How to blood type and cross-match

Abstract

In the UK blood components (products), packed red blood cells, fresh frozen plasma, frozen plasma, cryo-precipitate and cryo-supernatant have only been available since the emergence of blood banks 5 years ago. This has led to advancement in transfusion medicine and a positive outcome for thousands of patients whose previous treatment options were restricted due to the lack of available blood. To date in the UK only canine blood products are available with feline transfusion patients being restricted to the option of fresh whole blood donated from emergency donors at the time.

With blood products now more widely available a greater understanding of using them becomes ever more important not only to ensure transfusions are being carried out safely but also to make best use of the blood donated by donors volunteered by their owners. Blood typing and cross-matching are two screening tests recommended to be performed prior to canine and feline transfusions.

Key words: blood type, antigen, compatibility, cross-match, alloantibody

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Wendy Barnett DipAVN (Surgical) RVN is Executive Director of Pet Blood Bank UK

Blood typing background

Dogs and cats, like humans, have blood types — these are species specific.

Dogs

The different canine blood types are described as Dog Erythrocyte Antigens (DEA). In simple terms these are markers on the surface of the red cells. There are eight DEA antigen systems 1.0, 3, 4, 5, 6, 7, 8 and *Dal* that have been determined through studies to have the potential to cause acute or delayed immunological transfusion reactions (*Table 1*). There may be many more that have not been defined. Dog blood group systems are inherited independently which allows them to coexist on the surface of the red cell allowing dogs to have more than one blood type.

For the purpose of this article the author will concentrate on the only canine blood type that testing is widely available for and that has the most transfusion significance in terms of acute immunologic transfusion reactions: DEA 1.1.

Dogs are either described as DEA 1.1 positive where there is 1.1 antigen on the surface of the red cells or DEA 1.1 negative where no 1.1 antigen is present on the surface of the red cells. Three commercial typing test kits are available to veterinary practices to use 'in house' with one of the most common being described in detail in the *Step-by-step guide* of this article. With regards to the other blood group systems, identification of these is limited to individual laboratories that are internationally distributed, mainly in the USA. Extended typing is of use when dealing with complex multiple transfusion patients and transplant patients. Most of the research into canine blood types used today was performed in 1949 (Young et al, 1949). The science of companion animal immunohaematology will continue to develop as there are a lot of unanswered questions making transfusion medicine an ever-evolving field in the veterinary profession.

Cats

Feline blood groups are inherited. Our understanding is that they have a relatively simple blood group system. Feline blood groups are described as A, B or AB blood type (Day, 2012).

There are three alleles that control the AB blood type. The *A* allele is dominant over the b allele and the phenotype AB is the result of the third allele (a^{ab}) this allows co-dominance expression of both A and B. Cat breeders are likely to ask for their breeding cats to be blood typed due to its importance in reducing neonatal isoerythrolysis in their kittens. The prevalence of blood types varies with breed and also by country, refer to *Table 2* supplied by Feline Advisory Bureau for Type B breeds and www.fabcats.org for more information.

Compatibility

Identifying blood type antigens is one part of compatibility; the other is ensuring any blood given is not going to be removed from the patient's circulatory system prematurely. Alloantibodies are antibodies that are made against antigens occurring naturally within the same species. Alloantibodies at-

tach to antigens on red cells and may initiate a process that causes destruction or removal of any transfused red cells containing that antigen. Alloantibodies can be naturally occurring or occur due to previous sensitizing to a foreign antigen generally 4–7 days post exposure.

Dogs

Dogs rarely have any naturally occurring alloantibodies in their circulation (*Table 1*). Sensitization from a previous transfusion with incompatible blood is the main cause of alloantibodies.

On first transfusion DEA 1.1 positive blood should only be administered to a DEA 1.1 positive patient to prevent sensitizing the DEA 1.1 negative patient to the foreign 1.1 antigen and thus creating the potential for production of alloantibodies. Subsequent administration of DEA 1.1 positive blood to a sensitized DEA 1.1 negative patient can produce an acute immune-mediated transfusion reaction which is potentially life threatening and must be avoided by ensuring the correct blood type is administered.

