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FELINE BLOOD TYPES & BLOOD TRANSFUSIONS

PART I:
INTRODUCTION & DONOR SELECTION

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In this edition:

The administration of blood to feline patients can be a lifesaving procedure. In order that blood transfusions are performed properly, knowledge of feline blood types and the methods of blood collection and administration is required. This article will focus on the reasons why feline blood types are so important in feline transfusion medicine as well as donor cat selection.

Indications for blood transfusion

Given the number of practical steps involved, the process takes time and it is not without risk to both donor and recipient, so careful patient selection is necessary. A blood transfusion must be the most appropriate treatment and other treatment options should have been exhausted first.

The most common indications for feline transfusion are:

- **Severe anaemia** in a patient showing clinical signs due to reduced oxygen carrying capacity (e.g. tachycardia, tachypnoea, lethargy, inappetence). This is especially likely with anaemia that develops acutely. In cases of haemolysis, the patient is likely to be normovolaemic as there has been no loss of plasma volume, and therefore packed red blood cells (pRBCs) would be the most appropriate transfusion. Unfortunately, these are not readily available and so whole blood is usually given, with care taken over the rate of administration.

- **Haemorrhage** either due to trauma or surgical intervention. A transfusion may be performed in response to trauma, surgery or pre-operatively if haemorrhage is anticipated during a complicated procedure. In acute haemorrhage, the concurrent loss of both red blood cells (RBC) and plasma volume means measurement of the patient's packed cell volume (PCV) may be initially normal, with the anaemia only becoming apparent once fluid redistributes to the intravascular compartment.

- **Coagulopathy** as a result of severe hepatic disease, rodenticide toxicity or an inherited coagulopathic condition. A whole blood transfusion will provide clotting factors within the plasma. Separation of the RBCs from the plasma could be considered, but centrifugation is not usually available, and plasma alone is not readily available to purchase. Clotting factors degrade within hours, even with refrigeration, and so the transfusion must be performed in a timely fashion.

It should be noted that a whole blood transfusion is a poor source of platelets for thrombocytopenic patients, especially with ongoing immune-mediated destruction. Similarly, whole blood or plasma transfusions will not significantly improve the serum protein levels in a hypoproteinaemic patient as transfused proteins do not remain long within the intravascular compartment and are not in high concentrations (compared to concentrated albumin products used in human transfusion medicine).

Possible transfusion triggers that can be used in conjunction with clinical signs suggestive of reduced oxygen carrying capacity are *(Barfield and Adamantos, 2011)*:

- Chronic non-regenerative anaemia with a PCV < 10 %
- Ongoing haemorrhage with a PCV < 15 %
- Acute haemorrhage with hypoperfusion with a PCV < 15 %

Feline blood types

Blood types result from the presence of genetically determined antigenic markers on the surface of the RBCs. The blood type antigens are alloantigens as they exist in alternative (allelic) forms in different cats and can induce an

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immune response when one blood type is transferred to a cat that lacks it.
Cats have one blood group system called the AB system. Within this system, there are 3 blood type phenotypes; type A, type B and type AB.

The frequency of the different blood types in cat populations shows great geographical variation

(Gunn-Moore et al, 2009; Forcada et al, 2007; Knottenbelt et al, 1999):

- Type A is the most common blood type. Studies have identified a prevalence of 67 – 87 % in non-pedigree cats and high prevalence in some pedigree breeds, e.g. Siamese, Bengal, Birman and Persian.
  Type B is less common, although it may be prevalent in some pedigree breeds. Studies have identified a prevalence of 7.9 – 30 % in non-pedigree cats and a high prevalence in some pedigree breeds, e.g. British Shorthair, Ragdoll, Birman and Rex.
- Type AB is regarded as rare. Studies have identified a prevalence of 1.9 – 5 % and 2.6 – 25.4 % in non-pedigree and pedigree cats, respectively.

In contrast to dogs, cats can possess naturally occurring alloantibodies against the blood type antigen (alloantigen) that they are lacking. Kittens develop these antibodies between 6 to 8 weeks of age.

