Original Study

Comparison of a commercial blood cross-matching kit to the standard laboratory method for establishing blood transfusion compatibility in dogs

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Abstract

Objective – To evaluate the accuracy of a commercial blood transfusion cross-match kit when compared to the standard laboratory method for establishing blood transfusion compatibility.

Design – A prospective observational in intro study performed from July 2009 to July 2013.

Setting – Private referral veterinary center.

Animals – Ten healthy dogs, 11 anemic dogs, and 24 previously transfused dogs.

Interventions – None.

Measurement and Main Results – Forty-five dogs were enrolled in a prospective study in order to compare the standard blood transfusion cross-match technique to a commercial blood transfusion cross-matching kit. These dogs were divided into 3 different groups that included 10 healthy dogs (control group), 11 anemic dogs in need of a blood transfusion, and 24 sick dogs that were previously transfused. Thirty-five dogs diagnosed with anemia secondary to multiple disease processes were cross-matched using both techniques. All dogs cross-matched via the kit had a compatible major and minor result, whereas 16 dogs out of 45 (35%) had an incompatible cross-match result when the standard laboratory technique was performed. The average time to perform the commercial kit was 15 minutes and this was 3 times shorter than the manual cross-match laboratory technique that averaged 45–50 minutes to complete.

Conclusions – While the gel-based cross-match kit is quicker and less technically demanding than standard laboratory cross-match procedures, microagglutination and low-grade hemolysis are difficult to identify by using the gel-based kits. This could result in transfusion reactions if the gel-based kits are used as the sole determinant of blood compatibility prior to transfusion. Based on our results, the standard manual cross-match technique remains the gold standard test to determine blood transfusion compatibility.

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Keywords: blood incompatibility, hemolytic reaction, point-of-care tests, transfusion reaction

Abbreviations

pRBC	packed red blood cells
PTG	previously transfused group
TG	transfusion group

Introduction

Blood transfusion in critically ill animals has increased tremendously since the 1980s; it has become a standard of care in patients with life threatening anemia.[1–4] The most common causes of anemia leading to transfusion include red blood cell destruction, decreased red blood cell production, or whole blood loss such as encountered during invasive surgical procedures.[1–4] Blood transfusion is not a benign procedure. Transfusions carry the risk of transmission of infectious diseases and may promote fluid overload or trigger adverse reactions.[1–8] These reactions include acute lung injury and hemolytic red

blood cell destruction, among others.[1–9] Most transfusion reactions can be avoided by using proper technique for collection and storage of donor blood, appropriate donor selection, selecting the appropriate blood type for the recipient and by cross-matching the donor and recipient appropriately.[6]

The blood cross-match detects the serologic compatibility between the recipient and the potential blood donor.[1,2,9] The goal of the test is to identify the presence or absence of alloantibodies in dogs in the effort to reduce the potential for adverse transfusion reactions. Adverse transfusion reactions are most commonly seen in cats and previously transfused dogs.[1] Dogs do not have naturally occurring alloantibodies; therefore, a dog that has never been transfused does not necessarily require a cross-match.[1,6] Blood cross-match is recommended in previously transfused dogs or dogs with an unknown transfusion history. Cats have naturally occurring alloantibodies; therefore, blood type and cross-match are recommended prior to administrations of blood transfusions. When blood typing is not available a cross-match can detect an A-B mismatch.

There are 2 different parts of the cross-match: the major and minor crossmatch.[1,9] The major cross-match tests for alloantibodies in the recipient's plasma against donor red cells and the minor cross-match tests for alloantibodies in the donor's plasma against the recipient's red blood cells. The presence of agglutination on either test implies that the recipient is not compatible to the donor's red blood cells (major) or to the donor's plasma (minor).[1,10] Incompatibility to a major or minor, cross-match is seen when macroaggluitination, microagglutination, or hemolysis are present. The presence of macroagglutination and high-grade hemolysis precludes the use of the donor's red blood cell. In contrast, the presence of microagglutination may not necessarily indicate that the patient will have an adverse transfusion reaction.[8]