DEA 1.1 negative blood should be administered to DEA 1.1 negative patients. Although no adverse effects occur when administering DEA 1.1 negative blood to a DEA 1.1 positive patient, DEA 1.1 negative donors make up just over 30% of the dog population presented as donors. Using DEA 1.1 negative blood on DEA 1.1 positive patients has ethical and welfare implications, most poignantly it means stocks of DEA 1.1 negative blood could subsequently be depleted to a level that makes it unavailable to DEA 1.1 negative patients (*Table 3*).

Table 1. Canine blood group systems showing their antigen phenotypes and prevalence in the population. Null phenotype means the dog does not carry genes for the expression of the antigen

Canine blood group system	Antigen phenotypes	Population prevalence	Incidence of naturally occurring antibody	Comments
1.0	1.1, 1.2, 1.3, null	62%, 2%, 0.1%	< 2%	Commercial typing system available for 1.1 only
3	3, null	5%	8–15%	
4	4, null	98%	Rare	
5	5, null	15%	8–12%	
6	6, null	96%	Unknown	No available typing system
7	7, null	40–55%	10–40%	
8	8, null	20–40%	Unknown	No available typing system
Dal	Dal, null	99%	Rare	No commercial typing system

***Data to be published in 2012 by Pet Blood Bank UK indicates a higher percentage of DEA 1.1 positive dogs in their donor population.**

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Table 2 Estimated frequency of type B cats in various breeds

NB: For some breeds only small numbers of cats have been tested, so the figures may not be as accurate as they would be if results were available for larger numbers of cats. The proportion of group B cats within a breed may change with time, depending on breeding choices and patterns within that breed.

Only type A	Low type B frequency (1-10%)	Intermediate type B frequency (10-25%)	High type B frequency >25%)
Siamese*	American Shorthair*	Abyssinian*	British Shorthair* ^
Tonkinese*	Maine Coon*	Birman* ^ †	Cornish Rex*
Oriental Shorthair*	Manx*	Burmese^	Devon Rex* †
	Norwegian Forest*	Himalayan*	Exotic*
	Bengal**	Persian* ^	Ragdoll*
		Scottish Fold*	Turkish Van*
		Somali*	Turkish Angora *
		Sphynx* †	

*** Figures supplied by Dr Giger, University of Pennsylvania**
^ Figures from a study of UK cats conducted by C Knottenbelt, University of Glasgow
† Figures supplied by Dr Addie, University of Glasgow
**** Figures supplied by Professor D Gunn-Moore, Edinburgh University**

Cats

Unlike dogs, A and B cats develop naturally alloantibodies to other blood groups within the first few months of life (AB cats do not) (Knottenbelt et al, 1999). This means cats are at great risk of transfusion reaction if blood typing is not performed prior to the initial transfusion to allow the correct blood type to be administered. Basic compatibility in cats is illustrated in *Table 4*.

Type A cats generally have low levels of anti-B alloantibodies in their serum whereas type B cats usually have high levels of anti-A alloantibodies in their serum; AB type cats have no alloantibodies to either A or B however donors are hard to find. Due to the low level of anti-B alloantibodies in serum if a search for a rare AB type matched donor is unsuccessful, it is recommended to use a type A donor for transfusion, some reaction will be seen. Type A packed red cells or washed type A red blood cells would be even better — however these are not usually available or produceable in general veterinary practice.

In-house available blood typing tests

- **Alvedia** (France) **Feline and Canine Quick Typing Tests** (single units) work in the presence of agglutination (such as immune-mediated haemolytic anaemia and autoimmune haemolytic anaemia) and at low packed cell volume levels. Their immuno-chromatography test strips are impregnated with monoclonal antibodies

Step-by-step guide to typing

Step 1 *(Figure 1)*

- *Write down the name of the patient.*

Step 2 *(Figure 2)*

- Add three drops of buffer into the well.

Step 3 *(Figure 3)*

- Dip the blood collector strip into the blood tube or rub it on the cat's umbilical cord. The extremity of the collector strip must be fully impregnated.

Step 4 *(Figure 4)*

- Dip the extremity of the collector strip into well and mix gently (7 secs).

Step 5 *(Figure 5)*

- Separate the plastic device in two parts by pulling out the membrane holder.