In the UK, over 70 % of type A cats have anti-B alloantibodies, mostly in low titres, whilst all type B cats have anti-A alloantibodies, often in high titres *(Knottenbelt et al, 1999)*. Type AB cats are not thought to have alloantibodies to either type A or type B antigens, but *Weinstein et al (2007)* postulated the presence of other, non-AB blood group systems and identified a novel feline RBC antigen named Mik. Subsequent studies, however, found no positive cross-matches between AB-matched blood samples and transfusion-naïve cats (*Hourani, Weingart and Kohn, 2017; Tasker et al, 2014).*

Reactions associated with feline blood types

Alloantibodies are responsible for potentially fatal feline blood transfusion reactions that can arise when cats undergo their first blood transfusion, as alloantibodies are already present in the cat's circulation, ready to destroy RBCs from a different blood phenotype. The two main types of reactions seen in cats are haemolysis due to incompatible blood transfusion reactions and neonatal isoerythrolysis (NI).

Incompatible blood transfusions

All blood type B cats have been shown to have anti-A haemolysins and haemagglutinins, often in high titres. These can cause premature destruction of the transfused RBCs, but also a very severe, acute and potentially fatal haemolytic transfusion reaction even with a small volume of type A blood. Over 70 % of blood type A cats have weaker haemolysins and haemagglutinins. If blood type B is administered to a blood type A cat, there will be premature destruction of the transfused RBCs, but the transfusion reaction will be milder, delayed and unlikely to be fatal.
The presence of these naturally occurring alloantibodies means there is no feline universal donor (unlike blood group O in man). Consequently, type A cats need type A blood transfusion, type B cats need type B blood transfusions and type AB cats should ideally receive type AB blood or, if not available, type A blood after cross-matching.

Neonatal isoerythrolysis

Neonatal isoerythrolysis can occur when type B queens give birth to type A or type AB kittens and, because of the existence of naturally occurring alloantibodies in cats, NI can occur even in first-time queens. The feline placenta does not allow significant passage of antibody during gestation, however high concentrations of antibodies are transferred to the kittens in the colostrum, which are absorbed by the kitten during the first 24 hours of life.

Neonatal kittens born to type B queens therefore acquire maternal alloantibodies during the first day of life in the colostrum, and it is these alloantibodies that can cause NI by haemolysing the kittens' type A or type AB RBCs.

The severity of NI depends upon the quantity and nature of the anti-A alloantibodies ingested, thus clinical signs or anaemia may not always develop. These can vary from peracute to subclinical and include haemoglobinaemia, haemoglobinuria, anaemia, icterus, failure to thrive, tail tip necrosis or sudden death.

Treatment of severe anaemia due to NI may involve administration of washed type B RBCs during the first 3 days of the kitten's life. The anti-A alloantibodies will have diminished in the kitten's circulation after 3 days, whereupon type A blood can be given.

Preventing neonatal isoerythrolysis

Prevention of NI involves avoiding incompatible matings between type B queens and type A toms which can be achieved by blood typing or genotyping cats before mating.

A simple strategy is to mate type B queens only with type B toms. If incompatible matings occur between type B queens and type A toms, kittens at risk for NI should not be allowed to nurse from their type B mother for the first 24 hours when absorption of colostral antibodies occurs and, if available, should receive colostrum from a type A queen at this time.

Cord blood can be used to determine the kitten's blood type at birth, or to perform a crossmatch with serum from the queen. Type B kittens can be immediately placed back with their queen.

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Blood typing; phenotyping and genotyping

Blood type phenotyping, commonly referred as blood typing, can be performed on submitted EDTA blood samples to a commercial laboratory or by using an in-house test kit. The in-house tests require only a small volume of blood and the result, based on the visualisation of an agglutination reaction, is available within minutes, making them an invaluable tool in an emergency setting.

Examples of commercially available, validated in-house kits:

RapidVet®-H blood typing cards *(DMS Laboratories)*, needing 150 µl of blood.

RapidVet®-H immunochromatographic (IC) tests *(DMS Laboratories)*, needing 30 µl of blood.

Quick Test A+B *(Alvedia)*, also using an IC methodology, needing 10 µl of blood.

Numerous studies have evaluated these tests *(Spada et al, 2015; Hourani, Weingart and Kohn, 2014; Seth, Jackson and Giger, 2011)*

- Cards may mistype blood type AB cats as type B and, less often, blood type A cats as type AB.
- IC tests appear to be more reliable than card tests.
- Anaemic cats infected with Feline Leukaemia Virus (FeLV) may be mistyped using a number of blood typing methods.

