# Feline transfusion medicine: current Australian blood banking and transfusion practices and the effect of storage lesion

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# Statement of originality

This is to certify that to the best of my knowledge; the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Chapter four has been published in the Journal of Feline Medicine and Surgery. I am the primary author of this manuscript; the contributions of my co-supervisor are outlined in the author attribution statement. Formatting of this section was in accordance with journal publishing guidelines and therefore may differ from the remainder of the thesis.

Ethics approval for the published research study contained within chapter four was granted by the University of Sydney Animal Ethics Committee, project number: 2018/1358.

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# Authorship attribution statement

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I co-designed the study with the co-authors, interpreted the statistical analysis done by Professor Michael Ward and wrote the drafts of the study.

In addition to the statements above, in cases where I am not the corresponding author of a published item, permission to include the published material has been granted by the corresponding author.

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# Abstract

**Introduction**

Blood transfusions are an essential and potentially lifesaving therapeutic intervention in feline emergency and critical care medicine. While recent research has expanded understanding of species-specific differences in haemorheology and transfusion medicine, there are still significant and clinically important deficits in the evidence-based literature.

Geographical differences in commercial feline blood product access and blood banking equipment mean that results from the limited published studies characterising feline transfusion practice outside of Australia may not reflect current local practices. To date, there are no published studies characterising methods of feline blood collection or storage within Australia.

Many blood banking and transfusion practices are extrapolated from canine or human studies with few experimental or clinical studies evaluating their suitability to feline blood. While there are an increasing number of feline-specific studies investigating the effects of storage lesion, the impact of transfusion technique, including filter passage and blood delivery method, remains largely unexplored. It is unknown if the common transfusion method of syringe driver and 18 µm microaggregate filter passage induces greater injury in whole blood affected by storage lesion versus freshly collected whole blood.

**Objectives**

1. To describe how Australian emergency and specialty referral hospitals source feline blood products, approach collecting blood donations and to identify and characterise feline blood storage practices.
2. To determine whether passage through an 18 µm microaggregate filter damages feline RBCs as indicated by increased haemolysis percentage, mean osmotic fragility or haematological derangements and if these effects are compounded in blood that has been stored for 35 days.

3. To determine if a commercially available open blood collection system carries risk of bacterial contamination.

## Methods

An electronic survey was created and distributed to Australian small animal emergency and/or referral veterinary hospitals. Information collected from the survey included respondent demographics, whether feline blood transfusions were performed, source of feline blood donors and characteristics of blood product collection and storage including type of collection system, anticoagulant used and length of blood storage. Descriptive statistics were reported for demographics, donor source, blood collection systems and anticoagulant type. A chi-squared test was used to assess associations between hospital type and whether blood transfusions were performed or not. Ordinal logistic regressions were conducted to assess associations between type of hospital and donor source, collection system and anticoagulant type. For all analyses, a p value $< 0.05$ was considered significant.

The second objective was investigated with an experimental study using nine cats recruited for a single blood donation using an open collection system. A simulated transfusion using a syringe driver and microaggregate filter was performed over two hours with half the blood on the day of donation, and the second half after 35 days storage. Haematological analysis, haemolysis percentage calculation and osmotic fragility testing were performed on blood sampled on the day of donation pre-filter passage (D0-), the day of donation post-filter (D0+), day 35 storage pre-filter (D35-) and day 35 post-filter passage (D35+). The Wilcoxon signed ranks test was used to compare differences in the ranks of haematological parameters, percent haemolysis and osmotic fragility for D0- versus D0+, D0- versus D35-, D0- versus D35+ and D35- versus D35+. Statistical significance was determined using a z-statistic applied to sum of ranks, and differences were considered statistically significant when $p < 0.05$.

The third objective was achieved as part of the experimental study, with blood cultures performed at D0+ and D35+.

## Results

A total of 31 responses were included in the survey study. The majority (15/31) were from private emergency and referral hospitals followed by emergency-only (10/31) then university teaching hospitals (6/31). Nearly all respondents (29/31, 93.4%) performed feline blood transfusions. Most donors were sourced from a combination of staff- and client-owned cats. There were no significant associations between type of hospital and donor source. The majority of blood collections were performed using an open collection system, not sterilised after assembly (16/28, 57.1%) followed by an open, partially pre-assembled system not sterilised after assembly (9/28, 32.1%). Citrate phosphate dextrose adenine (CPDA-1) was the most frequently used anticoagulant either as the only anticoagulant used in the hospital (20/28, 71.4%) or alongside citrate phosphate dextrose (CPD) (1/28, 3.6%). No association was found between type of hospital and type of collection system or type of anticoagulant used. Three hospitals indicated routine storage of feline blood, with two storing whole blood and one storing blood components. Maximum storage times for blood were reportedly 28, 35 and 42 days.

Results of the simulated transfusion study found no differences in the D0- versus D0+ comparisons. In comparison to D0-, greater haemolysis percentage, red cell distribution width % and mean osmotic fragility were detected at D35-. For this same comparison, packed cell volume (PCV), red blood cell count, haemoglobin and haematocrit were lower at D35-. Similar results were noted for D0- versus D35+, with the addition of a higher mean corpuscular haemoglobin at D35+. For D35- versus D35+, only mean corpuscular haemoglobin was higher at D35+. At day 35, 6/9 units had haemolysis percentages that exceeded 1% (range 0.81 - 1.85%). This increased to 8/9 (haemolysis range 0.84 - 2.07%) of stored units post-filter passage. All blood units cultured negative.

