Evaluation of an immunochromatographic technique for feline AB blood group system typing

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Introduction and aim of the study - Administration of compatible blood is a prerequisite to prevent fatal transfusion reactions in cats. The purpose of this study was to evaluate the performance of a rapid immunochromatographic test for feline blood typing, comparing the results with the gold standard technique of tube agglutination.

Materials and methods - Fifty blood samples in EDTA were typed in duplicate by tube agglutination and by an immunochromatographic technique. Immunochromatography was also used to type diluted and concentrated blood samples, samples anticoagulated with CPDA, and samples stored at room temperature, -20°C and at 4-6°C.

Results - The tube technique typed 36 samples as group A, 9 as group B and 5 as group AB. Results were identical with the two methods in 45/50 samples with good concordance. The sensitivity and specificity of the immunochromatographic technique were respectively 97.2% and 71.4% for group A, 100% and 100% for group B, 20% and 97.7% for group AB. Concordance was good for group A, excellent for group B and poor for group AB. Immunochromatography was able to correctly type diluted and concentrated blood samples, samples anticoagulated with CPDA, and samples stored at room temperature for up to 7 days and at 4-6°C for up to 4 weeks.

Discussion - Immunochromatography is able to identify, with high sensitivity and specificity, both type A and type B blood groups, which are the most problematic in transfusion medicine. Using immunochromatography cats with the rare AB group are generally mistakenly typed as group A.

Keywords - Cat, transfusion, blood type, blood typing, immunochromatography.

INTRODUCTION

Veterinary transfusion medicine in Italy has progressed significantly over the last decade. Despite problems related to the characteristics of the species and to the limited availability of specific material, feline transfusion medicine has also undergone dramatic developments and ever more frequently a cat can now donate blood or receive a blood transfusion.

To reduce the occurrence of potentially fatal transfusion reactions in cats receiving a transfusion and to identify the most suitable blood donors, it is essential

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to have a blood test that is easy and fast to use and has proven ability to identify red blood cell antigens correctly.

The main blood group system in cats is called AB, and subjects can belong to group A, B or AB. In Italy the prevalence of group A varies from 87.1% to 92.3%[1-4] and can reach 100% in Maine Coon cats[4,5], the prevalence of group B ranges from 5.1% to 12.9%, while the AB group is the rarest, with a maximum prevalence of 5.7% in cats in Piedmont[1-4] and up to 18% in the Ragdoll breed[6]. There is also a recently discovered blood group called Mik[7] for which cats cannot currently be typed because of the absence of commercially available specific tests and reagents.

Cats have naturally occurring antibodies against non-self red blood cell antigens, called allo-antibodies, which are formed from a few months of age following stimulation by common environmental antigens. These alloantibodies, which are absent in cats with AB blood group, are present in all group B cats, in different proportions of group A subjects depending on the geographical origin of the animals, and in some Mik-negative cats. They may be responsible for serious transfusion reactions in the case of administration of incompatible blood, even at the first transfusion in subjects never previously transfused[7-12]. It is therefore essential to evaluate feline blood compatibility before each transfusion by determining the blood group and by administering group A blood to group A cats, group B blood to group B cats and group AB or A blood to group AB cats. Furthermore, even before the first transfusion in this species it is important to carry out cross-matching tests to identify any alloantibodies against blood groups that cannot be typed[13].

The available blood typing methods are based on agglutination reactions that can occur in a test-tube, on a slide, on a card, or on strips of material of a different nature. Test-tube agglutination technique is considered the gold standard test[14,15] with a 100% sensitivity and specificity in identifying AB blood system groups in the cat. However, this test requires hard-to-find reagents (such as group B plasma or Triticum vulgare lectin), experienced personnel for its implementation and long running times (about 55 minutes). For these reasons, it is generally used in transfusion centres to confirm blood groups that have raised major typing problems, such as group B and AB[14-18]. There are also more practical and quicker kits for outpatient use, including recently marketed tests based on immunochromatographic techniques[17,18].

It is essential to have a blood test that is easy and fast to use and has proven ability to identify red blood cell antigens correctly.