A new commercially available cross-match kit[a] utilizes gel tube technology and centrifugation. This technique works by trapping agglutinates within the matrix, while allowing free red cells to sink to the bottom during centrifugation, making it easier for the operator to determine the compatibility between donors and recipients. As the samples are mixed and centrifuged, if agglutination occurs, the clumped cells remain suspended in the matrix, while compatible cells travel completely through the gel precipitating at the bottom. There are kits for both minor and major cross-match. Each package contains a series of tubes (red, blue, green, and yellow) that are used as reactive tubes and control tubes (Figure 1). The results are interpreted based on accumulation of red blood cells on the top of the tube (positive) or if the red blood cells sink to the bottom (negative) (Figure 2).[3]

Figure 1: The gel-based cross-matching kit used in the study contains 7 different tubes including blood prep tube (blue top), red positive control (red top), green negative control (green top), and yellow reactive control (yellow top), clear positive control (red +), clear negative control (green), and clear reaction control (yellow R).

Figure 2: Depiction of cross-match results using the gel-based cross-match kit. The red label tube (positive control) shows an accumulation of red cell at the top of the matrix. The green label tube (negative control) shows an accumulation of cells at the bottom of the tube. The center tube (yellow) is the patient sample, which reveals accumulation of the red blood cells at the bottom of the tube that are consistent with a negative result, which implies a compatible cross-match.

The standard laboratory cross-match technique is considered the gold standard method but requires expertise, is time consuming, and its reliability has been shown to be operator dependent.[1–3] The gel-based cross-match kit evaluated in the current study is quick and easy to perform and requires less blood (1–2 mL) than a standard cross-match technique (3–4 mL). In addition, reactions are stable and the tubes can be reviewed by multiple people at a later time when using the gel-based cross-match kit.[2,3] The gel-based cross-match kit purportedly can be used even if the patient is auto-agglutinating,[2] whereas the standard cross-match can be difficult to interpret in animals with auto-agglutination. With shorter turnaround time to perform the cross-match procedure, smaller samples needed, and ease of interpretation, the gel-based cross-match kit is an appealing alternative to the standard cross-matching technique.

The purpose of this study was to compare the reliability of a commercial gel-based cross-matching kit to standard laboratory cross-match method and to examine the reliability of these assays when tested on samples obtained from healthy dogs, dogs previously transfused, and dogs with clinical anemia. We hypothesized that the standard laboratory cross-match results would correspond with the gel-based cross-match kit technique.

Materials and Methods

In this study, 3 different groups of dogs were initially evaluated (a control group, a transfusion group, and a previously transfused group, PTG). For each group the patient breed, age, sex, and weight were obtained. The first group consisted of 10 staff-owned healthy dogs with no evidence of disease and these dogs were used as a control group. The second group of dogs consisted of 11 dogs with anemia that were in need of a blood transfusion (transfusion group, TG) and the third group consisted of 24 dogs that were previously transfused but that were not in need of a transfusion (PTG). All blood samples were collected at the research institution with exception of 10 dogs in the PTG in which blood samples were collected at 2 local referral institutions.

Data collection for the TG also included date of transfusion, amount of packed red blood cells (pRBC) transfused and number of transfusions. Animals that received a pRBC transfusion were monitored closely for any sign of an acute reaction (eg, tachycardia, tachypnea, hyperthermia, and vomiting). All animals transfused were administered Dog Erythrocyte Antigen (DEA) 1.1 negative pRBC. Transfusions were administered using standard hospital protocol with monitoring performed every 15 minutes at the start of transfusion. Monitoring was tapered if there were no adverse reactions noted. If signs of transfusion reaction were noted, the transfusion administration was altered by the clinician of record. Owner consent was obtained for all dogs enrolled in the study.

Cross-matching

Blood samples were collected from each dog in the same manner regardless of group or disease process. For the standard laboratory cross-match technique, 4 mL of whole blood was collected and placed into an EDTA tube. Each sample was cross-matched (major and minor) to a single canine sample that was known to be DEA 1.1 negative using the standard laboratory cross-match method.[9] The degree of agglutination was graded from 1+ to 4+ (Table 1). In the anemic group, animals were cross-matched to the unit of blood they were to receive. Determination of transfusion compatibility was made based on results of the commercial cross-match kit as was hospital protocol at the time of the study.

