagglutination using the methods described.

Anti-*Mik* alloantibodies might have also been involved in rejection of the transplanted kidney. Many red cell antigens in humans are expressed on vascular endothelial cells; both naturally occurring and induced alloantibodies against these antigens can result in antibody-mediated transplant rejection in blood type-mismatched renal transplants.<sup>18–20</sup> Expression of red cell antigens on renal vascular endothelium in cats has also been alluded to in the veterinary literature.<sup>21</sup> As such, anti-*Mik* alloantibodies, present in the recipient, could have contributed to failure of the renal allograft from a *Mik*-positive cat.

The positive direct antiglobulin test in donor 1 was surprising although did not seem clinically relevant given the lack of anemia and poikilocytosis. A positive direct antiglobulin test in cats may indicate a primary idiopathic immune response or may be secondary to infectious, inflammatory, or neoplastic conditions; anemia may or may not result.<sup>22–24</sup> Cats are also reported to have spontaneous arising, cold-reacting IgM antibodies at low titers.<sup>22</sup> The direct antiglobulin test in donor 1 was performed at 37°C, which suggests warm rather than cold-reacting antibodies. In humans, positive direct antiglobulin tests have been recognized at a low frequency through routine screening of otherwise healthy blood donors. Although often idiopathic, potential causes for a positive direct antiglobulin test in these donors include prior administration of certain drugs, increased age of the donor, and antiphospholipid antibodies.<sup>25–27</sup>

Donors 1, 2, and 3 came from the same research cat supplier<sup>b</sup> but were not closely related based upon a 2 generation pedigree. The recognition of the *Mik* antigen in this relatively small and potentially related group of cats suggests a genetic basis, but the mode of inheritance of the *Mik* antigen remains undefined as an insufficient number of relatives of these cats were available for testing. A wider distribution of the *Mik*-negative blood type is anticipated as the renal transplant patient came from a different geographic region and was not associated with the research colony cats. Additional testing of cats is necessary to determine mode of inheritance, geographical distribution, and frequency of the *Mik* antigen.

Naming a new blood group system is not uniformly standardized in human blood banking and uses various schemes.<sup>28</sup> One approach is to use a part (3 letters) of the name of the initial antibody producer or reactive donor. *Mik* was chosen based upon identification of an alloantibody in the 1st blood donor cat named Mike.

The misidentification of 3 blood donors as compatible for the renal transplant patient illustrates the complexity associated with the tube crossmatch test that was recently addressed in veterinary laboratory medicine<sup>g</sup>. Inherent variables in the tube crossmatch procedure include the strength of the red cell suspension, type of buffer used, the ratio of serum or plasma to erythrocytes, incubation time and temperature, and interpretation of the hemagglutination reaction. Incorrect crossmatch test results can be attributed to a weak red cell suspension, overzealous resuspension of the red blood cell button, improperly stored blood samples, or inadequate incubation. Also, antibody may be present at a concentration below the sensitivity threshold of the crossmatch test routinely used.<sup>29</sup> Upon re-examination of the crossmatch technique used by the routine laboratory in the feline transplant recipient, both a weak red cell suspension and overzealous resuspension of the red cell button were the likely explanations for the misidentification of compatible donors. During investigation of the hemolytic transfusion reaction, renal transplant recipient plasma collected before the VHUP blood transfusions and surgery did cause relatively weaker, although still identifiable, hemagglutination reactions when compared to plasma collected after transfusion. Additionally, use of the gel column technique plasma collected either before or after transfusion resulted in positive cross-match reactions. Use of the saline gel column for crossmatching has recently been described and clearly identified both AB blood group and non-AB blood group incompatibilities in cats<sup>d</sup>. Gel column methodology has been used extensively and successfully in human medicine since its introduction in the late 1980s for blood typing and for serologic evaluation of auto- and alloantibodies.<sup>30</sup> The gel column itself and standardization of the crossmatch protocol and interpretation circumvent many of the problems associated with the tube method. Hemagglutination, which is stabilized in the gel matrix, can be evaluated up to 24 hours after centrifugation, and, as in the tube method, follows a standard grading system. Nevertheless, crossmatch tests with gel column technology, as with tube methods, cannot correct for improperly handled or mislabeled blood samples. Red cell suspensions were made with LISS in the gel column procedure and PBS in the tube method. Although LISS may enhance antigen-antibody binding by decreasing steric hindrance, its use in gel column methodology is not without controversy in human blood banking, and the potential for error in compatibility testing exists.<sup>31,32</sup>