## Conclusions and clinical relevance

While most hospitals performed feline blood transfusions, very few stored feline blood, and the means of blood collection, storage times and subsequent processing differed across sites. The lack of uniformity in responses may reflect variations in resource access and a lack

of evidence-based recommendations and protocols to guide feline blood banking in the veterinary literature.

Fresh feline red blood cells exhibited no in vitro evidence of injury following passage through an 18 µm microaggregate filter. Higher mean corpuscular haemoglobin was observed in the stored feline blood and may represent haemolysis induced by the filter. All other changes could be explained by storage lesion rather than filter passage. The findings highlight the importance of blood banking quality controls and the need for further in vivo research to assess the effects of transfusion technique, specifically filter passage, on storage lesion-affected feline blood.

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# List of Abbreviations

| | |
|---|---|
| **2,3-DPG** | 2,3-diphosphoglycerate |
| **ABRI** | Animal Blood Resources International |
| **ABS** | Australian Bureau of Statistics |
| **ACD** | Acid citrate dextrose |
| **ACVIM** | American College of Veterinary Internal Medicine |
| **AVHTM** | Association of Veterinary Hematology and Transfusion Medicine |
| **AP** | Anticoagulant-preservative |
| **APVMA** | Australian Pesticides and Veterinary Medicines Authority |
| **ARDS** | Acute respiratory distress syndrome |
| **ATP** | Adenosine triphosphate |
| **BRM** | Biologic response modifier |
| **CBC** | Complete blood count |
| **CPD** | Citrate phosphate dextrose |
| **CPDA-1** | Citrate phosphate dextrose adenine - 1 |
| **CRP** | C-reactive protein |
| **CXCL** | Chemokine ligand |
| **D0-** | Day of blood donation pre-filter |
| **D0+** | Day of blood donation post-filter |
| **D35-** | Day 35 of storage pre-filter |
| **D35+** | Day 35 of storage post-filter |
| **DNA** | Deoxyribonucleic acid |
| **FDA** | Food and Drug Administration |
| **FEA** | Feline erythrocyte antigen |
| **FeLV** | Feline leukaemia virus |
| **FFP** | Fresh frozen plasma |
| **FIP** | Feline infectious peritonitis |
| **FIV** | Feline immunodeficiency virus |
| **FNHTR** | Febrile non-haemolytic transfusion reaction |
| **FWB** | Feline whole blood |

| | |
|---|---|
| **GP** | General practice |
| **Hb** | Haemoglobin |
| **HCT** | Haematocrit |
| **HTR** | Haemolytic transfusion reaction |
| **IMHA** | Immune mediated haemolytic anaemia |
| **ISFM** | International Society of Feline Medicine |
| **IL** | Interleukin |
| **IM** | Intramuscular |
| **IV** | Intravascular |
| **LR** | Leukoreduced |
| **MAC** | Membrane attack complexes |
| **MCH** | Mean corpuscular haemoglobin |
| **MCHC** | Mean corpuscular haemoglobin concentration |
| **MCV** | Mean corpuscular volume |
| **MOF** | Mean osmotic fragility |
| **MP** | Microparticle |
| **NET** | Neutrophil extracellular trap |
| **NLR** | Non-leukoreduced |
| **NTBI** | Non-transferrin bound iron |
| **NT-ProBNP** | N terminal pro-B-type natriuretic peptide |
| **OF** | Osmotic fragility |
| **PCR** | Polymerase chain reaction |
| **PCV** | Packed cell volume |
| **PRBC** | Packed red blood cell |
| **REDS** | Recipient Epidemiology Donor Evaluation Study |
| **RBC** | Red blood cell |
| **RCT** | Randomised controlled trial |
| **RDW** | Red blood cell distribution width |
| **ROS** | Reactive oxygen species |
| **SAGM** | Saline adenine glucose mannitol |
| **TACO** | Transfusion associated circulatory overload |
| **TNF-alpha** | Tumour necrosis factor alpha |

| | |
|---|---|
| **TRACS** | Transfusion reaction small animal consensus statement |
| **TRALI** | Transfusion-related acute lung injury |
| **TRIM** | Transfusion associated immunomodulation |
| **UK** | United Kingdom |
| **USA** | United States of America |
| **VAP** | Vascular access port |
| **WB** | Whole blood |

# Chapter 1: Literature Review

## 1.1 Introduction

Blood product transfusion is a vital and potentially lifesaving therapeutic intervention for critically ill cats. Advances in veterinary transfusion medicine have resulted in more widespread access to blood products and increased safety for both donor and recipient cats. In the last decade, publication of studies investigating novel feline blood groups [1], typing [2-5], pre-transfusion crossmatching [6-10], donor screening [11-13], adverse transfusion reactions [14-16], blood collection, storage and transfusion methodologies [17-29] continue to broaden the evidence base used to inform and evaluate current clinical practice. The expansion of feline blood banking outside of hospital run donor programs to commercially available component therapy has also increased the accessibility of blood products. With geographical limits to commercial product access, feline blood still remains a valuable and often limited resource.