Blood type genotyping at Langford Vets Diagnostic Laboratories uses new molecular methods, specifically PCR and sequencing of the cytidine monophosphate-N-acetylneuraminic acid hydroxylase gene (CMAH) from DNA isolated from mouth swabs or blood samples of cats. However, the relationship between blood type genotype and phenotype is not straightforward and this method has a 97 % accuracy for phenotype. Although this method cannot yet distinguish between blood type A and AB cats or replace blood type phenotyping, it can be used by breeders to direct mating by identifying b carriers and, thus, help prevent neonatal isoerythrolysis.

Cross-matching

Blood type phenotyping identifies blood type antigens present on feline RBCs, whilst cross-matching detects alloantibodies against those antigens. These alloantibodies may have been induced by a previous blood transfusion or, as is common in cats, could be naturally occurring against other erythrocyte cell surface antigens.

Genotype test result	Phenotype possibilities
AA	Type A or AB
Ab	Type A or AB
Bb	Type B

In an emergency, providing the donor and recipient are of the same blood type and there is no history of a previous blood transfusion, cross-matching is generally not performed. No studies to date have shown a clinically significant increase in post-transfusion PCV when cross-matching occurred prior to blood transfusion administration *(Sylvane et al, 2018; Weltman, Fletcher and Rogers, 2014)*.

However, cross-matching should always be performed if the recipient has an unknown transfusion history, has shown a previous transfusion reaction or had a blood transfusion 4 or more days previously. Interestingly, the 4-day rule may need to be modified as, in a recent study, alloantibodies developed as early as 2 days after a blood transfusion had been given *(Hourani, Weingart and Kohn, 2017)*.

An in-house cross-matching gel tube system is available (RapidVet®-H companion animal major and minor cross-match tests), although cross-matching can be performed in-house without this. However, cross-matching is a complex and time-consuming process, so should ideally be performed by an external laboratory in the situations outlined above.

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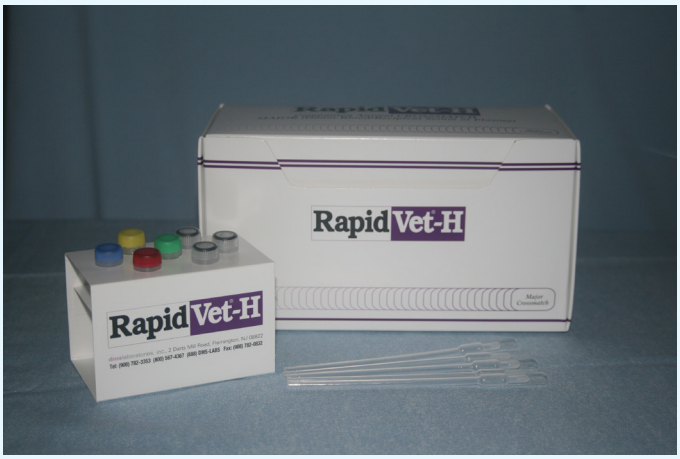
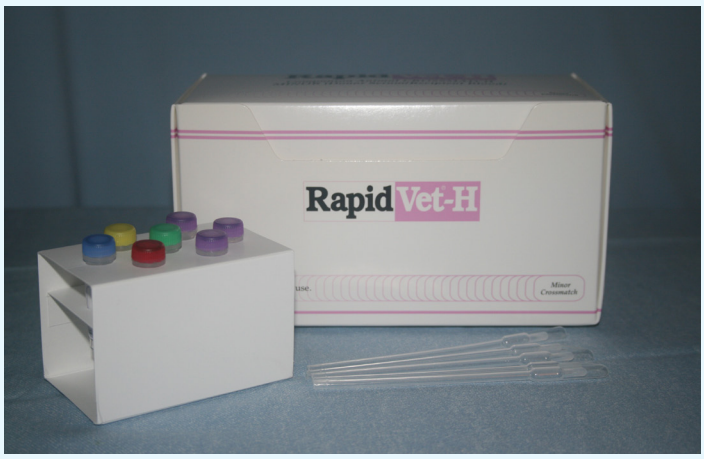
The **major crossmatch** tests for alloantibodies in the recipient's plasma against donor RBCs. An incompatible major crossmatch can result in an acute haemolytic transfusion reaction when donor RBCs are destroyed by alloantibodies in the recipient's plasma.

