Evaluation of an immunochromatographic test for feline AB system blood typing

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Abstract: Objective – To determine the accuracy of an immunochromatographic cartridge (IC) test for blood typing feline type A, B, and AB blood samples. Design – Prospective observational study. Setting – University teaching hospital. Samples – Fifty-one nonanemic and 19 anemic feline blood samples. Interventions – Samples were blood typed by both card agglutination (CA) and IC techniques. Discordant results were analyzed using a back-typing technique for the presence of alloantibodies. Repeatability and reproducibility of the IC method were evaluated. Accuracy of the IC method was determined for feline whole blood anticoagulated with EDTA and citrate phosphate dextrose adenine (CPDA1), for feline-packed RBCs with CPDA1 and saline adenine glucose-mannitol, and for autoagglutinated and hemolytic samples. Accuracy of IC testing was determined for feline blood after room temperature, 4 ± 2, −20, and −80°C storage following 24, 48, 72, and 96 hours, and after 1, 2, 3, and 4 weeks of storage. Measurements and Main Results – In anemic and nonanemic samples the IC technique had a specificity, sensitivity, positive predictive value, and negative predictive value of 100% in detecting feline blood types A, B, and AB, outperforming a CA test. Results were repeatable and reproducible. Using IC it was possible to blood type samples anticoagulated with EDTA and CPDA1, packed RBC samples with CPDA1 and saline adenine glucose-mannitol, autoagglutinated and hemolytic samples, and samples stored at 4 ± 2°C and at room temperature for up to 1 month. Conclusions – The IC technique is an accurate assay for the identification of A, B, and AB blood types in anemic and nonanemic feline blood. It has a higher sensitivity and specificity than the CA test, and can be used in samples stored with common anticoagulants or preservative solutions used in feline transfusion medicine.

Keywords: cat, feline neonatal isoerythrolysis, transfusion medicine, transfusion reaction

Abbreviations: CA card agglutination CPDA1 citrate phosphate dextrose adenine FNI feline neonatal isoerythrolysis IC immunochromatographic cartridge PBS phosphate buffered saline

Introduction

In feline medicine, AB blood system typing and crossmatching are vitally important to determine blood compatibility to prevent hemolytic transfusion reactions.(1,2) Feline blood typing also plays an important role in breeding programs to prevent feline neonatal isoerythrolysis (FNI).(3) Several methods are available for feline blood typing including card agglutination (CA), gel column agglutination, tube, and plate techniques.(4–7) The blood-typing card has been shown to reliably identify both type A and type B cats, but can show weak reactions with type AB blood, resulting in mistyping of AB cats.(4,5,7) The gel column agglutination technique can correctly identify type A, B, and type AB cats, and complete concordance between results using this technique and from back typing have been demonstrated.(5,7,8) Unfortunately, the gel column technique is no longer commercially available, and although the reagents used in the tube and plate methods are readily available, these assays are cumbersome to perform and difficult to standardize.(4,6) A new immunochromatographic cartridge (IC) test, based on immunochromatographic diffusion of RBCs passing through monoclonal antibody-containing strips, is commercially available for laboratory and clinical use. The aim of this study was to assess the accuracy of IC for blood typing nonanemic and anemic samples in clinical and laboratory settings; to compare its performance with the CA method; and to evaluate the effects of hemolysis, autoagglutination, different anticoagulant and preservative solutions, temperature, and time of storage on IC performance. Material and Methods Surplus blood collected from cats presented to the Veterinary Transfusion Unit and Internal Medicine Clinic of the University of Milan were used in this study. All cat owners provided written informed consent. A volume of 1–2.5 mL blood was drawn from 51 nonanemic and 19 anemic cats and placed in an EDTA anticoagulant tube. For each blood sample, age, breed, sex, and health status or underlying disease of the cat were recorded. All samples were initially analyzed within 3 hours of collection. All samples were macroscopically evaluated for autoagglutination. To test for agglutination, a drop of EDTA blood was placed on a slide and mixed with a drop of phosphate buffered saline (PBS)a solution. If autoagglutination was present, the RBCs were washed 3 times with the same volume of saline as the discarded plasma; if autoagglutination persisted, it was considered true autoagglutination. Autoagglutinated samples were analyzed with IC before washing with PBS. If IC was not able to blood type the agglutinated sample, it was washed 3 times with PBS and retyped. Autoagglutinated samples were always washed with PBS before blood typing with CA, according to manufacturer's recommendations. Once the packed cell volume (PCV)b or hematocritc had been determined, and hemolysis was recorded as present or absent based on the color of the plasma, each sample was split and blood typing performed in parallel, with the operator blinded to results, using CAd and a "Lab" version of ICe Five blood type A, 3 type B, and 7 type AB samples were also tested with a "Quick" version of IC.f Testing was repeated twice in all inconclusive samples For the Lab version of the IC test, 3 drops of diluent were placed into a single well. A 10 L aliquot of sample was pipetted from the sample tube, added to the diluent, and gently mixed with the pipette. An immunochromatographic strip, with 3 impregnated lines of monoclonal anti-A and anti-B antibodies and a control lectin, was placed in the RBC suspension for 2 minutes and then immediately read as follows: a red band at position C (control) had to be present for result interpretation, a visible red band at

