Comparison of gel column, card, and cartridge techniques for dog erythrocyte antigen 1.1 blood typing

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Objective—To compare accuracy and ease of use of a card agglutination assay, an immunochromatographic cartridge method, and a gel-based method for canine blood typing.

Sample—Blood samples from 52 healthy blood donor dogs, 10 dogs with immune-mediated hemolytic anemia (IMHA), and 29 dogs with other diseases.

Procedures—Blood samples were tested in accordance with manufacturer guidelines. Samples with low PCVs were created by the addition of autologous plasma to separately assess the effects of anemia on test results.

Results—Compared with a composite reference standard of agreement between 2 methods, the gel-based method was found to be 100% accurate. The card agglutination assay was 89% to 91% accurate, depending on test interpretation, and the immunochromatographic cartridge method was 93% accurate but 100% specific. Errors were observed more frequently in samples from diseased dogs, particularly those with IMHA. In the presence of persistent autoagglutination, dog erythrocyte antigen (DEA) 1.1 typing was not possible, except with the immunochromatographic cartridge method.

Conclusions and Clinical Relevance—The card agglutination assay and immunochromatographic cartridge method, performed by trained personnel, were suitable for in-clinic emergency DEA 1.1 blood typing. There may be errors, particularly for samples from dogs with IMHA, and the immunochromatographic cartridge method may have an advantage of allowing typing of samples with persistent autoagglutination. The laboratory gel-based method would be preferred for routine DEA 1.1 typing of donors and patients if it is available and time permits. Current DEA 1.1 typing techniques appear to be appropriately standardized and easy to use. (*Am J Vet Res* 2012;73:213–219)

A lthough many blood group systems have been described in dogs, the DEA 1 blood group, with the DEA 1.1 antigen, is generally considered the clinically most important.¹⁻³ There are approximately equal numbers of DEA 1.1–positive and –negative dogs, but their frequencies differ geographically and among breeds.⁴ Although naturally occurring DEA 1.1 alloantibodies have not been detected, there is rapid sensitization of DEA 1.1–negative dogs after they have received a transfusion of DEA 1.1–positive blood, which can cause potentially fatal acute hemolytic reactions with sub-

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	ABBREVIATIONS
DEA	Dog erythrocyte antigen
MHA	Immune-mediated hemolytic anemia

sequent DEA 1.1–mismatched transfusions.^{5,6} Accordingly, DEA 1.1 blood typing of donor and patient prior to transfusion is generally recommended^{1,4}; however, extended DEA typing may not be helpful unless incompatibility reactions are further characterized.⁷ Originally, DEA 1.1 typing was performed in a tube assay with polyclonal alloantibodies derived from sensitized DEA 1.1–negative dogs.^{3–5} Agglutinating strength of the DEA 1.1 antibodies in the tube assay varies and frequently requires a canine antiglobulin (Coombs') reagent.^{7,8} The availability of anti–DEA 1.1 reagent is limited, and the assay is cumbersome to perform and difficult to standardize; thus, its use is restricted to a few larger clinical pathology laboratories.

More recently, several standardized DEA 1.1 typing techniques with monoclonal anti–DEA 1.1 antibodies⁹ and kit techniques have been developed. These include a card-based test¹⁰ (which has been commonly used in clinical practice since 1995) and a gel matrix column assay^{7,8,11} (which has been used since 2003, involves the use of specific equipment, and is best adapted to use in

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large laboratories). In 2007, a new immunochromatographic cartridge became available that has the same monoclonal antibody as is included in the gel-based method.

Although results for the card agglutination assay and gel-based method have been compared with results for the tube-based method,^{8,10} and all 3 techniques have been used in many laboratories and clinics and by researchers, the accuracy of these techniques for samples obtained from diseased dogs has not been evaluated. Furthermore, results for the recently introduced immunochromatographic cartridge method have not been compared with results for other techniques. The purpose of the study reported here was to compare the card agglutination assay, immunochromatographic cartridge method, and gel-based method for DEA 1.1 blood typing and to examine test accuracy and relative strengths and weaknesses of these assays for samples obtained from healthy dogs, dogs with IMHA, and dogs with other illnesses.

Materials and Methods

Sample—Small (1- to 2-mL) EDTA-anticoagulated blood samples from active and potential blood donors at the Penn Animal Blood Bank or from healthy and sick dogs submitted to the Transfusion Laboratory or the Clinical Laboratory at the Ryan Veterinary Hospital of the University of Pennsylvania were analyzed. Samples were preferentially selected from anemic dogs, particularly those with autoagglutination or IMHA, to assist in identifying potential issues with blood typing. The study was approved by the institutional animal care and use committee.