Table 1: Agglutination scale used in the laboratory method of determining cross-match compatibility

Agglutination	Description
4+	One solid clump of cells
3+	Several large clumps of cells
2+	Medium size clumps of cells with a clear background
1+	Hemolysis, no clumping of cells
Negative	Negative for hemolysis: negative for clumping of red blood cells

For the gel-based cross-match kit, a total of 2 mL of whole blood was collected from the recipient and placed into an EDTA tube. Each sample was cross-matched using the commercial gel-based canine cross-match kit instructions (major and minor) to a single canine sample that was known to be DEA 1.1 negative.

Cross-match interpretation was performed by hospital staff. Clinicians only performed the standard cross-match procedure, while the technical staff involved in the study performed the cross-match kits. A separate investigator performed the gel-based cross-match in order to limit bias. Each investigator was blinded to the results of the alternative method until the results were completed and interpreted. Results of the cross-matches were then recorded and the data were reviewed.

Due to the poor agreement between the standard laboratory cross-match and the cross-match kit during initial data analysis, a subgroup of the PTG was created. This subgroup was composed of 4 previously transfused dogs that were healthy at the time of the cross-matches. These dogs were cross-matched using both techniques to a single canine sample that was known to be DEA 1.1 positive. The goal of this last step was to increase the likelihood of cross-match reaction by introducing new antigens and thus determine the sensitivity of the kit versus the standard cross-match. Dogs included in the PTG were cross-matched 3 weeks to 6 years postinitial transfusion.

Results

Forty-five dogs were enrolled in the study between July 2009 and July 2013 including 18 neutered males and 27 spayed females. The control group was composed of the following breeds mixed breed ($n = 3$), Chihuahua ($n = 2$) and Pit Bull, Australian Cattle Dog, Miniature Schnauzer, Golden Retriever, and Doberman Pinscher ($n = 1$). The group in need of transfusion and the previously transfused dogs were composed of the following breeds Shih Tzu ($n = 5$), Terrier Mix ($n = 4$), Cocker Spaniel ($n = 3$), Border Collie, and Beagle ($n = 2$) and 1 each of a variety of other breeds. The median age of

dogs enrolled was 74 months (10–156 mo). The median weight was 18 kg (3–40 kg).

The anemic animals presented for a variety of clinical conditions. Disease processes encountered during the study included immune mediated hemolytic anemia (IMHA) ($n = 22$), blood loss during surgery ($n = 4$), aplastic anemia ($n = 3$), hemoabdomen ($n = 3$), zinc toxicity ($n = 2$), and disseminated intravascular coagulation (DIC) ($n = 1$).

All dogs included in the study had a compatible cross-match result on the gel-based cross-match kit, whereas 7 out of 11 (63%) in dogs in need of a blood transfusion and 9 out of 24 (38%) previously transfused dogs had a noncompatible standard laboratory cross-match result. The gel-based cross-match kit took under 17 minutes to be performed, whereas the manual cross-match took closer to 60 minutes. The time difference to perform the cross-matches was statistically significant between the 2 techniques.

Out of these 7 dogs in need of a blood transfusion that had an incompatible cross-match 2 were noncompatible to the manual major cross-match but compatible to the manual minor cross-match, 2 were noncompatible to the major and minor cross-match, and the rest of the dogs were noncompatible to the manual minor only. Three of these dogs were in need of blood products due to immune-mediated hemolytic anemia, 2 due to zinc toxicity, and 2 dogs were with hemoabdomen.

In this group of dogs in need of a blood transfusion the major cross-match revealed that 3 dogs had 2+ microscopic agglutination and 1 dog had 1+ microscopic agglutination. None of these dogs had macroscopic agglutination (Table 2). The minor cross-match revealed that 1 dog had 1+ macroscopic agglutination and 3 dogs had 1+ microscopic agglutination. Out of these 7 dogs 3 had 1+ hemolysis in the major and minor cross-match wells, whereas 1 dog had 1+ hemolysis in the minor cross-match well only (Table 2). In this group 1 dog with compatible standard laboratory and kit cross-match had 1+ hemolysis in the donor control well. Three dogs also had 1+ hemolysis in the recipient control well.