position A indicated the expression of the A antigen, and a red band at position B indicated expression of the B antigen on RBCs. Red bands at both A and B indicated the sample was of AB blood type. The Quick version IC kit differed from the Lab version as it has single use packaging and reagents. The supplied absorbent paper strip was briefly dipped into anticoagulated blood and then in the diluent-containing well to suspend RBCs. The cartridge was then placed in a holder and results read as indicated in the legend of the holder. The CA method was performed as previously described.7 The presence of plasma alloantibodies was tested in discordant samples using a back-typing technique as previously described7 with the operator blinded to the results of CA and IC. All discordant samples were back typed against all 3 blood types. To establish the repeatability of Lab and Quick IC results, the tests were run on 1 sample of type A, B, and AB 10 times on the same day in the same laboratory and interpreted by the same operator. The operator was blind to the previous results. To establish the reproducibility of Lab and Quick IC results, 5 samples of type A, B, and AB were tested in a blinded fashion by 3 different operators in 2 different locations and with 2 different kits. Five samples of each blood type stored at room temperature, 4 ± 2°C, −20°C, and −80°C were analyzed with Lab and Quick IC after 24, 48, 72, and 96 hours, and after 1, 2, 3, and 4 weeks of storage. IC blood typing was performed on 6 samples (5 type A and 1 type AB) of citrate phosphate dextrose adenine (CPDA1) anticoagulated blood drawn from packed RBC units preserved with saline adenine glucose-mannitol, both on the day of preparation (D0) and on the day of the expiration of the unit, D35. Finally, IC was performed on 3 samples (all type A) drawn from CPDA1 anticoagulated whole blood units on D0 and D35. As there is no gold standard technique in feline blood typing to confirm the results, a composite reference standard and discordant resolution analysis were used to determine the blood type of each sample.9 Agreement of results of CA and IC was considered to indicate the true blood type. In case of discordant results, the back-typing technique was used to identify the correct blood type.

Statistical Methods

Sensitivity, specificity, and positive and negative predictive values were calculated for IC. Cohen's Kappa statistic was calculated to evaluate agreement beyond chance alone between CA and IC in detecting A, B, and AB blood type. Statistical analyses were performed using a statistical software package.g

Results

Characteristics of 51 nonanemic and 19 anemic samples are shown in Table 1. The IC method could be used to determine blood type in all samples (51 nonanemic and 19 anemic samples) resulting in a specificity, sensitivity, positive predictive value, and negative predictive value of 100% in blood type A, B, and AB samples. Results of Kappa statistics comparing the agreement between CA and IC are reported in Table 2. Details of discordant results and untyped samples are shown in Table 3. Blood typing was possible in both 13 of 15 nonanemic and in 2 of 15 anemic samples (PCV of 0.17 L/L [17%] and 0.12 L/L [12%], respectively) using the Quick version of IC. The IC method showed 100% repeatability, as the single samples of type A, B, and AB typed multiple times were always correctly blood typed. Reproducibility of IC results was excellent, as the correct blood type was identified

in all 5 samples of type A, B, and AB tested. The IC method was able to correctly blood type samples stored at room temperature and at 4 ± 2°C for up 1 month, but not samples frozen at −20°C and −80°C. The IC method was able to determine the correct blood type in samples from whole blood units anticoagulated with CPDA1 and samples of packed RBC units anticoagulated with CPDA1 and preserved with saline adenine glucose-mannitol, on D0 and D35 of storage. The 5 hemolyzed samples and three autoagglutinated samples were correctly blood typed with IC. In 1 of the autoagglutinated anemic samples (PCV of 0.04 L/L [4%]) it was necessary to wash RBCs before blood typing, because the autoagglutinated sample did not migrate along the immunochromatographic strip to reach the 3 impregnated lines of monoclonal anti-A and anti-B antibodies and control lectin. After washing, the sample correctly migrated along the strip and could be correctly blood typed.