Blood typing—All samples were tested by use of a card-agglutination assay,^a a gel matrix column assay,^b and an immunochromatographic cartridge.^c Samples were tested in accordance with manufacturer instructions, as described elsewhere.^{7,8,10} All testing was conducted by the authors, who were experienced with use of these techniques and routinely conducted blood typing with various techniques. In addition, a few samples were also typed by use of the tube-based method at an external laboratory^d for further confirmation of blood typing results.

Card agglutination assay—One drop (approx 50 μ L) of PBS solution^e was placed in the 3 wells of the assay, which contained an agglutinating lectin (positive control well), nothing (negative control well), or monoclonal anti-DEA 1.1 antibody (patient test well). One drop of blood was added to each well and then spread over the well area. The card was gently mixed for 1 minute, and agglutination then was interpreted by use of the following scale: 0, no agglutination; 1+, many small agglutinates with RBCs in suspension; 2+, some larger agglutinates and many small agglutinates; 3+, a few large agglutinates in clear suspension; and 4+, 1 large agglutinate in clear suspension. If no agglutination or only fine agglutination was observed, a second drop of PBS solution was added to the patient test well to overcome potential prozone effects and enhance agglutination; the card was again gently mixed for 1 minute and then reevaluated. Agglutination reactions of

 \geq 2+ were considered positive results. If agglutination was detected in the negative control well, an aliquot of blood was washed 3 times in PBS solution by use of standard methods^{7,8} before being resuspended to a 25% to 35% RBC suspension. If autoagglutination was reduced to \leq 1+, the test was repeated by use of the washed RBC suspension.

Immunochromatographic cartridge method—For the immunochromatographic cartridge test kit, 3 drops of diluent were placed into a plastic well from the kit. An absorbent paper strip from the kit was dipped into anticoagulated blood and then swirled in the diluentcontaining well for 15 seconds to suspend RBCs. The tip of the immunochromatographic strip, which was linearly impregnated at 2 levels with a monoclonal anti-DEA 1.1 antibody and a control lectin (which would bind to any canine RBCs), was then placed into the RBC suspension for approximately 2 minutes until the RBC suspension had diffused to the top of the strip. The cartridge was then inserted into a holder and immediately assessed. A red band had to be evident at the control mark for the test to be considered valid for interpretation. Detection of a red band of any intensity at the DEA 1.1 mark indicated expression of the DEA 1.1 antigen on RBCs.

Gel matrix column method—An aliquot (50 μ L) of EDTA-anticoagulated blood (or 25 µL of washed packed RBCs if the dog was markedly anemic or the sample had strong autoagglutination) was added to 500 µL of modified bromelin solution^f and incubated at 20°C for 10 minutes. Then, 10 µL of this suspension was loaded on top of 2 gel columns (1 that contained anti-DEA 1.1 antibodies and 1 that contained no antibodies). Gel columns were centrifuged for 10 minutes in the manufacturer centrifuge. Retention of RBCs in the gel was graded by use of the following scale: 0, all RBCs at bottom of the gel; 1+, a few RBC agglutinates in the lower half of the gel but most RBCs at bottom of the gel; 2+, RBC agglutinates dispersed throughout the gel; 3+, RBC agglutinates throughout the gel and RBCs on the upper surface; and 4+, all RBCs on the upper surface of the gel. Retention of \geq 2+ was considered a positive test result. A column containing saline solution but no antibodies was used as a control sample; if the control sample had a positive result, samples were washed and the assay was repeated; if the control sample still had a positive result after washing, results were disregarded.

Evaluation of the effects of anemia—Blood samples from 2 healthy DEA 1.1–positive dogs and 2 healthy DEA 1.1–negative dogs were centrifuged for 3 minutes at 1,000 \times g. Packed RBCs from these samples were resuspended in plasma from the same samples to create samples with PCVs of 10%, 30%, and 50%. Blood typing via the card agglutination assay, immunochromatographic cartridge method, and gel-based method was performed on these PCV-adjusted samples, as described previously.

Data collection and analysis—For each blood sample, breed and health status or underlying disease of the dog were recorded. The strength of all test reactions (anti–DEA 1.1 and control samples) was recorded as well as the interpreted test result for each method. Because of the lack of an established criterion-referenced standard, sensitivity, specificity, and overall accuracy of test methods were calculated on the basis of a composite reference standard¹²; agreement of results for \geq 2 methods was considered to indicate the true blood type.