The aim of this study was to evaluate the diagnostic performance of a rapid immunochromatographic test for the determination of blood groups A, B and AB in the cat, comparing this test with the gold standard test-tube agglutination technique. The study evaluated the applicability of this kit to samples with different characteristics (haematocrit [Hct] within, below and above the normal range), mixed with various anticoagulant solutions (ethylene diaminotetra-acetic acid - EDTA - and citrate, phosphate, dextrose, adenine - CPDA) and stored at different temperatures for variable periods.

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MATERIALS AND METHODS

For the purposes of this study, 50 feline blood samples were typed double blind with a tube agglutination method and rapid immunochromatographic assay (Rapid-Vet® H-IC®, Feline Blood Typing, Agrolabo, Scarmagno, Turin, Italy). The 50 blood samples, collected into EDTA, were taken from 42 non-anaemic cats (mean Hct ± SD 34.3% ± 8.2; range, 24-45%) and eight anaemic cats (mean Hct ± SD 16.5% ± 5.1; range, 8.3-22%).

The blood groups of the cats typed as B or AB with the gold standard technique were confirmed using a technique that identifies alloantibodies (back-typing)[14,15]. Prior to determination of the blood group, the samples were evaluated for the presence of haemolysis and spontaneous agglutination.

The presence or absence of haemolysis was determined by evaluating the colour of the plasma of the whole blood sample after centrifugation at 1500 x g for 10 minutes. The presence or absence of agglutination was identified by macroscopic and/or microscopic (40X magnification) inspection of a drop of the blood sample previously mixed with a drop of physiological saline.

The performance of the immunochromatographic test was also evaluated by typing five samples artificially diluted with physiological saline (Hct <10%) and five samples that had been concentrated (Hct >45%)

Table 1 - Type of blood samples analysed to evaluate the performance of the rapid immunochromatographic technique for determining the AB blood group in cats
Type of sample analysed
42 samples with normal Hct (24-45%)
8 samples with Hct <24%
5 samples with Hct <10% (diluted with physiological saline)
5 samples with Hct > 45% (concentrated by reducing the proportion of plasma)
5 samples with CPDA anticoagulant (from feline blood bags)
3 samples stored at room temperature tested every 7 days for 4 weeks
3 samples stored at 4-6°C tested every 7 days for 4 weeks
3 samples stored at -20°C tested every 7 days for 4 weeks

by eliminating some of the plasma. Five blood samples from feline whole-blood bags containing CPDA anticoagulant (mean Hct ± SD 31% ± 7.2%; range, 27-42%) were also tested. Three EDTA blood samples stored at room temperature, three samples frozen at -20°C and three samples refrigerated at 4-6°C were tested every 7 days for 4 weeks. Finally, to evaluate the repeatability of the method a sample of each blood group was retested ten times. The characteristics of the samples analysed are summarised in Table I.

Surplus blood from samples typed for clinical purposes was used to carry out this study, so there was no need for ethics committee approval for its implementation. The patients' owners consented to the excess of the blood, taken for clinical purposes, being used for research aims.

RAPID IMMUNO-CHROMATOGRAPHIC TEST

The immunochromatographic test used is based on the migration of feline red blood cells on reactive strips in three distinct zones of a crescent-shaped plastic support, a strip for group A, one for group B and one for the control, on which specific anti-A, anti-B and anti-erythrocyte antibodies, respectively, are present. The red blood cells migrate on the membrane and, depending on their exposed antigens, bind to the specific antibody for the blood group, creating a red-coloured band. Each disposable kit is presented in a single wrapper to be stored at room temperature. The kit consists of a crescent-shaped device with a central well and three windows labelled "Group A", "Group B" and "Control", a test-tube containing a diluent, a disposable pipette and a bottle of diluent with a metered stopper. After placing the typing device on a flat surface, the pipette supplied in the kit is used to dispense one drop of a sample of anticoagulated blood (the manufacturer indicates using blood with EDTA or heparin anticoagulant) into the specific test-tube containing the diluent with which it is mixed. Three drops of the diluted blood sample and then three drops of diluent are taken and dispensed into the central well of the device.

At the end of 10 minutes to allow for red blood cell migration, the appearance of a vertical red line in the "Group A" window indicates that the blood group is A, a red vertical line in the "Group B" window indicates that the group is B, and vertical red lines in both windows indicate that the sample belongs to the AB blood group. A horizontal red line should always appear in the control window for results to be considered valid.