Out of the 8 dogs previously transfused that had an incompatible cross-match results, 7 were noncompatible on the manual major cross-match and 1 dog was incompatible to the manual minor cross-match. In this group the major cross-match revealed 1 dog had a 1+ macroscopic and 1+ microscopic agglutination, whereas the other 6 dogs had microscopic agglutination only ranging from 1+ to 2+ (Table 3). The minor cross-match showed 1 dog had 1+ microscopic agglutination. Out of these 8 dogs 2 dogs had 1+ hemolysis in both major and minor control wells (Table 3). None of the remaining 7 dogs had minor macroscopic agglutination. All dogs in this group had anemia due to IMHA except for 1 that was di-

Table 2: Major and minor cross-match results for dogs that required a blood transfusion

Manual major cross-match			Manual minor cross-match			Control results	
Macroagglutination	Microagglutination	Hemolysis tube	Macroagglutination	Microagglutination	Hemolysis tube	Hemolysis donor well	Hemolysis recipient well
—	2+	—	—	—	—	—	—
—	—	—	—	1+	1+	—	1+
—	1+	1+	1+	—	1+	—	1+
—	2+	1+	—	—	1+	—	1+
—	—	—	—	1+	—	—	—
—	—	—	—	1+	—	—	—
—	2+	1+	—	—	1+	1+	1+

Table 3: Major and minor cross-match results for dogs that were previously transfused

Manual major cross-match			Manual minor cross-match			Control results	
Macroagglutination	Microagglutination	Hemolysis	Macroagglutination	Microagglutination	Hemolysis	Hemolysis donor well	Hemolysis recipient well
–	2+	1+	–	–	–	–	–
–	2+	–	–	–	–	–	–
–	2+	1+	–	–	–	–	–
–	–	1+	–	1+	1+	1+	1+
–	2+	–	–	–	–	–	–
–	1+	–	–	–	–	–	–
–	2+	–	–	–	–	–	–
1+	1+	1+	–	–	1+	–	–

agnosed with red blood cell aplasia. None of these dogs had evidence of disease or were anemic at the time of the study.

In the subgroup of dogs that were previously transfused that were cross-matched to known DEA 1.1 positive blood, only 1 dog revealed a 2+ macroscopic agglutination and 3+ microscopic agglutination to the standard laboratory major cross-match. The standard laboratory minor cross-match revealed 2+ macroscopic agglutination and 3+ microscopic agglutination; 2+ hemolysis was observed in both control wells. This dog previously diagnosed with IMHA had a normal hematocrit with no evidence of auto-agglutination at the time of the cross-match. The rest of dogs in this subgroup had a compatible standard laboratory and kit cross-match with no signs of hemolysis.

Immune-mediated hemolytic anemia was present in 22 dogs and 10 of these dogs had a noncompatible cross-match. Three of these dogs had an incompatible major and minor standard laboratory cross-match whereas 7/10 had an incompatible major standard laboratory cross-match only. Out of these 10 dogs three had 1+ macroagglutination and 1 had 2+ microagglutination on the standard laboratory recipient control despite repeated saline washes.

There was no correlation between breed or age and the results of the cross-match. Out of the 16 dogs that had a cross-match reaction 13 were female and 3 were males. Three dogs in the PTG received multiple transfusions during hospitalization. Out of these, 1 dog had a major manual noncompatible cross-match result. All the other dogs with incompatible cross-matches had received only 1 transfusion.

Only 1 dog of the 24 dogs that received a transfusion had clinical signs consistent with an adverse reaction (fever, tachypnea, and tachycardia). The blood transfusion rate was decreased and due to persistent clinical signs the transfusion was discontinued. This dog had a compatible result to the standard laboratory and kit cross-match.