Results

Sample population—Blood samples from 52 healthy large-breed dogs (potential blood donors) and 39 spare blood samples from canine patients at the veterinary hospital of the University of Pennsylvania were used in the study. There were 16 mixed-breed dogs, 15 Labrador Retrievers, 11 Borzois, 6 Boxers, 4 Doberman Pinchers, 3 German Wirehaired Pointers, 3 Golden Retrievers, 3 Poodles, 3 Rottweilers, 2 Border Collies, 2 Cocker Spaniels, 2 Dachshunds, 2 Greyhounds, 2 Scottish Terriers, and 1 dog each of 17 other breeds.

Among the 39 canine patients, 10 had IMHA, 6 had lymphoma, 6 had other neoplasias, 5 had various gastrointestinal tract diseases, 5 had renal disease, 3 had other hematologic problems, and 2 had trauma injuries; the diagnosis of the condition or disease was undetermined for 2 dogs. Autoagglutination was evident in 5 samples from the 10 dogs with IMHA. In 3 of the 5 samples, autoagglutination was persistent and prevented determination of the blood type by use of the card agglutination assay and gel-based method (and the

tube-based method as well). Of the remaining 88 blood samples, 48 (55%) had positive results for DEA 1.1 and 40 (45%) had negative results for DEA 1.1, as determined on the basis of the composite reference standard.

Test comparison—Identical results for blood type by all techniques were detected for 69 of 88 (78%) samples (**Table 1**). Potential test discrepancies were detected in 4 of 7 samples from dogs with IMHA, 6 of 29 samples from dogs with other diseases, and 9 of 52 samples from healthy dogs (**Table 2**).

The gel-based method was 100% accurate for all 88 samples that could be tested by use of this method (Table 3). All DEA 1.1-negative samples had no RBC retention in the gel-based method, except for 3 samples with weak 1+ agglutination. The 3 samples that could not be blood typed with the gel-based method and the card agglutination assay because of autoagglutination had negative results for DEA 1.1 when tested by use of the immunochromatographic cartridge method, as determined by use of whole blood samples (Table 2). There were 6 falsenegative results with the immunochromatographic cartridge method (Table 1). Among the DEA 1.1-positive samples as determined by use of the immunochromatographic cartridge method, the intensity of the DEA 1.1 band on the test strip ranged from weak to intensely dark red. Moreover, a band occasionally was detected in DEA 1.1-negative samples after the test dried, which highlighted the importance of conducting the interpretation and obtaining archival information (eg, photograph of the test result) immediately after the test was performed.

Table 1—Agreement of results* for a gel-based method, immunochromatographic cartridge method, and card agglutination assay with actual DEA 1.1 blood type of samples obtained from dogs.

Technique	Test result	n	True-positive results	True-negative results	False-positive results	False-negative results
Gel-based method	Pos	88	48	40	0	0
Immunochromatographic cartridge method	Pos	88	42	40	0	6
Card agglutination as sayt	≥1+	87	47	30	10	0
	\geq 2+	87	44	35	5	3
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*Agreement of results was determined as the agreement for at least 2 methods (which included the tube-based method for 7/88 samples). †Use of 1+ or 2+ agglutination as the cutoff between samples with positive results and negative results. n = Number of samples. Pos = Positive result for DEA 1.1 blood type.

Table 2—Results for samples obtained from 22 dogs in which blood typing could not be performed because of autoagglutination or because there were discrepancies between test results.

No. of dogs	Card agglutination assay	Immunochromatographic cartridge method	Gel-based method	Tube-based method	True DEA 1.1 blood type	Animal disease status
3	Auto	Negative	Auto		Unknown	IMHA
1	Auto	Positive	Positive	_	Positive	IMHA
1	1+	Negative	Negative	_	Negative	Healthy
1	1+	Negative	Positive	Positive	Positive	Healthy
2	1+	Positive	Positive	Positive	Positive	Gastrointestinal tract disease (1) and healthy (1)
4	1+	Negative	Negative	Negative	Negative	Healthy (1), IMHA (1), and lymphoma (1)
5	Positive	Negative	Negative	—	Negative	Healthy (2), IMHA (1), lymphoma (1), and trauma (1)
5	Positive	Negative	Positive	—	Positive	Healthy (3), hematologic disease (1), and renal disease (1)

For the card agglutination assay and the gel-based method, positive indicates a reaction $\ge 2+$. True blood type is based on the composite reference standard of the agreement for results of ≥ 2 test methods. Numbers in parentheses are the number of dogs with a particular health status.

