

# Accuracy of point-of-care crossmatching methods and crossmatch incompatibility in critically ill dogs

Hayden Marshall<sup>1</sup> | Shauna L. Blois<sup>1</sup>  | Anthony C. G. Abrams-Ogg<sup>1</sup> |  
 Alexa M. Bersenas<sup>1</sup> | Kristiina Ruotsalo<sup>2</sup> | Gabrielle Monteith<sup>1</sup>

<sup>1</sup>Department of Clinical Studies, University of Guelph, Guelph, Ontario, Canada

<sup>2</sup>Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada

#### Correspondence

Shauna L. Blois, 50 Stone Road East, Guelph, ON N1G 2W1, Canada.  
 Email: sblois@uoguelph.ca

#### Funding information

Ontario Veterinary College Pet Trust

## Abstract

**Background:** The performance of commercial point-of-care crossmatch (CM) tests compared to laboratory tube agglutination CM is unknown. Additionally, there is limited information regarding CM incompatibility in ill dogs.

**Objectives:** To determine if point-of-care major CM methods are accurate in detecting compatible and incompatible tests when compared to laboratory CM methods, and to identify factors associated with CM incompatibility in dogs.

**Animals:** Part 1 (prospective) included 63 client-owned dogs potentially requiring blood transfusion. Part 2 (retrospective) included all dogs from part 1, plus medical records of 141 dogs with major CM results.

**Methods:** For part 1, major CM was performed using a tube agglutination assay (LAB-CM), a gel-based point-of-care test (GEL-CM), and an immunochromatographic point-of-care test (IC-CM). For part 2, medical record data were collected to determine rates of and risk factors for CM incompatibility.

**Results:** Kappa agreement between the LAB-CM and GEL-CM methods could not be calculated due to a relative lack of incompatible results. Kappa agreement between the LAB-CM and IC-CM methods was 0.16 (95% confidence interval [CI] = 0-0.31,  $P = .007$ ) indicating no agreement. The LAB-CM incompatibility in transfusion-naïve vs dogs that had a transfusion was 25% and 35%, ( $P = .3$ ).

**Conclusions and Clinical Importance:** Compared to laboratory methods, point-of-care methods evaluated in our study lacked sensitivity for detecting incompatibilities. Dogs had similar rates of major CM incompatibility regardless of transfusion history. This suggests CM testing prior to transfusion be considered in all dogs however our study did not investigate clinical relevancy of incompatible LAB-CM.

## KEYWORDS

anemia, blood compatibility, canine, hemolysis, transfusion medicine

**Abbreviations:** CM, crossmatch; DEA, dog erythrocyte antigen; GEL-CM, gel-based point-of-care crossmatch; IC-CM, immunochromatographic point-of-care crossmatch; LAB-CM, laboratory tube agglutination crossmatch; RBC, red blood cell.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. *Journal of Veterinary Internal Medicine* published by Wiley Periodicals LLC, on behalf of the American College of Veterinary Internal Medicine.

## 1 | INTRODUCTION

Blood transfusions are an important tool in the treatment of critically ill anemic dogs. Blood transfusions can be lifesaving; however, they are not without risks and transfusion reactions can occur. Transfusion reactions in dogs range from 3% to 28%, with the most common reaction being a febrile nonhemolytic reaction.<sup>1-5</sup>

The risk of immunologic transfusion reactions can be decreased by compatibility testing before transfusion including blood typing and crossmatch (CM) testing.<sup>6-8</sup> Blood typing to detect the presence or absence of dog erythrocyte antigen (DEA) 1 is frequently performed before transfusion. Red blood cell (RBC) antigens other than DEA 1 exist, and incompatibilities related to these antigens can cause transfusion reactions.<sup>2,9,10</sup> Immunologic compatibility can be detected through CM.<sup>11</sup> A major CM test is used to detect recipient antibodies against potential donor RBC antigens. A minor CM test is used to detect antibodies in the donor serum against the recipient's RBC antigens. The tube agglutination assay performed in a reference laboratory is considered the gold standard CM method.<sup>12-14</sup> If agglutination, hemolysis, or both are present, the donor-recipient pairing is considered incompatible.<sup>12</sup> The test is time-consuming, not always accessible in an emergency, and its reliability depends on the expertise of the operator.<sup>12</sup>