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TUBE AGGLUTINATION TEST

The reagent used in this test to identify group A red blood cells is the plasma of a group B cat, whereas the reagent to identify group B red blood cells is the lectin of Triticum vulgare, a substance that binds to the sialoglycoproteins contained in N-acetylneuraminic acid (the only neuraminic acid present in group B cats and present in limited quantities on group A red blood cells) but not to those contained in N-glycolylneuraminic acid (present on group A red blood cells)[19,20].

Briefly, the sample of blood to be tested is centrifuged to separate the plasma from the red blood cell pellets. The red blood cells are washed three times with physiological saline and at the end of the washing a 5% erythrocyte suspension is prepared for testing (475 µL of saline and 25 µL of washed red blood cells). Fifty microlitres of a 5% suspension of the washed red blood cells to be typed are placed in each of three glass test-tubes before adding 100 µL of group B feline plasma to the first tube (identified as A), 100 µL of a solution containing Triticum vulgare lectin to the second tube (identified as B), and 100 µL of physiological saline to the last test-tube (identified as the

control). The tubes are then incubated at room temperature for 15 minutes and centrifuged at 1200 x g for 15 seconds. After centrifugation, the red blood cells are re-suspended by gently shaking the test-tube and the presence or absence of agglutination is evaluated macroscopically.

The blood samples are typed as group A if the agglutination occurs in the tube identified by A, group B if it occurs in test-tube B and group AB if it occurs in both tubes.

THE ALLOANTIBODY TEST (BACK-TYPING)

This test is used to confirm that a blood sample belongs to group B or AB. The principle is based on the search for alloantibodies targeting group A red blood cell antigens, which are always present in group B subjects, and absent in group AB cats. Briefly, 50 µL of plasma from cats typed as group B or AB are added to a test-tube containing 25 µL of a 5% suspension of group A red blood cells. After incubation at room temperature for 15 minutes and subsequent centrifugation at 1200 x g for 15 seconds, the red blood cells are re-suspended by gently shaking the test-tube. Macroscopically visible agglutination will be present in the case of group B plasma and absent in the case of plasma from a group AB cat.

STATISTICAL ANALYSIS

The results of both techniques were analysed statistically in order to calculate the sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the immunochromatographic method versus tube agglutination test. The concordance of the results was evaluated by Cohen's test (unweighted Kappa test). Kappa (K) values have been attributed the following degree of concordance[21]:

- <0.40 poor
- 0.41-0.60 moderate
- 0.61-0.80 good
- 0.81-1.00 excellent

The statistical analysis was performed using MedClac statistical software (version 16.4.3). The results are expressed as percentages with 95% confidence intervals (95% CI).

The results of both techniques were analysed statistically in order to calculate the sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the immunochromatographic method.

RESULTS

No sample showed haemolysis or spontaneous agglutination. According to the tube agglutination test 36 samples (72%) were group A, 9 (18%) were group B and 5 (10%) were group AB.

The immunochromatographic test was able to type all the samples, showing a good overall concordance with the results of the gold-standard technique (K = 0.75; 95% CI: 0.55-0.95) with identical results being obtained in 45 of the 50 samples (90%) (Table 2). Examples of the results for group A and group B samples are shown in Figure 1A and 1B, respectively.

The five samples that gave non-concordant results were four AB samples typed as A by immunochromatography and one group A sample mistakenly identified as group AB (Table 2).

The Se, Sp, PPV, NPV, and Kappa values for each blood group (Table 3) indicated that the performance of the immunochromatographic test was good for the identification of group A blood and excellent for group B. However, the ability to type group AB sam-