Discussion

Feline blood typing is vital prior to blood transfusion. High sensitivity and specificity methods are particularly important for the feline AB blood group system, which is characterized by the presence of naturally occurring alloantibodies against the missing blood antigen.1 The current investigation found 100% sensitivity and specificity in detecting both A and B antigens. A previous study of the immunochromatographic method6 demonstrated a sensitivity and specificity of 97.7% and 100%, respectively, for A antigen detection, and a sensitivity and specificity of 95.7% and 97.1, respectively, for B antigen detection. That study compared more methods of blood typing and had a higher number of more challenging cases in terms of diseased cats and cats with B or AB blood types. These differences could account for the different accuracies reported for the IC method. The current study added information regarding accuracy of IC, and on the effects of hemolysis, autoagglutination, storage temperature, storage time, and different anticoagulant or preservative solutions on IC performance. The CA and IC methods evaluated in our study produced discordant results in 15 of 70 (21.4%) cases. In all of these discordant samples, the IC test correctly identified erythrocyte antigens. Seven of the discordant results were of type AB blood. The difficulty in making a correct identification of type AB samples using CA could arise from the different ganglioside patterns of type A and AB cats,10 and from the presence of 2 phenotypes of type AB (11) since some antibodies may be unable to correctly identify the different patterns. The CA and IC methods use different antibodies against type A and type B antigens. The CA tests use murine monoclonal antibody as anti-A reagent and lectin from Triticum vulgaris as an anti-B reagent. The IC methods use monoclonal antibodies, but no information is available about their composition. The contradictory results provided by the 2 methods may be a consequence of the different antibodies used and differences in antibody-antigen affinity. Accurate blood typing is vital in breeding programs to prevent FNI, an important cause of fading kitten syndrome and mortality in kittens. If there is a need to mate a type B queen with a type A tom, the best way to prevent FNI is to blood type the kittens (using umbilical cord blood) and prevent the A and AB kittens ingesting colostrum.3,12 Blood typing using IC could be performed on samples from kittens since volumes of blood required are very low (10 L). 4 There are numerous advantages to the IC method. The volume of sample required is low; IC tests require only 10 L as opposed to the 150 L needed for CA. The IC method can determine blood types in

only 2 min versus the 10 min required to complete a CA test, which is useful in emergency situations. Using IC testing, blood type can be determined in samples that have been refrigerated or stored at room temperature for up to 1 month, and so this method can be used for stored blood or samples shipped by mail. The IC method can be used in samples stored with the typical anticoagulants or preservative solution used in veterinary transfusion medicine, which is useful for quality control testing in veterinary blood banks. The IC test was also able to blood type autoagglutinated samples. Finally, the Quick version of IC was able to determine blood types cageside, which is useful for emergency situations. The weaknesses of this study are the limited number of samples in which blood typing was challenging, such as anemic, hemolyzed, or autoagglutinated samples. Additionally, back typing used to type blood with discordant results is an imperfect reference test because it cannot discriminate between type AB blood and type A blood (found in 2 of 3 of the type A cat population) without naturally occurring, macroscopically agglutinating antiB alloantibodies.(1,2)

Conclusions

These data suggest that the IC test is an accurate assay for the identification of A, B, and AB blood types in cats, with higher sensitivity and specificity than CA testing. It can be used in samples stored with the typical anticoagulants or preservative solutions used in feline transfusion medicine.

Footnotes:

a PBS, BioMerieux, Lyon, France. Composition: sodium chloride 7.650 disodium phosphate 0.724 g, monopotassium phosphate 0.210

b Microliter Centrifuge Z 233M-2, Hermle Labortechnik GmbH, Wehingen, Germany

c Cell-Dyn 3500, Abbott Diagnostic Laboratories, Abbott Park, Illinois, USA

d RapidVet-H Feline, Agrolabo, Torino, Italy

e LabTEST A+B, Alvedia, Lyon, France f QuickTEST A+B, Alvedia, Lyon, France

g MedCalc software, version 12.7.0, Mariakerke, Belgium

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Table 1: Characteristics of 51 nonanemic samples and 19 anemic feline blood samples blood typed with card agglutination and immunochromatographic cartridge techniques