While CM helps assess immunological compatibility between donor and recipient, it is not routinely performed in all dogs before transfusion. Most hospitals only perform CM in certain situations, such as in dogs with a history of blood transfusion.<sup>15</sup> However, 1 study found major CM incompatibilities in 17% of dogs that have never received a blood transfusion.<sup>16</sup> Moreover, there are naturally occurring antibodies against various DEA types making incompatibilities to non-DEA 1 blood antigens potentially clinically important.<sup>9,17</sup>

A point-of-care CM test would allow for rapid results. The currently available point-of-care tests use either gel-based or immunochromatographic methods. Recent studies had conflicting results with respect to the agreement of the reference laboratory-based CM method compared to the gel-based kit.<sup>11,18</sup> Another study reported that the immunochromatographic CM kit had high agreement with a gel test card technique; both CM methods in that study used the same anti-canine antiglobulin reagent.<sup>19</sup>

The first objective of our study was to compare the results of 2 point-of-care major CM tests and a major CM performed by a reference laboratory in a group of dogs potentially requiring blood transfusion. The second objective was to report the incidence of, and risk factors for, a major laboratory CM incompatibility in recipient dogs with and without a previous history of transfusion.

## 2 | MATERIALS AND METHODS

### 2.1 | Part 1

Dogs were prospectively enrolled in the observational study between June 2016 and February 2018 at the Ontario Veterinary College Health Sciences Centre. To be eligible for inclusion, the dog had to be

considered by the attending clinician to be a possible RBC transfusion candidate either due to moderate-marked anemia or due to a planned invasive procedure with the potential for blood loss. Informed client consent was obtained before enrolment and institutional animal care approval was obtained before the study onset. Dogs were excluded if sufficient blood volume for at least 1 of the point-of-care CM tests was not obtained, or if body weight was <4 kg. All dogs had a major CM using a laboratory tube-based method (LAB-CM), and 1 or both of a gel-based (GEL-CM; Rapid-Vet Companion Animal Major Crossmatch, DMS Laboratories, Inc, Flemington, New Jersey) and immunochromatographic (IC-CM; Quick Test XM Canine, Alvedia, Lyon, France) point-of-care CM kit.

### 2.1.1 | Laboratory crossmatch method

Major LAB-CM was performed by a reference laboratory (Animal Health Laboratory, Guelph, Canada). Hemolysis of the serum sample upon arrival at the lab was subjectively graded as negative, mild, moderate, or marked. Then, donor RBCs were washed 3 times with phosphate-buffered saline (PBS). 5% RBC suspensions were added to aliquots of recipient serum in 2 separate tubes and centrifuged at low speed (2350-2450 rpm; Sero-fuge 2002, BD Diagnostics, Mississauga, Canada). After incubation of 1 tube for 30 to 60 minutes at room temperature and the second at 37°C, the samples were centrifuged at low speed and assessed for macroscopic agglutination or hemolysis. The sample previously incubated at 37°C was washed again in PBS, canine-specific antiglobulin (Polyvalent Antisera, Canine Coombs Reagent, Veterinary Medical Research and Development, Pullman, Washington) was added, and the tube was then incubated for an additional 30 minutes at 37°C. After each low-speed centrifugation in the CM protocol, each tube was assessed for macroscopic agglutination (graded 0 to 4+) and hemolysis, presence of either was considered an incompatible reaction. Hemolysis in the reaction tube was not routinely graded but reported as present if subjectively greater compared to the control tube; some hemolytic reactions were subjectively graded as mild (1+), moderate (2+), or marked (3+). Macroscopic agglutination was graded on a 0 (absent) to 4+ scale based on appearance after manual agitation of the tube: 1+, red blood cell button breaks into many small fragments with a cloudy background; 2+, button breaks into many medium-sized fragments with a slightly cloudy background; 3+, button breaks into 4 to 6 fragments with a clear background; 4+, button breaks into 2 to 3 fragments after being dislodged from tube wall with a clear background. If agglutination or hemolysis were not detected in the preceding steps, a portion of the cell suspension from each tube was transferred to a microscope slide and the cells were observed as they moved freely across the microscopic field of view. Presence of microscopic agglutination from reaction tube samples was compared to that in control tubes. If microscopic agglutination from reaction tubes subjectively exceeded that from control tubes, it was reported as positive and further subjectively characterized as rare, occasional, or frequent clumps of RBCs. Control tubes containing washed donor RBC suspensions and PBS were subjected to the same process.