Step 6 *(Figure 6)*

- Insert the legs of the membrane holder into the holes beside the well, so that the membrane end reaches the bottom of the well containing the diluted blood.

Step 7 *(Figure 7)*

- Allow to stand until the blood suspension is soaked up by the membrane and the control line becomes clearly visible (2 mins).

Step 8 *(Figure 8)*

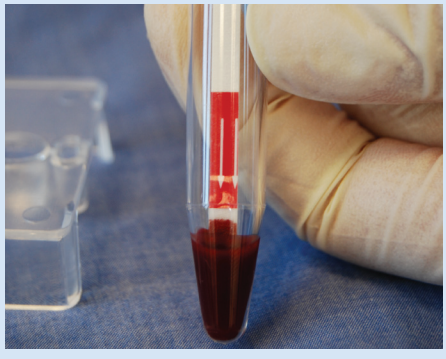
- Click back the membrane holder into the plastic device in order to read the results.

Step 9 *(Figure 9)*

- Read the results immediately after testing.

Figure 1.


Figure 2.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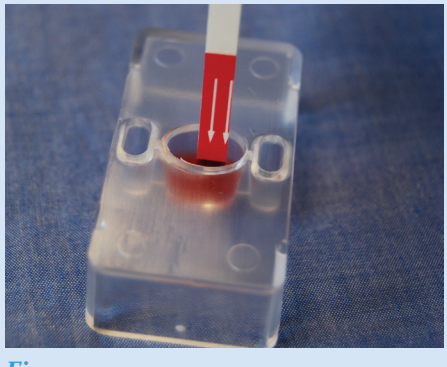


Figure 4.


Figure 5.

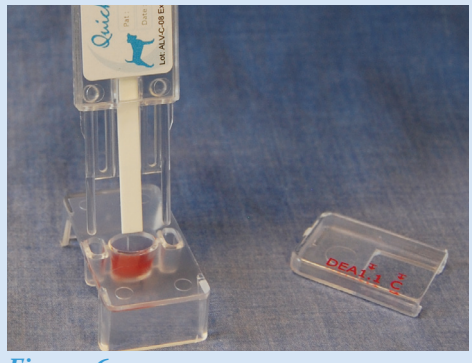


Figure 6

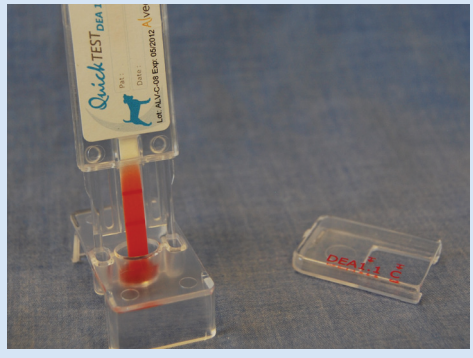


Figure 7.

Figure 8

Figure 9.

and display results in 5 minutes (see *Step-by-step guide*).

- **Woodley** (UK) **(DMS) Rapid Vet H Feline and Canine Tests (pack of 5)** uses a murine monoclonal antibody on a test card to create an agglutination reaction to show results — this however makes them problematic to use in patients that are auto agglutinating.
- **Woodley (DMS) Rapid Vet H IC Feline and Canine Tests (pack of 5 or 10)** are likely to replace the original Woodley (DMS) Rapid Vet H agglutination test with its newer technology they work in the presence of agglutination and low PCV levels. These new test kits use immuno-chromatographic technology impregnated with a monoclonal antibody displaying results in 5–10 minutes.

Cross-matching

Dogs

As discussed in the introductory typing section above, dogs have many blood types that can coexist on the surface of their red cells and with the absence of in-house testing kits for all of these blood types, DEA 1.1 is the only status a general practitioner can detect quickly and easily. It must be assumed that even when DEA 1.1 typed blood is given correctly to patients there is still the potential to introduce other antigens that may be seen as foreign and that in turn the patient may create antibodies against them (Young et al, 1949). It is therefore recommended in dogs to perform a cross-match prior to transfusion if:

- The transfusion history of the dog is unknown
- Previous transfusions have caused a reaction
- A transfusion has been administered previously — >4days after a transfusion. A cross-match should be carried out prior to transfusion for the lifetime of the dog.