— = Not tested via the tube-based method. Auto = Autoagglutination.

Table 3—Calculated test characteristics of a gel-based method, immunochromatographic cartridge method, and card agglutination assay for DEA 1.1 blood typing of dogs.

Technique result	Sensitivity (%)	Specificity (%)	value (%)	value (%)	Accuracy (%)
Gel-based method Pos	100	100	100	100	100
$\begin{array}{llllllllllllllllllllllllllllllllllll$	100	75	82	87 100	93 89
≥ 2+	94	88	90	92	91



Figure 1—The effect of alterations in PCV on results for a DEA 1.1–positive sample blood typed by use of the immunochromatographic cartridge method. The PCV was 10%, 30%, and 50% in the top, middle, and bottom cartridges, respectively. The red control band (arrow) is clearly visible for all PCVs, but the DEA 1.1 band (arrowhead) is less visible as the PCV decreases.

One sample from a dog with IMHA that could be blood typed by use of the gel-based method and immunochromatographic cartridge method could not be blood typed by use of the card agglutination assay because of persistent autoagglutination in the negative control well for this assay after washing of RBCs (Table 2). Of the remaining 87 samples tested by use of the card agglutination assay, 30 had no agglutination, 8 had 1+ agglutination, and 49 had \geq 2+ agglutination. Results were analyzed with 1+ agglutination as a negative result and then with 1+ agglutination as a positive result. Blood typing by use of the tube-based method was performed on 7 of 8 samples with 1+ agglutination as determined by use of the card agglutination assay; insufficient blood was available to conduct the tube-based test for the other sample. There was agreement between results of the gel-based method and the tubebased method for all 7 of these samples (3 DEA 1.1positive samples and 4 DEA 1.1-negative samples). One sample, which had 1+ agglutination by use of the card agglutination assay and negative results for DEA 1.1 by use of the immunochromatographic cartridge method, had positive results for DEA 1.1 when tested by use of the gel-based method and tube-based method; therefore, it was considered a true DEA 1.1positive sample in the analysis.

Photography or photocopying could be readily used to archive results for the immunochromatographic cartridge method and gel-based method. Photography also could be used to archive results of the card agglutination assay. Subjectively, the authors considered that the results of the immunochromatographic cartridge method and gel-based method were easy to interpret, whereas the distinction between negative and 1+ results for the card agglutination assay required closer scrutiny.

Effects of anemia—Alterations of the PCV from 10% to 50% in 4 samples had no effect on blood typing results for the gel-based method and card agglutination assay. The degree of agglutination or RBC retention was the same, but the number of RBCs present in the test was visibly reduced. When the 4 samples were tested by use of the immunochromatographic cartridge method, the expected result was obtained for the 2 DEA 1.1–negative samples but for only 1 DEA 1.1–positive sample. In the other DEA 1.1–positive sample, intensity of the DEA 1.1 band faded with reductions in the PCV, until it was barely visible in the sample with a PCV of 10%. Intensity of the control band was less affected by changes in PCV (Figure 1).

Discussion

As transfusions become more commonplace in canine medicine, there is an increasing need to rapidly and reliably determine the DEA 1.1 blood type of dogs to reduce the risk of hemolytic transfusion reactions.^{1,2,4-6,13} In the present study, we compared the accuracy of 3 commercially manufactured assays. The gel-based method was developed for use by trained personnel in a laboratory that frequently performs blood typing,^{8,11} whereas the card agglutination assay and immunochromatographic cartridge method are marketed for in-clinic use by veterinary staff who may conduct blood typing less frequently. Whereas the card agglutination assay and gel-based method have been compared with the historical criterion-referenced standard (ie, the tube-based method),^{7,8,10} to our knowledge, the study reported here is the first evaluation and comparison of the immunochromatographic cartridge method with any other method. Furthermore, the present study was conducted to examine the change in test reliability that may result from obtaining samples from diseased patients rather than healthy animals. We determined that the card agglutination test and immunochromatographic cartridge method had clinically adequate accuracy and that the immunochromatographic cartridge method also may have the advantage of allowing the typing of agglutinating samples.