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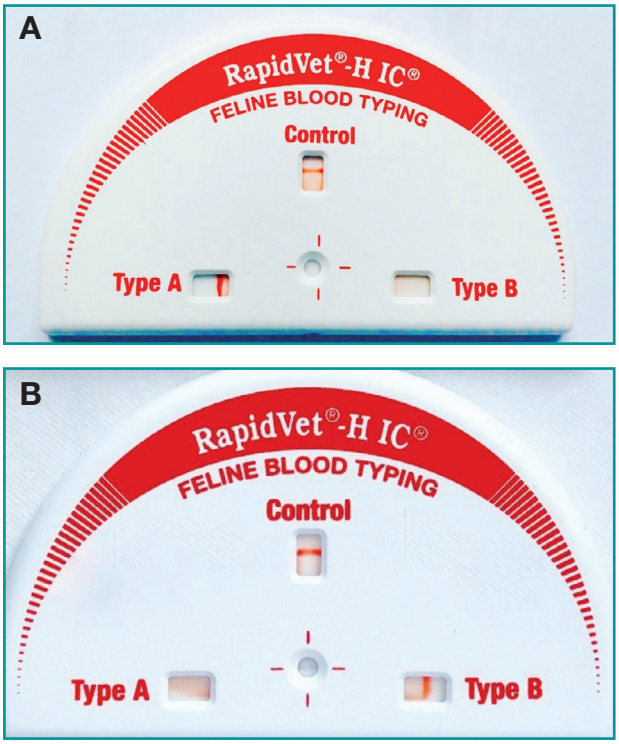

Figura 1 - Typing results of feline blood in EDTA analysed using the rapid immunochromatographic technique: (**A**) group A and (**B**) group B.

Table 2 - Results of typing 50 EDTA feline blood samples using the gold-standard, tube agglutination test and the immunochromatographic technique

	Tube agglutination test			
Immunochromatography	**A**	**B**	**AB**	**Total**
A	35	0	4	39
B	0	9	0	9
AB	1	0	1	2
Total	36	9	5	50

ples correctly was poor and most such cases (4/5) were identified as group A by the immunochromatographic technique.

The immunochromatographic test was able to type all the diluted, concentrated, and EDTA- and CPDA-anticoagulated samples as well as those stored at room temperature for up to 7 days and samples stored at 4-6°C for up to 4 weeks. The test was not able to type frozen samples. The results of testing the same sample ten times showed that the technique had a repeatability of 100%.

DISCUSSION

In feline transfusion medicine, just as in canine medicine, it is crucial to have a rapid and easy-to-use test to determine an animal's blood group in emergency situations. However, the speed with which the results are provided should not be at the cost of the accuracy of the test, as errors in typing could increase the occurrence of fatal transfusion reactions, which may develop in felines even at the first transfusion of a group B cat given group A blood[8-12].

The most serious immunological transfusion reactions in cats are caused by the presence, in group B individuals, of alloantibodies to group A red blood cell antigens. These antibodies are high-titre immunoglobulins (from >1:32 up to 1:2048) with haemolytic (IgG and IgM) and agglutinating (IgM) effects and may be responsible for serious reactions even after the inoculation of only 1 mL of group A blood[8]. The prevalence of blood group B in cats surveyed in Italy varies, ranging from 5.1% in donor cats from northern and central Italy[4] to 12.9% among cats in the centre of Italy[1].

These group B subjects can develop fatal transfusion reactions if not properly typed. It is therefore crucial not only to have rapid tests, but above all tests that have 100% sensitivity and specificity for identifying group B cats, characteristics which the quick immunochromatography test evaluated in this study has been demonstrated to possess.

Given the presence and characteristics of the alloantibodies, cats must be transfused with blood of the same blood group for which cross-matching tests have been negative for agglutination and/or haemolysis. The AB blood group is very rare in the general feline population with a prevalence ranging from 0% in

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Table 3- Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) as percentages and the Kappa value of the rapid immunochromatographic test compared to tube agglutination test for typing 50 samples of feline whole blood in EDTA anticoagulant

Group	**Se** (95%CI)	**Sp** (95%CI)	**VPP** (95%CI)	**VPN** (95%CI)	**KAPPA**
A	97.2 (85.4-99.9)	71.4 (41.9-91.6)	89.7 (79.2-95.2)	90.9 (58.4-98.6)	0.73
B	100 (66.3-100)	100 (91.4-100)	100	100	1.00
AB	20 (0.5-71.6)	97.7 (88.2-99.9)	50 (6.8-93.1)	91.6 (87.6-94.4)	0.24

cats in the centre of Italy[1] to 5.7% in cats in northern Italy[2]. This epidemiological feature often makes it extremely difficult to obtain donor cats or bags of AB group blood. Given the absence of alloantibodies in these subjects, they can be transfused with group A blood provided that cross-matching tests are negative[13]. Cross-matching tests could give a positive result and, therefore, indicate incompatibility between a group AB recipient and a group A donor if this latter has anti-B alloantibodies. In this case, a group A packed red blood cells concentrate can be given, avoiding administration of the plasma component containing the anti-B alloantibodies.