51 Nonanemic cats		19 Anemic cats
Reasons for blood typing	20 Blood donors	13 Blood transfusion recipients (anemia due to: neoplasia [1], hemorrhage [1], bone marrow aplasia [2], chronic renal failure [1], FIV infection [1], Mycoplasma hemofelis and FeLV infection [1], unknown origin [6]) 6 4-month-old kittens
	16 Breeding cats	
	15 Preoperative evaluation (neutering)	
Breed	33 DSH	11 DSH
	11 Ragdoll	4 Ragdoll
	5 Devon Rex	1 British Shorthair
	2 Persian	1 Sphynx
		1 Norwegian Forest Cat
		1 Persian
PCV range (mean	0.24 (24%)–0.42 L/L (42%) (0.31 L/L [31%])	0.04 (4%)–0.22 L/L (22%) (0.14 L/L [14%])
Hemolysis	4 Samples	1 Sample
Autoagglutination	0 Samples	3 Samples (one with true autoagglutination)
Concordant results:	43/51 Samples (83%)	12/19 Samples (63.2%)
	33/43 (76.7%) blood type A	9/12 (75%) blood type A
	9/43 (20.9%) blood type B	3/12 (25%) blood type B
	1/43 (2.3%) blood type AB	0/12 (0%) blood type AB
Discordant results	7 Samples	2 Samples
Untyped samples	1 Sample with CA	5 Samples with CA

CA, card agglutination; DSH, domestic shorthair cat; FIV, feline immunodeficiency virus; IC, immunochromatographic cartridge; FeLV, feline leukemia virus.

Table 2: Results of Kappa statistics comparing the agreement between card agglutination and immunochromatographic cartridge methods in detecting blood type in 51 nonanemic and 19 anemic samples

Nonanemic samples			Anemic samples	
Blood group	Kappa value (95% CI)	Agreement	Kappa value (95% CI)	Agreement
A	0.957 (0.872–1.000)	Very good	0.289 (−0.006–0.583)	Fair
B	0.638 (0.406–0.870)	Good	1.000 (1.000–1.000)	Very good
AB	0.194 (−0.134–0.522)	Poor	0.000 (0.000–0.000)	Poor

CA, card agglutination; IC, immunochromatographic cartridge.

Table 3: Untyped samples and discordant results for card agglutination and immunochromatographic cartridge methods of blood typing in 15 of 70 (21.4%) nonanemic and anemic feline samples

Alloantibodies detected								
Cat number	CA test	IC test	with back-typing technique (strength of agglutination)	True blood type	PCV/ hematocrit	Breed	Diagnosis	Reason for blood typing
1	ND	A		A	0.27 L/L (27%)	Devon Rex	Healthy	Breeding
2	B	AB	None	AB	0.26 L/L (26%)	Ragdoll	Healthy	Breeding
3	B	AB	None	AB	0.28 L/L (28%)	DSH	Healthy	Blood donor
4	B	AB	None	AB	0.38 L/L (38%)	Ragdoll	Healthy	Breeding
5	B	AB	None	AB	0.42 L/L (42%)	Ragdoll	Healthy	Breeding
6	B	AB	None	AB	0.34 L/L (34%)	Ragdoll	Healthy	Breeding
7	B	AB	None	AB	0.28 L/L (28%)	DSH	Healthy	Blood donor
8	B	AB	None	AB	0.24 L/L (24%)	DSH	Healthy	Pre-surgery evaluation
9	AB	A	Anti-B (2+)	A	0.16 L/L (16%)	DSH	Anemic (intestinal lymphoma)	Transfusion
10	AB	A	Anti-B (1+)	A	0.10 L/L (10%)	DSH	Anemic (FIV infection)	Transfusion
11	ND	A	Anti-B (2+)	A	0.04 L/L (4%)	BSH	Anemic (FeLV and CMhm infections)	Transfusion
12	ND	A	None	A	0.17 L/L (17%)	Ragdoll	Anemic kitten	Breeding
13	ND	A	None	A	0.22 L/L (22%)	Ragdoll	Anemic kitten	Breeding
14	ND	A	None	A	0.21 L/L (21%)	Ragdoll	Anemic kitten	Breeding
15	ND	A	Anti-B (1+)	A	0.06 L/L (6%)	DSH	anemic (pIMHA)	Transfusion

BSH, British Shorthair; CA, card agglutination; CMhm, Candidatus Mycoplasma hemominutum; DSH, domestic shorthair; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; IC, immunochromatographic cartridge; ND, not determined; PCV, packed cell volume; pIMHA, primary immune-mediated hemolytic anemia.