Although the tube-based method has been used for many years, it cannot necessarily be considered the de facto criterion-referenced standard because it is based on use of polyclonal serum, often yields weak agglutination reactions, and is difficult to standardize.^{5,8} There is the potential for cross-reactivity with other RBC antigens, and the strength of anti-DÉA 1.1 reactions varies among reagent batches, which thus frequently requires additional antiglobulins to cause appreciable agglutination. Nonetheless, investigators in another comparative study⁸ found complete agreement between results of the tube-based method and gel-based method, and in the present study, we found complete agreement between results of the gel-based method and the standard of 2 test results, which suggested that the gel-based method is an appropriate and standardized typing benchmark for dogs. Currently, availability of the DEA 1.1 gelbased method is limited, and it is uncertain whether manufacturing of this test will resume.

In contrast to results of the aforementioned comparative study,8 samples from healthy and diseased dogs (preferentially those from patients with IMHA) were included in the present study to highlight difficulties with blood typing. Although the overall agreement was good between the gel-based method and in-clinic tests (the card agglutination assay and immunochromatographic cartridge method), difficulties and discordant results were detected more frequently with samples obtained from diseased dogs, and discrepancies were most frequently detected for samples obtained from dogs with IMHA. It should be mentioned that this bias has influenced the reported accuracy of the test methods in the present study; if analyses had been restricted to samples obtained from healthy dogs, accuracy of both the card agglutination assay and immunochromatographic cartridge method would have been 92%.

Overall, there was good agreement between results for the various methods, but discrepancies in test results were apparent. Discrepancies between results of the gel-based and tube-based methods and the card agglutination assay have been reported in another study.8 Those discrepancies were associated with 1+ agglutination reactions for the card agglutination assay, which were detected in DEA 1.2-positive samples. In the present study, blood typing for DEA 1.2 was not performed, but discrepancies were not limited to 1+ agglutination reactions, with 5 samples with false-positive results having $\geq 2 +$ agglutination reactions for the card agglutination assay. Interpretation of the card agglutination assay was based on the scoring of agglutination in an RBC suspension, which can be a variable and subjective factor in a number of assays,¹⁴ although discrepancies may also be attributable to disease status of patients.¹⁵

The discordant results for the immunochromatographic cartridge method were all false-negative results, with no false-positive results reported. The gel-based method and the immunochromatographic cartridge method used the same monoclonal antibody, but the immunochromatographic cartridge method appeared to be less sensitive than the gel-based method on the basis of the observation of some extremely faint test bands for the immunochromatographic cartridge method, which may have been worsened by anemia. In the dilution experiment, a lower PCV weakened the intensity of the band obtained with the immunochromatographic cartridge method, but a lower PCV did not affect the agglutination for the card agglutination assay or gel-based method. Nevertheless, the manufacturer of the card agglutination assay reports that anemia may change the characteristics of observed agglutination and may lead to equivocal results.^a In the present study, the agglutinates appeared smaller in samples with a lower PCV; however, when these samples were closely scrutinized, the test result was unaltered. The manufacturer of the gel-based method provides protocols that involve the use of whole blood or standardized RBC suspensions made from packed RBCs. Use of the latter adjusts the PCV and thus would negate any effects of anemia. However, even when whole blood was used, we did not observe any change in the degree of RBC retention as the sample PCV was decreased to 10%. From a practical standpoint, it appears that the sensitivity of the immunochromatographic cartridge method and the readability of the card agglutination assay and gel-based method may all be improved in samples obtained from anemic animals by performing the test on blood that has had some plasma removed to concentrate RBCs to within the PCV reference range. We did not assay samples with PCV > 50% and thus cannot predict the impact of erythrocytosis, but it may also be prudent to adjust the PCV of such samples to within the PCV reference range before analysis.

In addition to anemia, IMHA may be associated with autoagglutination,¹⁶ as was detected in 5 of 10 samples in the present study. The card agglutination assay, gel-based method, and tube-based method all use agglutination as the endpoint for a positive test result. Thus, persistent autoagglutination (which cannot be abolished by triplicate washing of RBCs) prevents blood

typing by use of these methods.¹ Screening for autoagglutination is conducted by use of the negative control sample incorporated into each of these assays, except for the immunochromatographic cartridge method. It is worthy of mention that 1 sample had persistent autoagglutination for the negative control well of the card agglutination assay, but RBC washing of that sample caused the negative control sample of the gel-based method to appear negative. This may indicate that the gel-based method is less sensitive to interference from agglutination than is the card agglutination assay. For the immunochromatographic cartridge method, it appears likely that only free separate cells migrate up the strip to bind at the test sites, so there is no reason to expect the test to be altered by autoagglutination. Indeed, the 3 samples with persistent autoagglutination in the present study all yielded apparently valid (as indicated by a positive result for the control band) DEA 1.1negative test results for the immunochromatographic cartridge method. Follow-up samples from the same dogs after treatment and resolution of autoagglutination were not available to confirm the accuracy of these blood types by a second method.