Cats

Cats are likely to have additional blood types recognized as transfusion medicine advances, as an example in 2007 the *Mik* antigen was discovered (Weinstein et al, 2007) and no in-house testing kits are available to date. For these potential rare instances cross-matching cats prior to any transfusion is recommended.

Cross-matching techniques

Cross-matching is an in vitro test that looks for potential reactions between a donor's and patient's blood, these show as agglutination or haemolysis. Agglutination is more commonly seen in canine incompatibilities — haemolysis is less common. In cats both agglutination and haemolysis can be seen.

Performing a cross-match for dogs with immune-mediated haemolytic anaemia can be challenging due to background agglutination and haemolysis. It is therefore recommended to transfuse the patient with red cells that are the 'most' cross-match compatible in these circumstances.

The cross-match test required depends on the type of product being transfused:

Step-by-step guide to cross-matching

Test requirements *(Figure 1)*
- Blood samples from both donor (EDTA 0.5 ml) and recipent (Serum 2.0 ml)
- A centrifuge to hold 1.3 ml blood tubes
- Stopwatch.

Step 1 *(Figure 2)*
- Add 10 drops (0.5 ml) donor sample to the blue top preparation tube using a clean pipette
- Cap tube and invert several times to mix thoroughly.

Step 2 *(Figure 3)*
- Transfer 4 drops (200 µl) recipient serum to yellow top reaction tube using a clean pipette.

Step 3 *(Figure 4)*
- Transfer 2 drops (100 µl) from the blue top donor preparation tube into the red top positive control tube using a clean pipette.

Step 4 *(Figure 5)*
- Transfer 2 drops (100 µl) from the blue top donor preparation tube into the green top negative control tube using a clean pipette.

Step 5 *(Figure 6)*
- Transfer 2 drops (100 µl) from the blue top donor preparation tube into the yellow top reaction tube using a clean pipette.

Step 6 *(Figure 7)*
- Incubate
- Cap the tubes tightly and invert several times to mix thoroughly
- Let all tubes stand for 5 minutes at room temperature (20–27°C).

Step 7 *(Figure 8)*
- Transfer 1 drop (50 µl) from the yellow top reaction tube to the clear top reaction gel tube (yellow label) using a clean pipette
- Cap tightly.

Step 8 *(Figure 9)*
- Transfer 1 drop (50 µl) from the green top negative control tube to the clear top reaction gel tube (green label) using a clean pipette
- Cap tightly.

Step 9 *(Figure 10)*
- Transfer 1 drop (50 µl) from the red top reaction tube to the clear top positive reaction gel tube (red label) using a clean pipette
- Cap tightly.

Step 10 *(Figure 11)*
- Centrifuge gel tubes
- Correct centrifugation is essential.

Step 11 *(Figure 12)*
- Following centrifugation remove the gel tubes.

Step 12 *(Figure 13)*
- Interpret and report results — first interpret positive and negative control using the photo identifies provided
- Negative control — gel tube (green label) should demonstrate a collection of red blood cells at the bottom of the gel column
- Positive control — gel tube (red label) should demonstrate a collection of red blood cells at the top of the gel column
- If positive and negative controls do not react as stated, DO NOT proceed with the interpretation of the test.

Step 13 *(Figure 14)*
- Cross-match interpretation — Interpret clear top reaction tube (yellow label) using the photo identifier provided. Record results on the report card
- A positive cross-match indicates the patient is at risk from a transfusion reaction from the donor, DO NOT transfuse using this donor
- A negative cross-match indicates the recipient is likely NOT at risk from a major transfusion reaction from the donor.

Figure 1.