Discussion

When critical patients in need of blood products present to the hospital, delay in administration of appropriate blood products is detrimental to their condition and potentially to their survival.[1] Rapid assessment and stabilization play a key role in survival, response to treatment and length of hospitalization. As blood products become more common in veterinary medicine there is an increased need to perform a rapid cross-match and to reduce the risk of adverse transfusion reactions.

Blood transfusions are commonly used in the critical care unit, and although this therapy is lifesaving, it can be associated with immunologic and nonimmunologic

reactions such as acute immune-mediated hemolysis, febrile nonhemolytic reactions, allergic reactions, transfusion-related acute lung injury, transfusion-associated sepsis, transfusion-associated circulatory overload, and delayed transfusion reactions.[2–4] These factors make pretransfusion cross-matching essential in previously transfused dogs. Cross-matching is only one aspect of good quality transfusion medicine and other aspects include blood type compatibility, infectious disease screening, adequate blood collection, processing, storage techniques, and close patient monitoring during transfusion.[6]

To the authors' knowledge this is the first study that compared a commercial gel-based cross-match kit to the standard laboratory cross-match method. In this study statistical comparison of the manual cross-match to the gel-based kit was not possible as all the samples tested via this method were compatible. As the manual cross-match was used as the gold standard, this suggests that all samples that were incompatible on manual cross-match were falsely compatible on the gel-based commercial kit.

Sixteen dogs out of 45 (35%) had some degree of agglutination that was not observed on the commercial cross-match kit; suggesting there was not good agreement for the results between the standard laboratory cross-match and the commercial kit cross-match. Discrepancies have been reported between the manual cross-match and a gel-based cross-match kit.[5] In a previous study evaluating the lack of Dal antigen in Dalmatians, Blais et al reported an overall agreement between a gel-based cross-match kit (DiaMed Gel Column Technique) and standard cross-match when the agglutination was 3+ or 4+, cross-matches that showed an agglutination of 1+ (9 out of 80 dogs) were positive on the standard tube technique and negative on the gel-based cross-match.[5] These data are supported by the current study results.

In the current study few dogs had macroscopic agglutination and when present it was mild. The majority of incompatible samples were detected with microscopic agglutination only. With the presence of microscopic agglutination in most of the incompatible cross-matches, the small red cell agglutinate makes its way through the gel matrix moving to the bottom of the tube and the lack of presence of red blood cell within the matrix is interpreted as a compatible result. We suspect that if a more strongly incompatible degree of agglutination was present, the kit may have been able to identify the incompatibility; however, the smaller degree of microscopic agglutination could not be detected by the kit during this study. Additional studies would be needed to confirm this suspicion.

When comparing the data among groups we noticed that 7/11 (63%) of the dogs in need of a blood transfusion

and 9/24 (38%) of previously transfused dogs had a non-compatible standard laboratory cross-match. One would expect that previously transfused dogs would have had a greater incompatibility results due to the presence of alloantibodies. The most likely explanation for this discrepancy is that 5 of 7 dogs in the group in need for a blood transfusion had an active hemolytic process and 3 of those 5 were actively agglutinating due to their underlying disease, whereas the posttransfused dogs had no evidence of agglutination or hemolysis at the time of the standard laboratory cross-match. Of the dogs previously transfused 1 dog had 1+ hemolysis in the control well and recipient well, and this was thought to be secondary to blood collection or sample handling. If we eliminate this dog from the study it will decrease the noncompatible percentage from 38% to 33%. Similarly, if we eliminate the 5 dogs in need of a blood transfusion that had persistent agglutination or hemolysis, the number of cases decreases to 2/6 (33%).

In the present study, only 1 dog had clinical signs consistent with adverse transfusion reaction, despite a compatible manual cross-match and kit cross-match. This dog was diagnosed with immune-mediated hemolytic anemia and had received a pRBC unit 4 days prior without incident. This adverse reaction could have been secondary to infectious diseases, blood processing, chemical blood components, and storage or host reaction to leukocytes or plasma proteins.