The transfusion of red blood cells (RBC) in the form of whole blood (WB) or packed red blood cells (PRBCs) and/or blood component therapy such as plasma transfusion is indicated for the treatment of clinically significant anaemias and/or coagulopathies. Replacement of lost or poorly regenerated red cell mass through RBC transfusion increases blood oxygen carrying capacity, ensuring continued oxygen supply to vital organs. Uncommonly, dyshaemoglobinaemias like methaemoglobinaemia may also necessitate red cell transfusion [30]. Acute blood loss is a commonly reported cause of anaemia in the cat and may occur secondary to traumatic haemorrhage, neoplasia, surgical loss, sepsis, coagulopathies and gastrointestinal or urinary bleeding. Ineffective erythropoiesis or erythropoietic failure is often reported as the next most common cause of anaemia [31-35]. Causes of ineffective erythropoiesis include renal disease [31] or primary bone marrow disease secondary to feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV), feline infectious peritonitis (FIP), haematopoietic neoplasia, precursor cell immune mediated anaemia, myelodysplastic disease, and uncommonly toxic insults. Anaemia may also result from RBC destruction due to immune mediated haemolytic anaemia (IMHA), *Mycoplasma* spp. infection, Heinz body anaemia or hypophosphataemia [32, 33]. Cats are also susceptible to

developing hospital acquired anaemia, a finding that has been linked to both the frequency [36] and volume [37] of blood sampling.

Blood product transfusion is not without risk and should be limited to recipients that will clinically benefit from receiving them [28, 38, 39]. Standardized guidelines that establish a threshold packed cell volume (PCV) or haemoglobin that necessitates RBC transfusion are currently lacking for either human or veterinary patients. The concept of a 'critical haemoglobin' level has been established in both experimental dog [40] and human studies [41]. Critical haemoglobin refers to the haemoglobin value in an anaesthetised, euvolaemic, anaemic patient that corresponds to the point oxygen delivery becomes insufficient to meet oxygen demand, resulting in tissue hypoxia [41]. Human studies have established a critical haemoglobin value of 2 to 4 g/dL (20 – 40 g/L) [41], similar to a canine experimental study value of 3.3 g/dL (33 g/L) [40]. There are no studies in cats. The clinical utility of a threshold value has not been established. Experimentally determined critical haemoglobin values do not assess oxygen delivery and extraction at the tissue level nor account for the potential impairment of compensatory responses to anaemia in the critically ill.

Comparison of a restrictive or lower haemoglobin threshold versus a liberal or higher haemoglobin threshold for RBC transfusion has been the subject of numerous human studies. The current evidence is summarised in a 2021 Cochrane review update comparing restrictive transfusion triggers (lower haemoglobin threshold, commonly 7.0 g/dL to 8.0 g/dL) versus liberal transfusion triggers (higher haemoglobin threshold, commonly 9.0 g/dL to 10.0 g/dL) in anaemic human patients across a range of clinical conditions [42]. Restrictive transfusion strategies resulted in a 41% decrease in the probability of receiving a RBC transfusion with no impact on 30-day mortality or morbidity compared to a liberal transfusion strategy [42]. Currently, there are no studies exploring restrictive versus liberal transfusion strategies in veterinary patients.

Ultimately, the decision to transfuse RBC products must be based on an assessment of the individual anaemic cat. Transfusion 'triggers' may be a combination of absolute PCV or haematocrit and/or the rate of any relative decrease, ongoing RBC losses and the presence

of clinical signs associated with impaired oxygen carrying capacity such as tachycardia or bradycardia, tachypnoea, reduced mentation and exercise intolerance [28, 43].

To date, three consensus statements or guidelines concerning aspects of feline transfusion medicine have been published by different veterinary working groups or organisations. The American College of Veterinary Internal Medicine (ACVIM) published the first consensus statement, entitled Canine and Feline Blood Donor Screening for Infectious Disease, in 2005, with an updated version released in 2015 [12]. In 2021, the International Society of Feline Medicine (ISFM) released 2021 ISFM Consensus Guidelines on the Collection and Administration of Blood and Blood Products in Cats, aimed at general practitioners [28]. That same year, the Association of Veterinary Hematology and Transfusion Medicine (AVHTM) released a three-part Transfusion Reaction Small Animal Consensus Statement (TRACS) [14-16]. Recommendations generated from these statements represent an expert-led interrogation of the current evidence-base, however they also reveal a multitude of knowledge gaps where feline specific evidence is lacking.

The following literature review provides an overview of the current evidence base for feline transfusion practice from how donors are selected and screened to the techniques and methods used for blood storage, processing, and transfusion delivery. A brief review of the literature regarding storage lesion, controversies surrounding blood storage, and blood transfusion techniques is also presented. Background information from both the human and veterinary literature is included, with human or canine studies providing a point of comparison to feline studies or for some topics are the only source of information where there are few to no feline studies published.