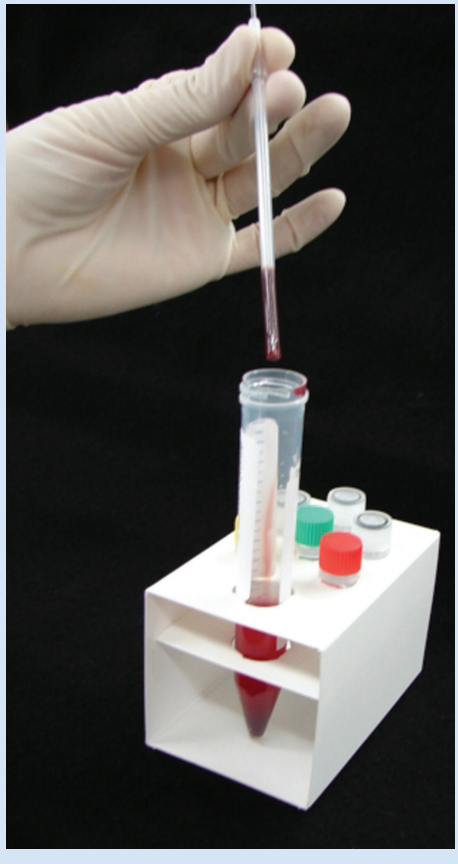


Figure 2.

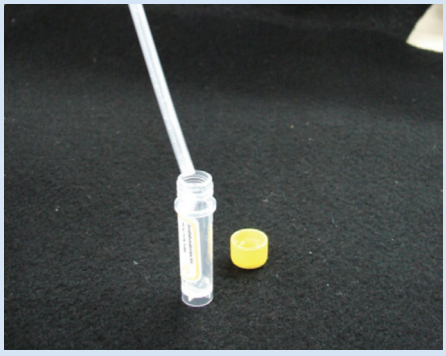


Figure 3.

Figure 7.

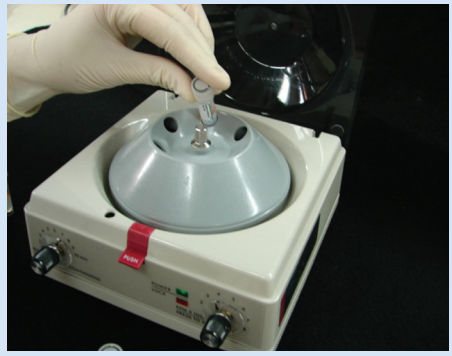
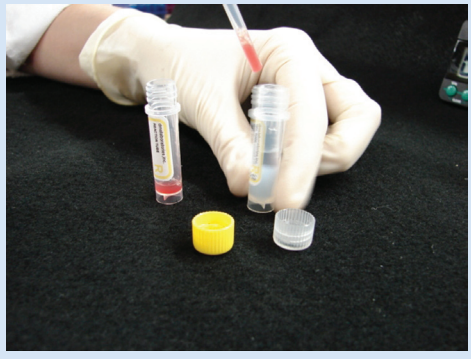


Figure 11.

Figure 4.



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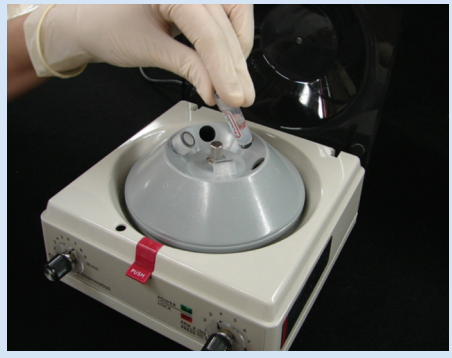

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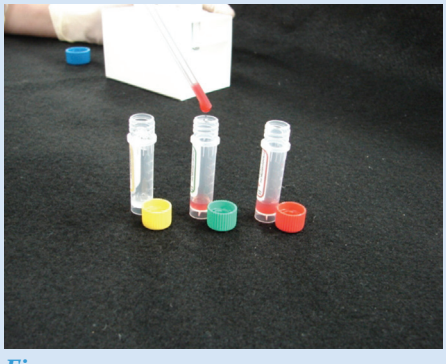

Figure 5.

Figure 9.

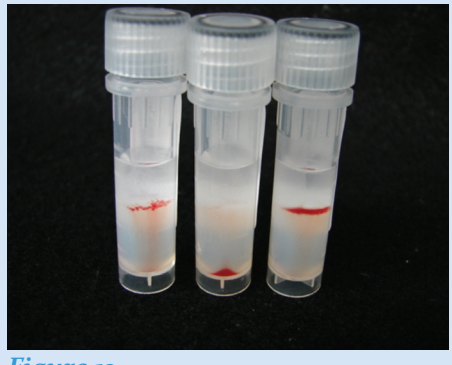

Figure 13.