The **minor crossmatch** tests for alloantibodies in the donor's plasma against recipient RBCs. A minor crossmatch incompatibility is less likely to cause a transfusion reaction because the volume of donor plasma is small and becomes markedly diluted in the recipient.





How to perform cross-matching

1. Obtain 1 ml EDTA blood and 1 ml plain blood from each donor and recipient. Label tubes.
2. Centrifuge (3,000 rpm for 5 minutes) and separate plasma and serum from RBCs. Discard the plasma if not required for other diagnostic investigations. Store serum in a separate tube and label.
3. Wash RBCs: add 2-3 ml of normal saline solution to the RBCs, mix gently, centrifuge (3,400 rpm for 1 minute) and remove the supernatant saline. Repeat twice.
4. After the 3rd wash, decant the supernatant and resuspend the RBCs with saline to give a 4 % RBC suspension (0.2 ml RBCs and 4.8 ml saline).
5. Label 4 tubes and place the following in each tube:
   I. Major crossmatch — 1 drop recipient serum & 1 drop donor RBC suspension
   II. Minor crossmatch — 1 drop donor serum & 1 drop recipient RBC suspension
   III. Recipient control — 1 drop recipient serum & 1 drop recipient RBC suspension
   IV. Donor control — 1 drop donor serum & 1 drop donor RBC suspension
6. Incubate the tubes (15 minutes at 37 oC).
7. Centrifuge the tubes (3,400 rpm for 15 seconds).
8. Read the tubes macroscopically (naked eye) and microscopically (under the microscope):

Macroscopic

In a compatible reaction, there should be no clumping or haemagglutination present; when the tubes are rotated, RBCs should be able to float off freely from the centrifuged "pellet" of RBCs.
The supernatant should be free of haemolysis.

Microscopic

A drop of the RBC/serum mixture is placed on a microscope slide, cover-slipped and viewed microscopically. The RBCs should be viewed as individual cells and not in clumps. Rouleaux formation, where RBCs appear as stacks of coins, can look macroscopically like agglutination, but its presence can be confirmed with microscopic examination.

This protocol has been modified to use plasma, rather than serum, along with a 3-5 % suspension of RBCs in phosphate buffered saline (PBS, rather than saline), and to not perform the donor control test *(Weinstein et al, 2007)*. This study also reported that cross-matching could be done with only 0.25 ml of blood.

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How to perform emergency cross-matching

1. Obtain 0.5 ml EDTA blood and 0.5 ml plain blood samples from donor and recipient. Label tubes.
2. Centrifuge (3,000 rpm for 5 minutes) and separate plasma and serum from RBCs. Discard the plasma (not used thereafter in the cross-matching). Store serum in a separate tube and label.
3. Place 2 drops of recipient serum and 1 drop of donor RBCs (similar to major cross-match) on a glass slide, then examine it microscopically for agglutination after 1-5 minutes.
4. Controls should also be performed using recipient serum and RBCs and, if possible, the donor serum and RBCS as described on the full cross-matching. Agglutination must be differentiated from rouleaux formation. Desiccation of the sample on the slide will result in rouleaux formation, but this takes > 5 minutes to occur.

Feline blood donor selection

Contrary to dogs, there are no available feline blood banks in the UK and finding a suitable donor cat can sometimes be difficult in an emergency situation or for blood types B and AB. Compiling a list of potential donor cats (e.g. staff or client pets) can be useful. There are potential risks to the donor cat associated with sedation and blood collection and these must be discussed before consent is given for blood donation. The recipient cat must also be considered to ensure blood collection is necessary; a blood transfusion must be the most appropriate treatment and other treatment options should have been exhausted. The diagnosis of a terminal disease in the recipient may make it difficult ethically to justify the risks of blood donation in another cat.