## 1.2 Feline blood donor criteria and blood characteristics

### 1.2.1 Donor screening

Rigorous donor screening is important to ensure a safe blood product for the transfusion recipient and a safe donor experience during blood donation. The recently updated ACVIM veterinary blood banking consensus statement outlines blood-borne pathogen screening recommendations for canine and feline blood donors [12]. At a minimum and subject to

geographic location, cat donors should be PCR negative for *Anaplasma* spp*., Bartonella henselae* and *Mycoplasma haemofelis,* antigen negative FeLV and antibody negative for FIV [12]. The 2021 ISFM Consensus Guidelines on the Collection and Administration of Blood and Blood Products in Cats recommend donors are PCR tested for FeLV rather than antigen tested to ensure an absence of proviral DNA [28]. This recommendation arises from an experimental study that documented FeLV transmission and subsequent infection in cats transfused antigen-negative, FeLV provirus-positive blood [11]. A recent study assessing prevalence of subclinical infectious agents in healthy, client-owned, indoor and blood donor eligible cats in Spain and Portugal found 5.2% of 173 cats tested were positive for feline leukaemia provirus [13]. Additionally, of the 4880 SNAP test (IDEXX Laboratories) FIV antibody- and FeLV antigen-negative cats, 1.3% were positive for *Mycoplasma haemofelis*, 2.3% for *Candidatus Mycoplasma haemominutum* and 0.12% for *Candidatus Mycoplasma turicensis* [13]. As highlighted in the ISFM consensus guidelines, the lower pathogenicity of the latter two organisms may not strictly exclude a positive cat from donating especially in situations of scarcity [28]. Depending on geographic location and the prevalence of potentially transfusion-transmissible haematological infectious agents, extended PCR screening panels for potential blood donors may be warranted [13].

Authors of both the ACVIM and ISFM consensus statements advocate for any potential blood donor cat to ideally be housed exclusively indoors to prevent risk of disease exposure [12, 28]. Other blood donor selection criteria include age, weight, physical examination findings, complete bloodwork, regular endo- and ectoparasitic use, current vaccinations and lack of recent medication administration [28, 44]. The ISFM consensus guidelines for example, specify an eligible donor age range of one to eight years and a lean body weight of greater than 4.5 kg [28]. Cats that have received a prior blood transfusion or are currently pregnant are not eligible to be blood donors [28]. Due to the associated increase in anaesthetic and donation risks, donor programs may exclude potential donors based on abnormal cardiac auscultation findings alone such as the identification of a murmur, gallop or arrhythmia [28]. With structural heart disease found in up to 30% of apparently healthy cats with no audible heart murmur in one study [45] some donation programs require an echocardiogram for all potential donors. Echocardiographic evaluation may also help reduce the unnecessary exclusion of cats with structurally normal hearts and benign physiologic

murmurs. In a recent retrospective study of 856 cats referred for specialist cardiologist evaluation of a heart murmur, 6.9% were ultimately diagnosed with an innocent flow murmur [46]. Alternatively, quantitative N terminal pro-B-type natriuretic peptide (NT-proBNP) serum testing has been shown to consistently differentiate normal cats from those with occult cardiomyopathy [47] and may be used by some in place of echocardiography.

The financial and time costs of establishing a blood bank are not only limited to initial donor screening and blood bank establishment but entail a process of ongoing maintenance. Annual reassessment of donor suitability via repeated physical examination, history assessment, haematology, and biochemistry profile are recommended to ensure ongoing donor suitability and safety [28].

### 1.2.2 Chemical restraint and blood collection methods

While conscious donation is successfully practiced in some institutions [22], it is more common for cats to be heavily sedated or anaesthetised for blood donation [44, 48]. Donors must be sufficiently fasted and at a minimum have history taken, a physical examination, body weight and PCV or haemoglobin performed prior to each donation. Donor screening criteria and factors related to the donation process including donor temperament and relative anaesthetic risk may therefore considerably narrow accessibility to suitable donor candidates.

The ISFM consensus guidelines provide evidence-based recommendations for the safe chemical restraint of donor cats [28]. Recommended drug protocols for donation have a predictable short onset and duration of action, provide adequate anaesthetic depth and a smooth, rapid recovery [28]. A number of studies have been published assessing different drug combinations for feline anaesthesia and their effect on blood analytes [49-55]. Ketamine and diazepam [53] or midazolam with butorphanol [49, 54] provide variable periods of chemical restraint and recovery quality based on dose and route of administration. An anaesthetic protocol using ketamine (10 mg/kg intravascular (IV)), midazolam (0.5 mg/kg IV) and buprenorphine (10 ug/kg intramuscular (IM)) has been shown to cause a temporal 24-25% average reduction in RBC count, haemoglobin concentration and PCV in samples collected from anaesthetised cats [55]. As noted by the authors, this

may lead to a false diagnosis of anaemia. Of note, the ketamine and midazolam doses in this study [55] are higher than another study which used 5 mg/kg ketamine and 0.2 mg/kg midazolam in combination with 0.3 mg/kg of butorphanol for the purpose of performing feline blood donation [54]. Tiletamine and zolazepam represent an alternative anaesthetic combination that has also been shown to rapidly induce adequate sedation for feline blood donation with no statistically significant alterations in donor cat pre versus post donation haematological variables [52].