### 2.1.2 | Point-of-care crossmatch tests

Point-of-care GEL-CM and IC-CM major CM tests were performed according to manufacturers' instructions. For the GEL-CM, 100  $\mu$ L of donor whole blood was diluted with 900  $\mu$ L of saline. A 100  $\mu$ L aliquot of diluted donor blood was incubated with 200  $\mu$ L of recipient serum for 5 minutes at room temperature. After incubation, 50  $\mu$ L of the sample was added to the gel tube and centrifuged (Horizon easy-spin 12, Drucker Diagnostics, Inc, State College, Pennsylvania) at 3150 rpm for 5 minutes until a cumulative 6500G was achieved. Results were interpreted according to a guide provided by the manufacturer. Briefly, reactions were considered incompatible if the RBC suspension was at or near the top of the gel column, or if the RBC pellet was at the bottom of the tube but extended >50% up the gel column. In the latter case, a ruler was used to determine if the extension exceeded 50% of the gel column. For the IC-CM, 3 drops of buffer, 120  $\mu$ L of recipient serum or plasma, and 10  $\mu$ L of donor blood were incubated for 10 minutes at room temperature. The sample was washed 3 times using wash buffer, then centrifuged for 2 minutes at 2300 rpm. After that, 2 drops of a second buffer were added to the sample, and the immunochromatographic test membrane was placed into the sample tube. Results were read from this membrane within 2 to 5 minutes, according to the interpretation guide provided by the manufacturer. Briefly, presence (incompatible) or absence (compatible) of a line at the test site was recorded after the sample completely migrated across the test membrane.

### 2.2 | Part 2

To determine the incompatibility rates of both naïve and previously transfused dogs, the same dogs enrolled in part 1 of the study were also enrolled in this part of the study. To increase the numbers of dogs evaluated in the previously transfused group, the laboratory database of any dog with a laboratory-based major CM test during the period of January 2013 to May 2018 were retrospectively reviewed. Although the prospective and retrospective periods overlapped, individual dogs were enrolled only once. Medical records were reviewed to confirm transfusion history. Any dog that had been previously transfused with 1 or more DEA 1-type matched blood products >3 days prior to major CM was eligible for inclusion in the retrospective analysis of historical incompatibility rates in previously transfused dogs.

All dogs were blood-typed with a DEA 1 immunochromatographic cartridge test kit (Quick Test DEA 1, Alvedia, Lyon, France). Blood types were categorized as either DEA 1 negative, DEA 1 positive, or DEA 1 weak positive. For statistical analysis, the DEA 1 positive and weak positive dogs were grouped together. Dogs were divided into transfusion-naïve dogs that had never previously received a blood transfusion, and dogs that had previously been transfused >3 days prior. Dogs with anemia were subdivided into the following categories based on the cause of their anemia: blood loss, RBC destruction, or decreased RBC production. Nonanemic dogs were also included when blood loss was anticipated due to a planned intervention. Blood loss was defined as any dog with identified gross hemorrhage (eg, surgical,

traumatic, or other) and a concurrent reduction in total solids and hematocrit or PCV. Red blood cell destruction was identified as any dog with evidence of 2 or more of: hemoglobinemia/hemoglobinuria, hyperbilirubinemia/bilirubinuria in absence of liver disease; spherocytes; ghost cells; schistocytes; positive slide agglutination test or positive direct antiglobulin test. Decreased production was defined as any case with non-regenerative anemia without signs of blood loss or peripheral blood destruction, or multiple cytopenias, or diagnosis of bone marrow level disease on bone marrow cytology or histopathology.

### 2.3 | Sample size calculation

#### Part 1

There were no published reports describing the specificity of the point-of-care CM tests compared to the LAB-CM at the onset of the study. Therefore, an anticipated specificity of 90% was used to calculate the sample size. In addition, a 15% to 20% overall CM incompatibility rate at the study institution was anecdotally estimated. This rate represented a conservative estimate of the CM incompatibility across both naïve and previously transfused dogs. Using these anticipated values, a sample size calculation to test the accuracy of the point-of-care vs laboratory CM methods indicated that at least 60 unique donor-recipient pairings were required to evaluate the agreement between the CM methods. Before the onset of the study, the investigators aimed to recruit 60 unique transfusion recipients as each would have at least 1 donor-recipient pairing for CM testing performed.

#### Part 2

To compare the rates of CM incompatibility between transfusion-naïve dogs and those with a previous history of transfusion, the sample size was determined by using an anticipated incompatibility rate of 10% to 20% (transfusion-naïve) vs 30% to 40% (previously transfused),  $\alpha$  of .05 and  $\beta$  of .8. Anticipated sample sizes ranged from 32 to 119 individuals per group depending on differences in rates between the populations. A convenience sample of 141 dogs was used for the retrospective analysis of CM incompatibilities.