These cats must fulfil certain criteria for donor suitability as outlined below:

- Cats should be large, non-obese animals greater than 4.5 kg in body weight in order to donate a sufficient volume of blood safely.
- Ideally, donor cats should be aged 1 to 5 years (up to 8 years old).
- Repeated handling (accurate weighing, blood pressure measurement, blood collection for PCV, placement of an intravenous catheter) is required before blood collection and a calm temperament facilitates the process whilst stressing the cat minimally. Sedation is usually required for blood collection and the procedure takes 15-20 minutes, although blood collection from conscious cats has recently been described (Doolin *et al.*, 2017).
- Routine vaccinations as appropriate for the cat's environment, as well as regular ectoparasite and endoparasite preventative health care, including heartworm in prevalent areas (not the UK), should be up to date.
- Physical examination and clinical history should be unremarkable.
- Routine haematology and biochemistry should be unremarkable and ideally performed within the last 6 months. Blood typing should also be undertaken at the same time.
- Echocardiography should ideally be performed when screening donor cats to exclude occult cardiac disease which could result in hypotension or increased risk with sedation.
- Regular infectious agent testing should be undertaken according to published guidelines (Wardrop *et al*, 2016; Pennisi *et al*, 2015), taking the donor cat's environment under consideration. An indoor only, single cat household donor cat that has not travelled outside the UK would be ideal due to the reduced risk of infectious diseases.

Within the UK and in the Feline Centre, potential donor cats are screened for:

- FeLV: using PCR analysis, as provirus positive cats can transmit FeLV infection via blood (Nesina *et al*, 2015). In-house antigen testing can be done as an alternative, but this is not ideal.
- Feline Immunodeficiency Virus (FIV): using in-house antibody testing.
- Haemoplasma species: Some suggest testing only for the most pathogenic only (Mycoplasma haemofelis), but we recommend checking for all 3 haemoplasma species using PCR.
- Bartonella species: using PCR and serology, if possible

Other infectious agents which can be tested in other parts of the world or for travelled cats:

- *Cytauxzoon felis, Ehrlichia canis* and *Ehrlichia canis-like, Anaplasma platys* and *Anaplasma phagocytophilum, Neorickettsia risticii spp.*
- Feline coronavirus antibodies: Testing is a matter of debate *(Wardrop et al, 2016; Pennisi et al, 2015)*, but we do not feel it is necessary.

- Systolic blood pressure should be measured prior to donation and be within normal limits (120-150 mmHg). A patient with hypotension could be placed at risk by sedation and blood collection and must not be used for blood donation.
- Donor PCV should be ≥ 35 % prior to blood donation to ensure the donor does not become anaemic following blood collection and to provide the maximum benefit to the recipient after a single blood transfusion. A small volume of blood is collected from the cephalic vein and spun in a microhaematocrit tube to measure PCV. This preserves the jugular veins for blood collection later. Ideally, pet blood donors should not donate more frequently than every 3 months and more frequent donation than monthly may induce iron deficiency anaemia.
- Ideally, the donor would not have been fed in the previous 6 hours to minimise the risks of vomiting or regurgitation under sedation.
- The donor and recipient should be of the same blood type.

References and further reading can be found at the end of this doccument.

The second article of the Feline Blood Types and Blood Transfusion series, discussing blood collection and administration, will be published in the next edition of the Feline Update.

Feline Blood Types & Blood Transfusions - Part 1

Additional reading and online resources

- *Abcdcatsvets.org. (2018). | Advisory Board on Cat Diseases (ABCD). [online] Available at: http://www.abcdcatsvets.org/ [Accessed 2 Oct. 2018].*
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WELCOME TO...

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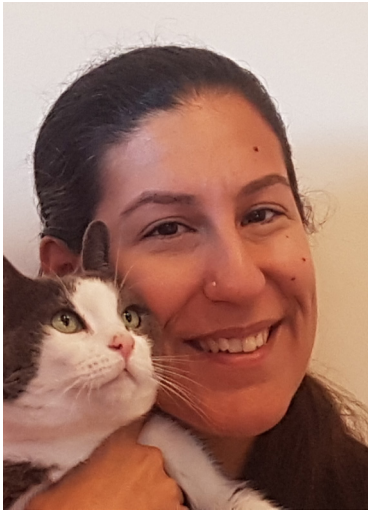


Evelyn joined the Feline Centre at Langford, University of Bristol as the Zoetis-sponsored Feline Scholar in May 2018.

Following graduation from University of Thessaly in Greece in 2009, she worked in small animal practice before completing an internship in small animal medicine and surgery at University of Bristol in 2016.

Evelyn continued to develop a strong interest in internal medicine and was awarded the RCVS Certificate in Advanced Veterinary Practice and the Postgraduate Certificate in Veterinary Professional Studies in 2017.