Combinations of alfaxalone and butorphanol at varied doses either as IV or IM injections have also been studied [50, 51]. Benefits of this combination include adequate sedation, rapid anaesthetic recoveries [50, 51] and minimal cardiorespiratory depression [50]. In contrast, authors of the ISFM consensus do not recommend the use of dexmedetomidine or other alpha-2-adrenergic receptor agonist sedative combinations due to the risk of associated adverse effects including decreased cardiac output, emesis induction and increased venepuncture difficulty [28, 49, 51]. The authors also counsel against the use of mask or 'box' induction using an inhalation agent such as sevoflurane due to the potential for stress to the donor cat and gas exposure to human personnel involved in the donation [28]. A sevoflurane anaesthetic protocol has also been associated with higher rates (84%) of hypotension in cats during blood donation compared to a ketamine, midazolam and butorphanol protocol (42%) [54].

The blood donor may be positioned in lateral, sternal or dorsal recumbency for donation. Blood is collected from a jugular vein after the fur is clipped and the skin aseptically prepared [28]. Frequent monitoring throughout the blood donation is strongly recommended and should ideally include pulse oximetry, blood pressure, body temperature, mucous membrane colour, capillary refill time, heart or pulse rate and respiratory rate. Supplemental oxygen should also be provided and balanced crystalloids fluids administered intravenously or subcutaneously after donation [28].

### 1.2.3 Blood types

Blood typing is a mandatory aspect of feline transfusion medicine due to the presence of naturally occurring alloantibodies that may cause fatal transfusion reactions in cats [38, 48,

56-59]. Blood types are defined by the presence or absence of specific RBC surface alloantigens that can induce an antigenic response in another individual's immune system when transfused [4]. The feline AB blood system has been well characterised and describes three blood type phenotypes, A, B and AB [4, 28]. A and B type cats have innately present anti-B and anti-A alloantibodies respectively. Transfusion of incompatible blood results in acute haemolytic reactions and reduced survival of the transfused RBCs [58]. Due to the presence of strong natural anti-A alloantibodies in type B cats, transfusion of type A blood to a type B cat produces more rapid haemolysis and life-threatening reactions compared to type A cats receiving type B blood [58]. Type AB cats lack alloantibodies and are considered universal recipients [60]. No universal cat donor exists. Type A cats predominate, though prevalence within cat populations varies widely and has been reported to be between 62 to 100% depending on geographic location [3, 58, 59, 61, 62]. Certain blood types also occur with greater frequency within some breeds, for example Siamese and Burmese cats are more likely to be type A [61].

Discovery of the novel *Mik* feline RBC antigen revealed the existence of naturally occurring alloantibodies outside the AB system [63]. The *Mik* antigen was discovered after a haemolytic transfusion reaction occurred in a transfusion naïve cat receiving type compatible blood [63]. Results from a more recent investigation seeking to identify and characterise naturally occurring alloantibodies outside the AB system support the presence of five seemingly distinct, novel feline erythrocyte antigens (FEA). It is postulated by the authors that the most commonly detected antigen designated FEA 1 in the study may correlate with the previously described *Mik* antigen. Crossmatching revealed a naturally occurring non-AB alloantibody frequency of 7% amongst a total of 258 type A cats, 2% of which proved incompatible to all RBCs against which they were tested [1]. Further work is needed to identify and characterise feline blood types outside the AB system to establish the clinical importance of novel, naturally occurring alloantibodies and how they may influence transfusion compatibility testing recommendations.

Unlike blood typing, which identifies the presence or absence of a known RBC alloantigen, crossmatching can only determine if incompatibility is present through general antibody/antigen RBC reactions [4]. Crossmatching is generally recommended prior to all

feline RBC transfusions, not just for cats receiving a subsequent transfusion two to five days after the first transfusion of a blood product [6, 14, 30]. There are conflicting results from the literature regarding the objective benefits of mandatory crossmatching prior to all feline RBC transfusions [6, 9, 10, 33]. Nonetheless, the requirement for type specific and crossmatch compatible blood may further limit available suitable feline donors or blood products for an intended recipient. Accessibility to suitable blood products for multiple transfusions and/or in emergency situations can present a challenge, especially in clinical settings where stored feline blood is not available. Where compatible feline blood is not available, successful xenotransfusion of dog blood to cats has been described in the emergency setting [64]. Xenotransfusion is a potentially lifesaving short term intervention, with delayed haemolysis of transfused RBCs expected to occur within the days following transfusion [64, 65]. As repeat xenotransfusion may cause fatal anaphylaxis, cats requiring an additional transfusion more than four days after receiving dog blood must be given compatible feline blood [65].

### 1.2.4 Ethical considerations

Cat blood products are a valuable and often limited resource. While the administration of blood products can be a lifesaving therapeutic intervention for the recipient, consideration must be given to the source of the blood and wellbeing of the donor cat. The term 'harvesting' rather than 'donation' has been suggested as a more appropriate descriptor of blood collection, emphasising the fact a cat cannot consent to being a blood donor [28]. Risks to the blood donor are primarily associated with sedation and blood removal, ranging from iatrogenic haematoma formation at the site of venepuncture [22] to hypotension [54, 66] and possibly cardiopulmonary arrest associated with sedation and/or venesection. Receiving a blood transfusion also carries risks, including the transmission of infectious disease and transfusion reactions. Many of the risks to both recipient and donor can be mitigated or even eliminated by adherence to evidence-based transfusion practice and adequate staff training.