All blood products utilized in this study were purchased from a certified veterinary blood bank. This blood bank utilizes donors free of infectious diseases. Storage could have been a factor for the adverse reaction, but this is considered less likely. The practice maintains the blood products in an upright position in a dedicated refrigerator, with tight temperature control, and the blood is inspected weekly for evidence of impropriety (eg, expiration date, blood separation). Leukoreduction may have prevented this adverse transfusion reaction.

In an attempt to increase the likelihood of agglutination, and to further test the sensitivity of the cross-match kit a separate subgroup was created. This subgroup consisted of 4 previously transfused dogs that were cross-matched to a DEA positive blood. Unfortunately, 1 dog had severe hemolysis in both control wells, and this was likely secondary to venipuncture or sample handling. The remaining dogs had a normal cross-match to both techniques. This subgroup was not included in the statistical analysis.

One of the clinical manifestations of IMHA is auto-agglutination. The major and minor manual standard laboratory cross-match uses agglutination as the endpoint for an incompatible result. Persistent auto-agglutination in certain cases cannot be abolished by

triple saline wash of red blood cells. This was observed in 4 of the 16 dogs, and this may have influenced the results obtained. It is possible that these cases with IMHA were truly compatible and due to the agglutination secondary to the disease process were interpreted as incompatible in the standard laboratory cross-match. If these animals are eliminated from analysis due to the persistent agglutination, the false negative value decreases to 26%. Nonetheless, this remains a significant amount of incompatible results.

Hemolysis was found in 9 out of 16 dogs during the standard laboratory cross-match. This hemolysis could be secondary to sample collection or cross-match reaction and agglutination. Hemolysis is observed in patients with IMHA and zinc toxicity; therefore, the hemolysis may have been a result of the underlying disease process and not an incompatible cross-match.

In human medicine the gel-based cross-matching technique has proven to have a sensitivity and specificity for potential antibodies of 92% and 96%, respectively.[12] In our study all samples tested on gel-based cross-matching were compatible regardless of disease or previous transfusion; therefore, a comparison cannot be made. However, based on results of the manual cross-match and previous studies, the kit does not appear to be in agreement with gold standard testing in many instances. This incompatibility allows for possible transfusions with incompatible blood products and may lead to a higher incidence of transfusion reactions. Compatible major cross-match, minor cross-match, or both does not guarantee normal RBC survival or completely eliminate the risk of the transfusion.[2] Cross-matching does not prevent transfusion reactions or leukocyte/plasma reactions.[2] Signs consistent with leukocyte/plasma reaction were observed in 1 dog in the current study.

Although transfusions reactions were uncommon in the current study, cross-match incompatibilities were noted. While incompatibility was detected, it was deemed minor and this may have been tolerated by the animals receiving the blood products. No evaluation regarding the need for future transfusion or red blood cell survivability was performed. It may be that in dogs in the study with incompatible results, transfusion reactions were delayed and not initially detected. This information would be important in future studies and imperative in the potential for use of the kit in future clinical venues.

This study had certain limitations. The study population included a low number of anemic dogs. Had a larger group of dogs been evaluated, additional macroagglutination and noncompatible results in the gel-based cross-match kit may have been detected. This would have potentially allowed further statistical

analysis and evaluation of the gel-based cross-match kit technique. Also, no dogs in the current study were blood typed prior to transfusion. This information may have been useful for administration of blood products and for cross-match purposes. Also, as stated above, no follow-up or outcome studies were performed that may also provide useful information.

For the study reported here, we concluded that the commercial gel-based cross-match kit is not suitable to detect major or minor microscopic agglutination. While the kit has a potential role in the use of screening for compatibility it should not be used as a sole method of blood compatibility assessment. In order to better determine this kit's reliability and accuracy, further studies with gross macroscopic agglutination should be performed and larger number of dogs evaluated. The presence of hemolytic disease may influence the results obtained on the manual cross-match or gel-based cross-match kit; therefore, caution should be exercised when using the kit in cases with hemolytic anemia.

Footnote

a   RapidVet-H Companion Animal Cross-match – Major and Minor. DMS Laboratories, Inc., Flemington, NJ.

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