Alongside her other feline-related duties, Evelyn is studying towards an MSc by research, the Feline Activity Study, and is involved in additional projects within the Bristol Cats Study, one of which will evaluate the environmental enrichment of indoor cats.

Evelyn is owned by two mischievous moggies called Lucy (pictured) and Smudge.

FELINE ACTIVITY STUDY

Bristol Cats

Feline degenerative joint disease (DJD) is a common condition in cats, with prevalence estimates ranging between 61% and 99% and increasing with age. There is little known about risk factors for this condition and diagnosis is far from straightforward as it primarily depends upon owners detecting subtle changes in the activity or behaviour of their cat, then seeking veterinary advice.

Early detection of DJD would allow a multimodal approach to delaying/halting progression of the disease by educating veterinary personnel and owners, thereby improving the quality of life of cats with this condition.

We are using activity monitors to study the effect of joint disease on feline activity levels and hope this study can further advance our understanding of this challenging condition.

We would be grateful to hear from any vets with suitable cases, including their own cats!

We are recruiting cats that are:

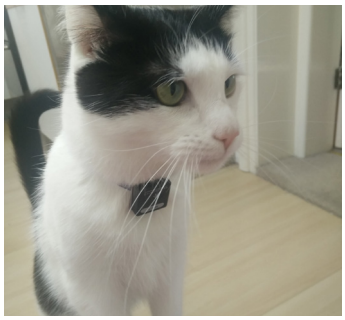
- 6 years old or older
- Indoors only, or with outdoors access within a closed run and/or on a lead
- Aren't on any pain medication
- Live within 1-2 hours' drive of Bristol or Bournemouth

As the cats' welfare is our top priority, included cats should also be happy to:

- Meet and be stroked by strangers
- Wear a breakaway (safety) collar
  (if the cat isn't wearing one already, this will be provided along with step by step instructions of how to slowly introduce the cat to it.)

What is required from the owner – and their cat?

- Completion of two short questionnaires (~15 minutes total)
- A 30-60-minute home visit to meet them and conduct a gentle examination of their cat's joints.
- The cat wearing a light activity monitor on their collar for 2 weeks.



Smudge, one of the study cats, wearing his activity monitor'

For further information, please see our Facebook page @feline.activity.study, or do not hesitate to contact us:

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FELINE BLOOD DONOR CHECKLIST

PATIENT STICKER

DONOR REQUIREMENTS

- ❑ Body weight > 4.5 kg
- ❑ Age 1 – 8 years (ideally 1 – 5 years)
- ❑ Indoor cat (ideally)
- ❑ No history of travel outside the UK
- ❑ No history of previous receipt of blood transfusion
- ❑ Normal echocardiography
- ❑ Calm temperament & not unduly stressed
- ❑ Discussed risks with owner (Check: https://icatcare.org/advice/cat-health/blood-donor-cats)

≤ 6 MONTHS BEFORE DONATION

- ❑ Up to date vaccination status, flea treatment / worming status
- ❑ Unremarkable history and physical examination (consider repeating echocardiography if > 6 months)
- ❑ Unremarkable haematology and biochemistry
- ❑ Blood typing
- ❑ Negative for all infectious agents
  - ❑ FeLV PCR
  - ❑ FIV antibody
  - ❑ Haemoplasma PCR for all three haemoplasma species
  - ❑ Bartonella spp PCR & serology

AT THE TIME OF DONATION

- ❑ No history of blood donation within the previous 3 (ideally) or 1 months
- ❑ Fasting for at least 6 hours (ideally)
- ❑ Unremarkable history & clinical examination
- ❑ Normal BP (120-150mmHg)
- ❑ Packed Cell Volume (PCV) ≥ 35%

CONSENT FORM

- ❑ Confirm history and fasting status
- ❑ Acknowledgement of risks
- ❑ Signature

BLOOD DONATION RECORDS

- ❑ Pre-donation temperature, pulse and respiratory rate)
- ❑ Volume of blood donated
- ❑ Drugs and doses used for sedation
- ❑ Recovery quality

KENNEL FORM

- ❑ Intravenous fluid therapy using Hartmann's for 2-3x the volume of blood collected administered over 1-2 hours.
- ❑ Monitor TPR and mucous membranes/capillary refill time every 2-4 hours.
- ❑ Food as soon as recovered from sedation, usually 2-3 hours.
- ❑ Home after around 8 hours