### 2.4 | Statistical analyses

Descriptive statistics were calculated using mean and SD (or median and range, for non-normally distributed values) using a commercial software product (Excel, Microsoft Office 365, 2016; Microsoft, Inc, Redmond, Washington). The remainder of the statistical analysis was performed using a commercial statistical program (SAS 9.4, SAS Institute, Cary, North Carolina). A general linear binomial mixed model was used to determine if blood type, cause of anemia, PCV before transfusion, or previous transfusion history were significant risk factors for a CM event/reaction. The frequency of transfusion reaction for each

animal was calculated by dividing the number of events by the total number of transfusions. The agreement between the 2 point-of-care CM methods (GEL-CM and IC-CM) and LAB-CM was tested with Cohen's kappa. The level of significance was set at  $P < .05$ .

### 3 | RESULTS

#### 3.1 | Part 1: Comparison of laboratory and point-of-care major crossmatch tests

A total of 162 (97 naïve; 65 previously transfused) unique donor-recipient pairings were evaluated using LAB-CM, 142 (85 naïve; 57 previously transfused) with GEL-CM, and 144 (89 naïve; 55 previously transfused) with IC-CM. Incompatible results were detected in 30/164 (18.3%) LAB-CM, 1/142 (0.7%) GEL-CM, and 3/144 (2.1%) IC-CM pairings (Table 1). There were 24 LAB-CM incompatibilities with a concurrent GEL-CM result, all of which were compatible with the latter method. The GEL-CM detected 1/142 incompatible donor-recipient pairings, and the corresponding LAB-CM and IC-CM results were compatible. Of the 29 CM incompatibilities detected by the LAB-CM method where there was a corresponding IC-CM result, 3 had incompatible IC-CM results. Each of these 3 LAB-CM incompatibilities was characterized by 3+ agglutination reactions after the addition of the antiglobulin reagent (Tables 2 and Table S1). The median number of LAB-CM incompatibilities for each donor in part 1 of the study was 1 (range, 1-2).

Kappa agreement between the LAB-CM and GEL-CM methods could not be calculated due to a relative lack of incompatible results. Kappa agreement between the LAB-CM and IC-CM methods was 0.16 (95% CI = 0-0.31,  $P = .007$ ) indicating no agreement. Compared to the LAB-CM, the GEL-CM sensitivity and specificity to detect an incompatibility were 0% and 99.2%, respectively, while sensitivity and specificity for the IC-CM were 10.3% and 100%, respectively.

#### 3.2 | Part 2: Rates of laboratory major crossmatch incompatibility in naïve and previously transfused dogs

Laboratory major CM incompatibility rates were evaluated in 63 dogs from part 1 and an additional 141 dogs included retrospectively, for a total of 204 dogs. Of this total, 40 dogs were considered naïve to any

previous blood product transfusion while 164 had a history of at least 1 previous whole blood or packed RBC transfusion administered at least 3 days prior. The primary cause of anemia was considered to be RBC destruction in 89/204 (43.6%), blood loss in 59/204 (28.9%), and decreased production in 54/204 (26.4%) of cases; 2/204 (1%) of dogs were not anemic. Recipients were crossmatched against a median of 3 donors (range, 1-3).

Ten out of 40 (25%) transfusion-naïve dogs had at least 1 major LAB-CM incompatibility reaction. In the previously transfused dogs, 57/164 (34.7%) dogs were LAB-CM incompatible with 1 or more donors. The number of dogs having at least 1 LAB-CM incompatibility was not significantly different between transfusion-naïve and previously-transfused dogs ( $P = .3$ , Table 3).

There was a total of 558 donor-recipient pairings. Of these, 157 (28%) were incompatible and 401 (72%) were compatible using the LAB-CM test. Of the incompatible reactions, 138 (87.9%) were graded: 47 (34%) were microscopic, 37 (26.8%) were 1+, 32 (23.2%) were 2+, 21 (15.2%) were 3+, and 1 (<1%) was 4+. Of the 157 incompatible reactions, 146 were classified as either agglutination or hemolysis: 124 (84.9%) were agglutination reactions, 20 (13.7%) were hemolytic reactions, and 2 (1.4%) were both agglutination and hemolysis. One hundred and forty five incompatibility reactions noted the phase of reaction where incompatibility was first detected: 24 (16.6%) were immediate, 28 (19.3%) were after room temperature incubation, 26 (17.9%) were after incubation at 37°C, and 67 (46.2%) were after the addition of antiglobulin reagent (Table S2). There was no significant difference in reaction type between transfusion-naïve and previously transfused and dogs ( $P = .25$ ).