Increased blood compatibility testing will likely identify even more red cell antigens in cats.<sup>a,15</sup> A non-AB, non-*Mik* alloantibody and corresponding antigen likely caused the incompatible tube crossmatch results seen with the 2 additional research cats.<sup>b</sup> Additional non-AB, non-*Mik* antigens are being recognized in both feline blood donors and clinical cases through continued crossmatch testing.

Conventional thinking in feline transfusion medicine has been that determination of AB blood type in a cat with no prior transfusion history was adequate, and the minor crossmatch, using plasma from an AB-compatible cat with no prior transfusions should be compatible. Crossmatch testing was performed only when additional transfusion support was necessary, usually more than 4 days after the 1st transfusion. Performing a crossmatch before any transfusion may now be necessary given the clinical relevance of naturally occurring anti-*Mik* alloantibodies and potentially additional alloantibodies. Crossmatch tests with AB typed donors have also been used historically to indirectly determine blood type.<sup>33</sup> In light of *Mik*, and possibly other red cell antigens, and associated alloantibodies, this alternative practice may give erroneous blood type results. AB blood typing using either in-house commercially available typing cards or reliance upon a reference laboratory is

advised. A non-AB blood group alloantibody should be suspected when repeated crossmatch tests between AB blood group compatible cats are incompatible, regardless of prior transfusion history. Conversely, identification of cats positive for a specific red cell antigen, such as *Mik*, can also be accomplished.

Since submission of this paper, additional *Mik*-negative cats have been identified. Two of these cats had a hemolytic transfusion reaction after receiving a first, AB-compatible blood transfusion, further emphasizing the clinical relevance of this newly recognized blood group.

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Table 1

Tube crossmatch (37°C) results for *Mik* negative donors, the renal transplant recipient, and other type A, B, and AB cats.

Red cells	Plasma					
	Donor 1	Donor 2	Donor 3	Transplant Recipient	Type A ( <i>Mik</i> +) n = 30	Type B n = 3
Donors 1, 2, 3 Type A ( <i>Mik</i> -)	-	-	-	-	-	4+
Transplant patient Type A ( <i>Mik</i> -)	-	-	nd	-	-	nd
Type A ( <i>Mik</i> +) <sup>a</sup>	3+	2+/3+	weak	2+/3+	-	4+
Type B n = 3	3+	2+/3+	weak	nd	weak	-
Type AB n = 2	3+	2+/3+	weak	nd	-	3+/4+

Nd, not done.

<sup>a</sup>Type A, *Mik*+ red cells from n = 55, 30, and 25 cats for donors 1, 2, and 3, respectively. Hemagglutination graded from negative (-) to 4+. Autocontrols are also shown and were always negative.

**Table 2**

Renal transplant recipient tube and gel test major (plasma) crossmatch results for the cats before and after transfusion.

Red cells	<u>Recipient Plasma Before Transfusion</u>		<u>Recipient Plasma After Transfusion</u>	
	Tube	Gel test	Tube	Gel test
Transplant recipient	–	–	–	–
Donor X	1+	3+	2+/3+	3+
Donor Y	2+	2+	2+/3+	3+
Donor Z	weak	3+	2+	3+
Donor 1	–	–	–	–
Donor 2	–	–	–	–