The ISFM consensus guidelines underline the importance of the ethical implications of transfusion medicine with inclusion of a dedicated appendix section discussing ethical considerations. The authors highlight a need for the equal weighting of clinician care and

responsibility towards both blood donor and recipient cat. Consideration of donor wellbeing includes not only in clinic donors but extends to commercially sourced blood products where due diligence necessitates an informed understanding of the welfare and sourcing of donor cats [28]. Clear clinical indications for blood usage should be established for all donor programs, with appropriate staff training and knowledge regarding current blood transfusion practices in place to ensure judicious blood usage and maximal safety for donor and recipient.

## 1.3 Blood storage solutions, quality controls and proposed storage time

### 1.3.1 Anticoagulant-preservative solutions and additives used for blood storage

Blood is stored in anticoagulant-preservative (AP) solutions designed to prevent clotting and preserve viability of the stored RBCs [67, 68]. Common components of AP solutions include citrate or acid citrate, dextrose, adenine and phosphate. Sodium citrate is the constituent anticoagulant used in most AP solutions. As a calcium chelator it inhibits the calcium dependent steps of the coagulation cascade, preventing blood clotting [67]. Dextrose provides nutrients for RBCs during storage, acting as an energy source for glycolytic adenosine triphosphate (ATP) production [68, 69]. Phosphate and adenine are also important for ATP generation. Phosphate reduces RBC phosphate loss and adenine provides a source of adenine nucleotide which helps maintain RBC shape and reduces osmotic fragility [67, 69].

Citrate phosphate dextrose adenine (CPDA) and acid citrate dextrose (ACD) are commonly used anticoagulants for feline WB storage [25, 26, 31, 48, 70, 71]. In some parts of the world, feline blood component therapy is increasingly accessible and more commonly utilised compared to WB storage and transfusion [31, 32, 48]. RBC additive solutions are designed to increase storage time, RBC viability and flow properties of packed RBC products by providing additional post processing nutrients and volume [69]. Citrate phosphate dextrose (CPD) in combination with additive solutions such as saline, adenine, glucose, and mannitol (SAGM) have been reported for the storage and preservation of feline RBC concentrates sourced from commercial animal blood banks [23, 29]. While there are a few

studies evaluating the quality and post-transfusion viability of canine PRBCs stored in RBC additive solutions [72-74], to date there are no equivalent feline investigations published.

### 1.3.2 Blood banking regulation and storage times

Human blood banking is strictly regulated, with procedures and processes falling under the purview of government health agencies [75]. Within the United States of America (USA) human blood products are regulated at a federal level by the Food and Drug Administration (FDA) [76]. Based on FDA and state government issued regulations, various accreditation organisations such as the International Association for the Advancement of Blood and Biotherapies (formerly the American Association of Blood Banks) set practice standards that must be met for blood banking accreditation [75, 76]. Animal blood banks located in the USA are not currently subject to federal regulation however some state legislation exists [77]. In Australia, veterinary blood products are regulated by federal government legislation, with all commercially available blood products requiring registration with the Australian Pesticides and Veterinary Medicines Authority (APVMA) [78].

Maximum RBC storage time or 'shelf life' for specific storage media is determined from in vivo RBC survival studies [67]. In vivo post-transfusion viability studies involve RBC labelling and tracking within recipient circulation to assess the percentage of cell survival 24 hours after transfusion [67]. Human blood banking regulations stipulated by both the Council of Europe European Directorate for the Quality of Medicines and Healthcare and United States FDA require that at 24 hours post-transfusion, there must be a minimum RBC survival rate of 75% in the recipient's circulation [79, 80].

Post transfusion survival thresholds for veterinary blood banking are currently extrapolated from the human literature. One of the first anticoagulant-preservative solutions used for blood storage, ACD, was validated for feline blood storage by Marion and Smith (1983) [70]. Using a viability threshold of >70% RBC survival 24-hours post-transfusion, this study concluded that feline blood anticoagulated in ACD could be stored for at least 30 days. With advances in transfusion medicine and the development of newer anticoagulants, citrate dextrose adenosine phosphate 1 (CPDA-1) is now commonly used for the storage of feline and canine blood [67]. A study only presented as a conference abstract reports 84.8% RBC

survival 24-hours post-transfusion using biotinylating cell labelling techniques for feline WB stored in CPDA-1 for 35 days [71]. There are no peer-reviewed publications supporting this in vivo survival study and with a lack of definitive shelf-life recommendations for CPDA-1 anticoagulated feline WB, studies investigating in vitro markers of RBC quality describe storage periods ranging from 21 to 35 days [25-27, 35, 81]. Studies investigating markers of in vitro quality control [23] and infusion pump mediated RBC haemolysis [29] describe storage times for feline PRBC units anticoagulated with CPD and using SAGM as an additive solution of up to 42 days. This is in line with cited storage times for canine PRBC units with SAGM added [74]. A multinational survey of transfusion practice in dogs and cats found 36% of hospitals stored feline PRBCs with the addition of RBC preservatives, with half using the preservative SAGM. Most hospitals surveyed stored feline PRBCs for less than 30 days with 11% storing them for up to 42 days [48].