**TABLE 2** Results of the gel-based (GEL-CM) and immunochromatographic (IC-CM) point-of-care major crossmatch tests compared to the laboratory tube agglutination crossmatch method (LAB-CM) in a group of dogs

	GEL-CM		IC-CM	
	+	-	+	-
LAB-CM +	0	24	3	26
LAB-CM -	1	117	0	115

Abbreviations: GEL-CM, gel-based point-of-care crossmatch; IC-CM, immunochromatographic point-of-care crossmatch; LAB-CM, laboratory tube agglutination crossmatch.

**TABLE 1** Number of compatible and incompatible laboratory, gel-based, and immunochromatographic CM tests for dogs with and without a history of previous blood transfusion

CM method	Compatible pairings, transfusion naïve dogs	Incompatible pairings, transfusion naïve dogs	Compatible pairings, previously transfused dogs	Incompatible pairings, previously transfused dogs	Compatible pairings, all dogs	Incompatible pairings, all dogs
Laboratory CM	78/97 (80%)	19/97 (20%)	55/65 (85%)	10/65 (15%)	134/164 (81.7%)	30/164 (18.3%)
Gel-based CM	84/85 (99%)	1/85 (1%)	57/57 (100%)	0/57 (0%)	141/142 (99.3%)	1/142 (0.7%)
Immunochromatographic CM	89/89 (100%)	0/89 (0%)	52/55 (95%)	3/55 (5%)	141/144 (97.9%)	3/144 (2.1%)

Abbreviation: CM, crossmatch.

**TABLE 3** Number of compatible and incompatible laboratory major CM tests in transfusion-naïve vs previously transfused dogs

	Transfusion-naïve (n = 40)	Previously transfused (n = 164)
Compatible	30	107
Incompatible	10	57

Abbreviation: CM, crossmatch.

Several risk factors for an incompatible major LAB-CM test were identified, including a DEA 1 positive blood type (odds ratio [OR] = 1.63, 95% CI = 1.06-2.51). Dogs with anemia due to destruction were 2.55 (95% CI = 1.47-4.42) times more likely to have an incompatibility compared to dogs with anemia due to decreased production, and were 3.1 (95% CI = 1.71-5.59) times more likely to have an incompatibility compared to dogs with anemia due to blood loss. Dogs with a PCV between 15% and 20% before transfusion had a decreased risk of incompatibility (OR = 0.19, 95% CI = 0.11-0.32), when compared to other PCV values. In those dogs, each unit increase in PCV up to 20% led to an 87.2% decrease in odds of incompatibility.

## 4 | DISCUSSION

A point-of-care kit CM kit would allow for rapid and widespread CM testing, but the accuracy of both point-of-care kits evaluated in the present study raises questions for their use. The GEL-CM method did not detect any of the incompatibilities detected by the LAB-CM method, while the IC-CM method detected 3 of the 29 incompatibilities detected by the LAB-CM method. Considering the LAB-CM method as the gold standard, both point-of-care tests lack sensitivity. This gives rise to the potential of a false negative result and subsequent transfusion using a major CM-incompatible donor. Similarly, a previous study comparing the performance of the major and minor GEL-CM vs a standard reference laboratory method found no GEL-CM incompatibilities compared to a 35% major LAB-CM incompatibility rate.<sup>11</sup> Similar to the present results, another study identified that the major GEL-CM had good agreement with a laboratory method when samples were compatible, but that the GEL-CM had both false positive and false negative results when compared with the laboratory tube method.<sup>18</sup> Additionally, the same study reported that the GEL-CM results was difficult to read 50% of the time due to sample spreading throughout the tube, possibly due to test error introduced by the centrifuge model, angle of rotation, and settings used.<sup>18</sup>

In contrast to the present study, a previous study found complete agreement between the IC-CM kit and a laboratory-based method that used a dextran-acrylamide gel test card method.<sup>19</sup> A possible reason for the discrepancy between that study and the present study's results is that the former study utilized the same anti-canine antiglobulin reagent in both CM methods, while the LAB-CM in the present study used a antiglobulin reagent and the GEL-CM did not have antiglobulin.