The immunochromatographic method evaluated in our study often confused group AB samples, incorrectly typing them as group A. This error may not have serious clinical consequences, but must be considered in breeds in which the prevalence of the AB group is high, such as Ragdoll cats[6], which could be selected as "universal" plasma donors. Moreover, this limitation makes the immunochromatographic method evaluated unsuitable for epidemiological studies in which the distribution of the three blood groups in the feline population is to be investigated since the real prevalence of blood groups A and AB would not be revealed.

The results of this study agree with an earlier evaluation of this technique performed by Hourani et al.[17]. In fact, as in this work, the performance regarding the identification of group A and B samples was good, but the same difficulties with AB typing emerged. The typing difficulties that this and other methods, such as card agglutination, have shown with regards to group AB cats[3,14-16] may arise because AB group erythrocytes have at least two phenotypes that differ by the amount of A antigen expressed on the surface of the red blood cells[22]. Unlike the study by Hourani et al.[17] in our study no sample showed inconclusive results. This may be due to recent improvements to the kits made by the manufacturer.

The only samples that the immunochromatographic technique was not able to type were those of frozen blood. This characteristic, also found using other methods based on the antigen and antibody reaction[18], may be explained by the fact that the erythrocyte membrane is completely lysed during freezing.

The immunochromatographic technique was able to type refrigerated samples for up to 1 month and samples stored at room temperature for up to 7 days. This feature may be useful if it becomes necessary to determine a patient's blood group at a later stage than the baseline analysis, or when the sample is shipped to an outside laboratory and it is not sure that the storage temperature has been maintained during the transport.

Finally, the possibility of typing blood collected into standard anticoagulants used in transfusion medicine, such as CPDA, may be useful if blood tests are to be carried out on blood bags. This ability to type CPDA samples may also be useful following fatal transfusion reactions in apparently blood group-compatible recipients, when it is desirable to perform control tests to exclude an error of typing donor blood that has been stored in blood bags.

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The main limitations of this study are the low number of samples evaluated and the failure to assess the performance of the immunochromatographic technique in spontaneously agglutinated or haemolysed samples. Both spontaneous agglutination and haemolysis may interfere with blood-typing methods and may occur in patients with anaemia of autoimmune origin (such as immune-mediated anaemia or anaemia caused by feline leukaemia virus or haemoplasma infections) or toxic nature (such as in paracetamol poisoning), pathological conditions that often require a blood transfusion as supportive therapy. Finally, the health of the subjects from which the typed samples came from was not analysed, although such information could have been useful to identify and characterise possible differences in the performance of the test under investigation. Indeed, it has emerged from some studies[15,17] that there are often difficulties in typing anaemic subjects infected by feline leukaemia virus.

In conclusion, the Rapid-Vet® H-IC® Immunochromatographic Feline Blood Typing Test is a fast, easy-to-perform test that can identify, with high sensitivity and specificity, group A, the most commonly found blood group in the feline population,[1-6] and group B, the most problematic in feline blood transfusions.[9-12] With this technique, most of the rare AB group cats were identified as belonging to group A.

The immunochromatographic identify sensitivity and specificity, group A, the most commonly found blood group in the feline population, and group B, the most problematic in feline blood transfusions.

KEY POINTS

- It is essential to administer compatible blood also at the first transfusion in order to prevent fatal transfusion reactions in the cat.
- Blood typing test should be quick and easy to use and have proven ability to identify the blood group correctly.
- The Rapid-Vet® H-IC® Immunochromatographic Feline Blood Typing kit showed good concordance with tube agglutination test (K = 0.75) giving identical results for 45 out of 50 samples (90%).
- Immunochromatography has a high sensitivity and specificity for identifying blood groups A and B
- Immunochromatography may mistakenly type rare AB group cats as group A.