The objective of DEA 1.1 blood typing is to avoid administration of DEA 1.1-positive blood to DEA 1.1-negative patients.^{1,2,4} This may be achieved through exclusive use of DEA 1.1-negative blood products or by collecting both DEA 1.1-positive and DEA 1.1-negative blood and administering type-matched blood products. Specificity is the most important test characteristic when typing recipients because a test with no or few false-positive results, such as the immunochromatographic cartridge method or gel-based method, will prevent transfusion of DEA 1.1positive blood to DEA 1.1-negative patients. Conversely, sensitivity can be considered the more important test characteristic when screening blood donors because a test with few false-negative results, such as the gel-based method or card agglutination assay by use of a 1+ cutoff, will prevent the misidentification of DEA 1.1-positive blood products as DEA 1.1-negative blood products. As such, the intended use of the blood typing information should be considered when choosing the appropriate blood typing test.

The blood typing methods evaluated here were all standardized and relatively simple to perform, compared with standardization and ease of performance for the tube-based method. Although the in-clinic assays (the card agglutination assay and immunochromatographic cartridge method) were sufficiently accurate to be used for patient-side screening of blood type, they were inferior to the laboratory gel-based method for accuracy of blood typing. Unfortunately, the gel-based method requires relatively costly equipment and reagents, involves a multiple-step process, and may no longer be commercially available. By contrast, the card agglutination assay and immunochromatographic cartridge method are packaged as kits that contain all necessary supplies and can be performed in minutes with little prior instruction. However, it should be emphasized that testing in the present study was performed by experienced personnel in a laboratory who routinely perform blood typing. The potential for increases in error rates when testing is performed by inexperienced personnel in a nonlaboratory setting should not be discounted. These observations are in keeping with experiences in human medicine, whereby patient-side typing of blood groups (ie, ABO typing) by nurses may have an error rate of up to 30%, which can be influenced by the experience of the nurse performing the test.¹⁷ By contrast, the error rate with ABO typing via modern laboratory methods, such as via the gel-based method, is approximately 1:3,400, with most errors being clerical rather than related to the test method.¹⁸ The prevalence of these errors has led to the recommendation that human patients be blood-typed and crossmatched prior to every donation or transfusion event, with the test preferably performed by trained personnel and, if time permits, in a clinical pathology or blood bank laboratory. The same recommendations could be made for veterinary patients, although this would add considerably to the expense of a transfusion¹³ and would limit transfusions to facilities with these resources.

For the study reported here, we concluded that the commercial card agglutination assay, immunochromatographic cartridge method, and gel-based method are all suitable for DEA 1.1 blood typing of donor and patient dogs, but some discrepancies in results exist among the test methods, especially for samples obtained from dogs with IMHA. The card agglutination assay is sensitive for the detection of DEA 1.1, but there is subjectivity in test interpretation; thus, this assay is appropriately suited for screening of blood donors in a blood bank program. The immunochromatographic cartridge method is specific, which makes it appropriately suited and safe for use in the screening of patients in emergency situations. It also may be particularly useful when a patient has autoagglutination. Results for the gel-based method and immunochromatographic cartridge method are easy to interpret and archive. The gel-based method (or the tube-based method) can be considered criterionreferenced standards and should be used to confirm a dog's blood type whenever possible to minimize the risk of potentially fatal hemolytic transfusion reactions.

- a. RapidVet-H Canine, provided by DMS Laboratories, Flemington, NJ.
- b. ID-Gel Test Canine DEA 1.1, provided by DiaMed, Cressier-sur-Morat, Switzerland.
- c. DME VET DEA 1.1, provided by Alvedia, Lyon, France.
- d. Midwest Animal Blood Services, Stockbridge, Mich.
- e. Dulbecco PBS solution without calcium, without magnesium, without phenol red, Thermo Scientific, Logan, Utah.
- f. ID-Diluent VET 1, DiaMed, Cressier-sur-Morat, Switzerland.

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