It is possible that the LAB-CM method is too sensitive and identifies clinically irrelevant incompatibilities compared to the point-of-care kits. A previous study compared a gel test card method, different from the GEL-CM in the present study, to a laboratory method and found that the gel method was only incompatible when the laboratory method reported strong (3+ or 4+) agglutination reactions.<sup>9</sup> In simulated incompatibilities created by adding anti-canine antiglobulin to RBC suspensions, another study found perfect agreement between the GEL-CM and a laboratory tube method at higher antibody titers, but weaker reactions caused by lower titers were more difficult to identify in both methods.<sup>18</sup> In contrast, in the present study, even pairings resulting in strong LAB-CM incompatibilities consistently resulted in compatible GEL-CM results, while the IC-CM method was able to identify these strong (3+) reactions on only 3 occasions. An additional four 3+ agglutination LAB-CM results had concurrent compatible IC-CM results.

It is difficult to know the grade or type of laboratory CM incompatibility that is predictive of in vivo incompatibility and transfusion reaction. In a recent study of cats, the GEL-CM method gave a lower rate of incompatibility compared to a laboratory method, but study results suggested that the GEL-CM was more clinically relevant than the laboratory method.<sup>20</sup> It is possible that some microscopic or lower grade LAB-CM reactions in the present study are not clinically relevant, and that only more severe reactions, which can sometimes be detected by point-of-care methods, are clinically relevant. In 1 study, dogs transfused with blood that was gel-based major and minor CM compatible but laboratory tube-method incompatible (1+ to 2+) did not show signs of acute transfusion reactions.<sup>11</sup> However, a previous case report described clinically appreciable hemolysis in a dog after receiving 2 separate transfusions using blood that was characterized as trace or 1+ incompatible using a laboratory tube-based major CM test.<sup>10</sup> In another report, a dog displayed acute hemolysis shortly after receiving laboratory tube-based major-CM incompatible blood, although the degree of CM incompatibility was not reported.<sup>7</sup> While tube laboratory methods detect incompatibilities due to either hemolysis or agglutination, both point-of-care kits evaluated in the present study are designed to primarily detect only agglutination, and therefore may be unable to detect an incompatibility due to hemolysis. Previous studies of dogs becoming sensitized to nonself RBC antigens have largely described incompatible CM tests due to presence of agglutination.<sup>6,19</sup> However, in our study and a previous study, hemolysis with or without agglutination was present in some laboratory CM tests and could signify immunologic incompatibility secondary to presence of hemolysin antibodies.<sup>11</sup> Further work is needed to determine the accuracy of point-of-care and laboratory CM methods in predicting clinically relevant incompatibilities. Additionally, the current lack of a standardized laboratory CM method makes it difficult to compare results between studies. Standardization of methods across laboratories could help alleviate this concern.

Based on previous studies, a possible advantage of the point-of-care kits compared to laboratory tube methods is that they might be less influenced by persistent autoagglutination caused by immune mediated hemolytic anemia (IMHA), resulting in fewer false positives



than a laboratory method.<sup>11,19</sup> The effect of persistent recipient RBC autoagglutination could not be investigated in the present study, as only major CM were performed. Further research to determine optimal CM methods in dogs with persistent autoagglutination is warranted.

In the present study, the rate of major CM incompatibility in transfusion-naïve dogs was not significantly different from the rate of incompatibility in previously transfused dogs, and dogs in each group had similar grades of incompatibility reactions. Similarly, a recent study identified that 17% of transfusion-naïve dogs had at least 1 incompatible CM result, while other reports show that up to 44% of dogs became CM incompatible following DEA 1-matched RBC transfusions.<sup>6,16,19</sup> This is in contrast to another report in which all of the 80 naïve dogs evaluated in the study had compatible major CM results.<sup>19</sup> While CM incompatibilities have been detected in transfusion-naïve dogs, the clinical relevance of these findings is not known. Dogs that have not been sensitized to other blood antigens have no, or only low levels of, alloantibodies. In 1 study, rates of alloantibodies to various DEA were: DEA 1, 0.3%; DEA 3, 1.2%, DEA 5, 0.8%, and DEA 7, 9.8%.<sup>21</sup> In other studies, up to 38% of dogs had naturally occurring alloantibodies to DEA 7.<sup>17,22</sup> The relevance of these naturally occurring alloantibodies with respect to immunologic transfusion reactions is uncertain. Acute hemolytic transfusion reactions have been reported in previously transfused dogs but not in transfusion-naïve dogs.<sup>7-10</sup> Additionally, while the grade of incompatibility reaction was similar between transfusion-naïve and previously transfused dogs in our study, the specific alloantibodies causing such incompatibilities were not determined. The results of the present study suggest that more widespread CM testing as well as investigation of specific alloantibodies is warranted to determine the importance of these findings.