Figure 6.

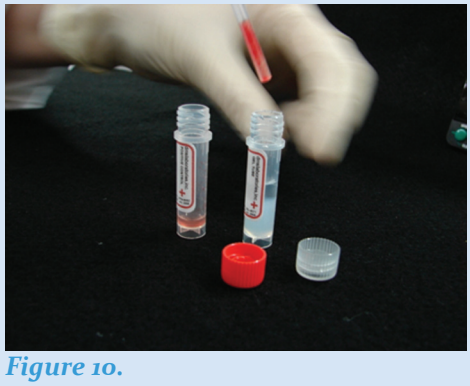

Figure 10.

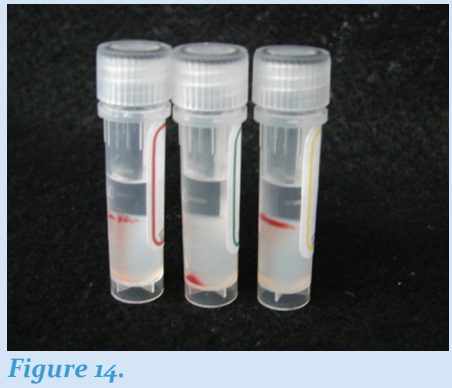

Figure 14.

major cross-match for red cell products and minor cross-match for plasma products. Anticoagulated whole blood contains both red blood cells and plasma therefore performing both a major and minor cross-match is recommended when administering whole blood:

- Major cross-match assesses the compatibility between donor red blood cells and patient plasma/serum
- Minor cross-match assesses the compatibility between donor plasma/serum and patient red blood cells.

Cross-matching can be performed in a laboratory with minimal equipment and takes up to 60 minutes or an in-house gel cross-match kit that identifies agglutination reactions is simple to use, and provide a quicker solution taking 15–20 minutes to perform. Alternatively blood from both donor and patient can be submitted to many commercial laboratories for cross-matching but this can take up to 24–48 hours before results are available.

In-house available cross-matching tests

- **Woodley (DMS) Rapid Vet H Major Cross-Match Tests — (3 in a box)** can be used for both feline and canine species in detecting red cell agglutination (see *Step-by-step guide*).
- **Manual slide and tube cross-match methods** — refer to **www.petbloodbankuk.org** for a full fact sheet with step-by-step guide.

Conclusion

In house, clinically relevant blood typing is a quick and easy procedure that reduces the risk of transfusion reactions and should be performed in both dogs and cats prior to initial transfusion. Cross-matching should be considered in all feline patients due to the discovery of the *Mik* antigen and other undiscovered antigens that are thought to exist. All canine patients where time allows should have a cross-match performed as gold standard however this is often not practical. It is however essential if a canine patient requires a subsequent transfusion and has received blood products more than 4 days previously. VN

Table 3. Canine blood type compatibility showing the red blood cell product that should be used when performing a red blood cell transfusion

Donor blood type	Patient blood type	
	DEA 1.1 negative	DEA 1.1 positive
DEA 1.1 negative	Yes	Emergency only — will deplete valuable stock levels
DEA 1.1 positive	No will sensitize patient	Yes

Table 4. Feline blood type compatibility

Donor blood type	Patient blood type		
	A	B	AB
A	OK	May be fatal	Possible reaction
B	Reaction	OK	Reaction
AB	Reaction	May be fatal	OK

Key Points

- Blood typing should be performed prior to the initial transfusion in both cats and dogs to ensure blood of the same type is used for the transfusion.
- Cats have naturally occurring alloantibodies and fatal transfusions reactions may be seen when blood of a different type is administered.
- Cross-matching must be performed in cats prior to all transfusions due to the potential presence of unknown antigens i.e *Mik* that cannot be detected on a typing kit.
- Antibodies can be produced 4 days post exposure to a foreign antigen therefore cross-matching must be performed in dogs prior to any subsequent transfusion after this time for the lifetime of the dog.
- Transfusion medicine is an ever evolving field and there is still much to learn about the antigens that have yet to be detected.

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