## 1.4 Blood banking

### 1.4.1 Blood components and processing

Blood may be stored as WB units or have post collection processing performed to separate it into individual components such as plasma and PRBCs. Post collection component processing is routine in human and canine blood banking, enabling maximum blood product utilisation and targeted component therapy [82]. Due to the small volumes of blood collected and the need for specialised equipment, feline blood component processing and transfusion has historically been uncommon [32, 44]. The expansion of commercial veterinary blood banking overseas has enabled increased access to feline blood components in some regions. In a survey of predominantly North American private referral and university teaching hospitals, feline PRBCs and fresh frozen plasma (FFP) were more likely to be stored compared to WB [48]. Of the hospitals surveyed, 22% sourced feline blood products solely from commercial blood banks with 49% using a combination of purchased blood and hospital sourced donors [48]. In contrast, a UK survey of referral and first opinion practice found only 2.5% of respondents, all referral clinics, had feline PRBCs or plasma stocked [83]. The resource demands for establishing clinic blood banks for component processing and regional UK and European specific animal blood storage restrictions may help explain this disparity [28, 83]. Despite a lack of commercial feline blood product access,

a recent survey of transfusion practice amongst Australian general practitioners, emergency and referral hospitals found 4.5% of respondents had access to feline PRBCs and 10% to feline FFP [8].

As part of processing for storage, it is a standard of care in human blood banking to perform leukoreduction of PRBC units [84]. Leukoreduction (LR) is the removal of white blood cells (WBC) from blood components via filtration or apheresis and can be performed pre or post storage [82]. Leukoreduction is generally performed prior to blood storage with the goal of attenuating subsequent in vitro production and accumulation of deleterious proinflammatory and immune modulating mediators [85]. Pre-storage LR is therefore thought to reduce the risk of transfusion associated inflammatory and immune responses in humans [39]. A human Cochrane review however was unable to make a recommendation for or against the use of leukoreduced PRBCs for prevention of transfusion-related acute lung injury (TRALI), infectious and other non-infectious adverse transfusion related events based on a lack of good quality evidence [86].

The benefits of LR in veterinary transfusion medicine are unclear, with few canine studies and no feline studies investigating the clinical impacts of transfusing leukoreduced blood products. A pilot study has demonstrated successful LR of chilled feline WB using human neonatal LR filters, however the volume of blood required for filtration is likely a limiting factor preventing current usage in feline blood banking [82, 87]. A recent study documenting neutrophil extracellular trap (NET) generation in stored canine PRBC units found that pre-storage LR lessened NETosis during storage [84]. Compared to non-LR units, pre-storage LR has also been found to decrease progressive, storage associated accumulation of procoagulant microparticles [88] and cytokines [89] in canine PRBC units. Transfusion of LR blood may be associated with a comparatively lower inflammatory responses in healthy recipient dogs as indicated by leukocyte count, fibrinogen and C reactive protein (CRP) levels [90]. Conversely, a study of critically ill dogs receiving LR or non LR PRBC units stored for less than 12 days failed to demonstrate significant increases in neutrophil count, CRP or fibrinogen in either group of dogs [85]. Neither study demonstrated any significant difference in transfusion associated adverse reaction rates between dogs receiving LR or non LR blood products [85, 90]. Based on a lack of evidence,

the recently published AVTHM TRACS were unable to make a recommendation for or against LR as a means to reduce or prevent transfusion associated adverse reactions in veterinary patients [14].

### 1.4.2 Collection systems

Use of a closed blood collection system is standard of care in human blood banking [91]. A closed collection system incorporates all necessary components for blood collection, anticoagulation and component processing into one contiguous, sterile system. The only open point in the system, representing the area vulnerable to bacterial entry and contamination is the blood collection needle. Due to unsuitability of human blood banking equipment, open or semi closed blood collection systems are routinely used for feline blood donations due to small donor size, vein diameter and volume of blood obtained per donation [30, 35, 44]. Feline blood collection systems are generally composed of a syringe, butterfly needle and small collection bag/s. There is no standardised nomenclature to define the various types of collection system used for feline blood donations. Collection systems may be designated as 'semi-closed' when all components, bar the anticoagulant, are packaged pre-assembled and sterilised. In contrast, open systems are assembled from individually packaged and sterilised components and do not commonly feature a storage bag as blood is intended for immediate use [92]. In both systems anticoagulant is added during assembly by the operator, effectively 'opening' the system.

A recent case series described the use of a novel closed collection system for feline blood donation intended for storage [25]. Custom 'in-house' manufactured closed collection systems where components are assembled in an aseptic manner have also been reported for feline blood collection [27, 93]. In a survey of feline and canine blood donation practices in private and university referral clinics, 56% of hospitals reported collecting feline blood using a commercially available closed system [48]. The vast majority of survey respondents were from the USA (85%), followed by Canada (10%) then the UK (1%) [48]. Switzerland and Australia each had a single respondent [48]. To the best of the author's knowledge, there are currently no commercially available closed collection systems suitable for feline blood donation in Australia.

Each additional step in collection system assembly represents a potential opportunity for bacterial contamination when an open or semi-closed collection system is used. This increased risk of bacterial contamination dictates the recommendation by some authors that blood collected using an open blood system be stored for less than 24 hours [28, 30]. However, many blood banking operations use open, modified 'semi-closed' or purpose 'in-house' created closed systems to collect feline blood for storage prior to transfusion, reporting minimal complication due to bacterial contamination [23, 26, 30, 31, 35, 93, 94].