There was an increased rate of LAB-CM incompatibility in dogs that were anemic due to RBC destruction, compared to dogs that were anemic due to blood loss or decreased RBC production. The most common cause of RBC destruction in our study was IMHA. The LAB-CM method uses agglutination as 1 endpoint for incompatibility. Dogs with IMHA have anti-RBC autoantibodies that could cause agglutination of donor RBCs. These antibodies interfere with routine compatibility testing before transfusion and could have increased the likelihood of an incompatible CM.<sup>23</sup> One explanation for the incompatible CM results in naïve dogs found in this present study could be due to circulating autoantibodies secondary to a disease process such as IMHA. However, consistent with a previous study, the present study demonstrated CM incompatibilities in naïve dogs with disease processes other than IMHA.<sup>16</sup>

The PCV before transfusion was also found to affect the rate of incompatibility such that, in a linear regression model, the rates of incompatibility were highest when dogs had a PCV < 15%, and that the risk decreased as PCV increased. Almost half (16/35) of the dogs with a PCV of <15% in the present study had RBC destruction as the cause of their anemia. As noted above, the diagnosis of destructive anemia was also a risk factor for incompatibility and could at least partially explain this finding.

An unexpected finding in our study was that the DEA 1+ blood type was associated with an increased risk of CM incompatibility. The reason for this is unclear, but it may be more important to perform a CM before transfusion in these dogs. It is possible that DEA 1+ dogs could lack certain unidentified common RBC antigens, making them more predisposed to have naturally occurring alloantibodies against some donors. However, the effect was relatively small and further work in larger studies is needed to determine if this is a true risk factor.

A limitation of our study is that blood-typing for DEA antigens other than DEA 1 was not performed. Similarly, as it is not routine practice in veterinary medicine, antibody screening of the recipients was not performed. Therefore, the causes of incompatible major CM in our study are not known. Additionally, it is possible that some transfusion-naïve dogs in the present study had a previous transfusion that was not reported at hospital admission, as this information relied on client recollection and medical records from 1 or more referring veterinarians. However, this likely does not account for all of the incompatibilities identified in this group. It is routine practice to perform crossmatching in previously transfused dogs at our institution, with rare performance of this test in transfusion-naïve dogs. As a result, part 2 of the present study was limited by relatively low numbers in the transfusion-naïve dog population. The present study examined only major CM, and further study should investigate the accuracy of the minor CM point-of-care kits, compared to laboratory tube-based methods. Finally, laboratory tube-based CM methods and reagents vary between facilities, and the results of the present study might not directly translate to another facility, depending on differences in methodology.

#### ACKNOWLEDGMENT

Funding for this study was provided by Ontario Veterinary College Pet Trust. We acknowledge the support from Helen Kocmarek and clinical pathology technicians at the Animal Health Laboratory for critically reviewing the study methodology, collecting data, and participating in technical editing of the manuscript.

#### CONFLICT OF INTEREST DECLARATION

Alvedia supplied the immunochromatographic crossmatch kits free of charge; DMS supplied the RapidVet gel crossmatch kits at a reduced rate for the investigators.

#### OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

#### INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the University of Guelph Animal Care Committee, animal use protocol number 3479.

#### HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

## ORCID

Shauna L. Blois  <https://orcid.org/0000-0002-4148-4617>

## REFERENCES

- Holowaychuk MK, Leader JL, Monteith G. Risk factors for transfusion-associated complications and nonsurvival in dogs receiving packed red blood cell transfusions: 211 cases (2008–2011). *J Am Vet Med Assoc.* 2014;244:431-437.
- Callan MB, Oakley DA, Shofer FS, Giger U. Canine red blood cell transfusion practice. *J Am Anim Hosp Assoc.* 1996;32:303-311.
- Maglaras CH, Koenig A, Bedard DL, et al. Retrospective evaluation of the effect of red blood cell product age on occurrence of acute transfusion-related complications in dogs: 210 cases (2010–2012). *J Vet Emerg Crit Care.* 2016;27:108-120.
- Bruce JA, Kriese-Anderson L, Bruce AM, Pittman JR. Effect of premedication and other factors on the occurrence of acute transfusion reactions in dogs. *J Vet Emerg Crit Care.* 2015;25:620-630.
- Kerl ME, Hohenhaus AE. Packed red blood cell transfusions in dogs: 131 cases (1989). *J Am Vet Med Assoc.* 1993;202(9):1495-1499.
- Kessler RJ, Reese J, Chang D, et al. Dog erythrocyte antigens 1.1, 1.2, 3, 4, 7, and Dal blood typing and cross-matching by gel column technique. *Vet Clin Pathol.* 2010;39:306-316.
- Callan MB, Giger U. Hemolytic transfusion reactions in a dog with an alloantibody to a common antigen. *J Vet Intern Med.* 1995;9:277-279.
- Giger U, Gelens CJ, Callan MB, Oakley DA. An acute hemolytic transfusion reaction caused by dog erythrocyte antigen 1.1 incompatibility in a previously sensitized dog. *J Am Vet Med Assoc.* 1995;206:1358-1362.
- Blais M-C, Berman L, Oakley DA, Giger U. Canine Dal blood type: a red cell antigen lacking in some Dalmatians. *J Vet Intern Med.* 2007;21:281-286.
- Melzer KJ, Wardrop KJ, Hale AS, Wong VM. A hemolytic transfusion reaction due to DEA 4 alloantibodies in a dog. *J Vet Intern Med.* 2003;17:931-933.
- Guzman LR, Streeter E, Malandra A. Comparison of a commercial blood cross-matching kit to the standard laboratory method for establishing blood transfusion compatibility in dogs. *J Vet Emerg Crit Care.* 2016;26:262-268.
- Tocci LJ. Transfusion medicine in small animal practice. *Vet Clin North Am Small Anim Pract.* 2010;40:485-494.
- Zaremba R, Brooks A, Thomovsky E. Transfusion medicine: an update on antigens, antibodies and serologic testing in dogs and cats. *Top Companion Anim Med.* 2019;34:36-46.
- Davidow B. Transfusion medicine in small animals. *Vet Clin North Am Small Anim Pract.* 2013;43:735-756.
- Jagodich TA, Holowaychuk MK. Transfusion practice in dogs and cats: an internet-based survey. *J Vet Emerg Crit Care.* 2016;26:360-372.
- Odonayo A, Garraway K, Rohrbach BW, Rainey A, Stokes J. Incidence of incompatible crossmatch results in dogs admitted to a veterinary teaching hospital with no history of prior red blood cell transfusion. *J Am Vet Med Assoc.* 2017;250:303-308.
- Spada E, Proverbio D, Flórez LMV, et al. Prevalence of naturally occurring antibodies against dog erythrocyte antigen 7 in a population of dog erythrocyte antigen 7-negative dogs from Spain and Italy. *Am J Vet Res.* 2016;77:877-881.
- Villarnovo D, Burton SA, Horney BS, MacKenzie AL, Vanderstichel R. Preliminary evaluation of a gel tube agglutination major cross-match method in dogs. *Vet Clin Pathol.* 2016;45:411-416.
- Goy-Thollot I, Giger U, Boisvineau C, et al. Pre- and post-transfusion alloimmunization in dogs characterized by 2 antiglobulin-enhanced cross-match tests. *J Vet Intern Med.* 2017;31:1420-1429.
- Humm KR, Chan DL. Prospective evaluation of the utility of cross-matching prior to first transfusion in cats: 101 cases. *J Small Anim Pract.* 2020;61:285-291.
- Hale AS, Werfelmann J. Incidence of canine serum antibody to known dog erythrocyte antigens in potential donor population [abstract]. *J Vet Intern Med.* 2006;20:768-769.
- Blais M-C, Rozanski EA, Hale AS, Shaw SP, Cotter SM. Lack of evidence of pregnancy-induced alloantibodies in dogs. *J Vet Intern Med.* 2009;23:462-465.
- Wheeler CA, Calhoun L, Blackall DP. Warm reactive autoantibodies: clinical and serologic correlations. *Am J Clin Pathol.* 2004;122:680-685.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Marshall H, Blois SL, Abrams-Ogg ACG, Bersenas AM, Ruotsalo K, Monteith G. Accuracy of point-of-care crossmatching methods and crossmatch incompatibility in critically ill dogs. *J Vet Intern Med.* 2021;35: 245–251. <https://doi.org/10.1111/jvim.15983>