BIBLIOGRAFIA

1. Continenza R, Lubas G, Gigliucci B. Indagini preliminari sul sistema di gruppo sanguigno AB nel gatto allevato in Italia. XLVI Congresso SISVet, Venezia, 1992, pp. 1470-1477.
2. Cavana P, Picco S, Bellino C *et al.* Distribuzione dei gruppi sanguigni nei gatti della regione Piemonte (Italia). 55° Congresso Nazionale SCIVAC-SIMEF Medicina Felina "Le nuove acquisizioni nella pratica clinica", Milano, 2007, pp. 439-440.
3. Proverbio D, Spada E, Baggiani L *et al.* Comparison of gel column agglutination with monoclonal antibodies and card agglutination methods for assessing the feline AB group system and a frequency study of feline blood types in northern Italy. Veterinary Clinical Pathology 40:32-39, 2011.
4. Spada E, Miglio A, Proverbio D *et al.* Signalment and blood types in cats being evaluated as blood donors at two italian university blood banks. Veterinary Medicine International Article ID:704836:1-3, 2014.
5. Spada E, Antognoni MT, Proverbio D *et al.* Haematological and biochemical reference intervals in adult Maine Coon cat blood donors. Journal of Feline Medicine and Surgery 17:1020-1027, 2015.
6. Proverbio D, Spada E, Perego R *et al.* Assessment of blood types of Ragdoll cats for transfusion purposes. Veterinary Clinical Pathology 42:157-162, 2013.
7. Weinstein NM, Blais M-C, Harris K *et al.* A newly recognized blood group in domestic shorthair cats: the mik red cell antigen. Journal of Veterinary Internal Medicine 21:287-292, 2007.
8. Bucheler J, Giger U. Alloantibodies against A and B blood types in cats. Veterinary Immunology and Immunopathology 38:283-295, 1993.
9. Giger U, Akol KG. Acute hemolytic transfusion reaction in an Abyssinian cat with blood type B. Journal of Veterinary Internal Medicine 4:315-316, 1990.
10. Giger U, Bucheler J. Transfusion of type-A and type-B blood to cats. Journal of the American Veterinary Medical Association 198:411-418, 1991.
11. Auer L, Bell K. Transfusion reactions in cats due to AB blood group incompatibility. Research in Veterinary Science 35:145-152, 1983.
12. Auer L, Bell K, Coates S. Blood transfusion reactions in the cat. Journal of the American Veterinary Medical Association 180:729-730, 1982.
13. Spada E, Baggiani L. Test di compatibilità pretrasfusionale. In: Proverbio D. Ed. Manuale di medicina trasfusionale del cane e del gatto. Milano: Point Veterinarie Italie, 2017, pp. 127-134.
14. Stieger K, Palos H, Giger U. Comparison of various blood-typing methods for the feline AB blood group system. American Journal of Veterinary Research 66:1393-1399, 2005.
15. Seth M, Jackson KV, Giger U. Comparison of five blood-typing methods for the feline AB blood group system. American Journal of Veterinary Research 72:203-209, 2011.
16. Proverbio D, Spada E, Baggiani L *et al.* Assessment of a gel column technique for feline blood typing. Veterinary Research Communications 33S:201-203, 2009.
17. Hourani L, Weingart C, Kohn B. Evaluation of a novel feline AB blood typing device. Journal of Feline Medicine and Surgery 16:826-831, 2014.
18. Spada E, Proverbio D, Baggiani L *et al.* Evaluation of an immunochromatographic test for feline AB system blood typing. Journal of Veterinary Emergency and Critical Care 26:137-141, 2016.
19. Bhavanandan VP, Katlic AW. The interaction of wheat germ agglutinin with sialoglycoproteins. The Journal of Biological Chemistry 254:4000-4008, 1979.
20. Butler M, Andrews GA, Smith JE. Reactivity of lectins with feline erythrocytes. Comparative Haematology International 1:217-219, 1991.
21. Cohen J. A coefficient of agreement for nominal scales. Educational and Psychological Measurement 20:37-46, 1960.
22. Green JL, Andrews GA, Wyatt CR. Phenotypic differences within the AB blood type of the feline AB blood group system. Comparative Clinical Pathology 14:138-145, 2005.