### 1.4.3 Bacterial contamination

Transfusion of blood contaminated by bacteria has been documented in the human literature as a cause of serious morbidity and mortality [81, 95]. Limited veterinary reports record bacterial contamination and growth within feline blood units collected by both 'semi-closed' [81] and 'in-house' manufactured closed collection systems [93]. The commonly employed open collection system for feline blood and potential lack of routine bacteriological surveillance means the overall prevalence of blood unit contamination in veterinary medicine is possibly underestimated [81, 96]. Potential sources of bacteria include donor blood or skin, non-sterile collection equipment or environmental contamination during blood collection, preparation and storage [81]. Hohenhaus and colleagues (1997) reported Serratia marcescens contamination of 29 of 174 units of feline WB collected over a seven-month period [97]. Of the 14 cats receiving 15 contaminated transfusions, 10 displayed signs of transfusion reaction and four died. Saline flush given intravenously to donor cats as part of the donation sedation protocol was identified as the primary source of bacterial contamination [97].

Closed blood collection systems are not infallible regarding bacterial contamination as demonstrated by case reports in human and canine blood banks [81, 95]. A case series evaluating the use of a new commercial closed system designed for cats reported positive bacterial contamination in one out of a total of eight WB units at day 35 of storage. Identified as *Serratia marcescens*, the authors hypothesised that contamination occurred during blood collection. However, stored WB units were also repeatedly sampled throughout storage via sterile built-in 'self-cleaning valves' [25]. Another recent study comparing an open system to a custom closed collection system for feline blood collection

also had 1/8 WB units from the closed system collections culture positive for bacteria [27]. This unit only cultured positive to *Staphylococcus* spp. on the day of blood collection and was later transfused without incident [27]. All eight WB units collected by an open system cultured negative on the day of collection and were transfused within 24 hours [27]. *Ralstonia* spp. DNA was detected by PCR in 5/16 units for at least one time point during storage in three closed and two open collection system units [27]. This finding was considered clinically insignificant as subsequent bacterial cultures failed to grow this species at any time point [27].

Negative aerobic and anaerobic culture results have been reported in both feline PRBC and WB units collected via open systems and stored for between 25 and 42 days [17, 24, 26, 94]. While these results are promising, only a small number of blood units were tested in each study making extrapolation to larger blood banking operations problematic. Similarly, Blasi Brugue and colleagues (2018) performed a series of in vitro quality control evaluations for a total of 489 units of feline PRBCs collected via a commercial blood bank using a modified, aseptically assembled semi-closed collection system. While bacterial contamination was not identified in any of the 489 units tested, bacterial culture was only performed 24 hours post collection [23]. Despite a relatively small body of evidence, storage of feline blood collected via open or modified open systems does not appear to have an inherently or intolerably high level of associated bacterial contamination.

### 1.4.4 Haemolysis

Red blood cell haemolysis percentage is routinely used in human blood banking as a quality control marker for stored blood. Haemolysis percentage increases during blood storage due to RBC rupture and release of intracellular haemoglobin or shedding of RBC microvesicles that contain membrane bound haemoglobin [98, 99]. Complement activation and subsequent increases in membrane attack complexes (MAC) are additional suggested mechanisms for enhanced RBC haemolysis during storage [100]. In vitro RBC haemolysis occurs as an end point of cell ageing and progressive injury during storage [99]. It can also result from improper blood handling during collection, processing and storage [57]. Exposure to temperature extremes such as freezing, or overheating may also cause irreversible thermal injury and subsequent RBC haemolysis [98, 101]. Mechanical RBC injury

and haemolysis may also arise from blood collection and transfusion factors related to the diameter of needles or intravenous tubing, pump type, in line filter occlusion and infusion rates [98, 101-103]. Susceptibility to mechanical injury and transfusion related shear stress has also been demonstrated to increase in aged, stored human blood [104-106].

At a high enough haemolysis percentage, free haemoglobin will cause visible pink or red discolouration to a RBC product supernatant [98]. While visual inspection of RBC units is an important aspect of routine blood banking quality controls, an accurate determination of haemolysis percentage is necessary to prevent over or underestimation based on visual inspection alone. A calculated haemolysis percentage can be determined by comparing the measured concentration of free plasma haemoglobin with the total haemoglobin, accounting for sample haematocrit [98]. Percentage haemolysis can be calculated using the formula:
Percent haemolysis (%) = (100 – haematocrit) x free haemoglobin in plasma or suspending medium (g/L) / total haemoglobin (g/L) [98].

A quantitative measurement of plasma free haemoglobin can be determined using either direct optical methods such as spectrophotometry or chemical techniques such as the cyanmethemoglobin method [98]. Considered the gold standard for measuring haemoglobin concentration, the cyanmethemoglobin method involves combining cyanide with the haemoglobin from lysed RBCs and then measuring the cyanmethemoglobin produced using photometry [107]. In contrast, spectrophotometric methods utilise absorbance peaks of oxyhaemoglobin at specified wavelengths to quantify haemoglobin concentration [98]. Designed to measure very low concentrations of free haemoglobin in plasma, portable machines such as the HemoCue Plasma Low Hb® utilise the azidemethemoglobin method. Plasma, serum or an aqueous sample is added to a microcuvette containing a dried reagent that converts free haemoglobin to azidemethoglobin [108]. This is then measured photometrically at dual wavelengths of 570nm to determine the haemoglobin concentration and 880 nm to correct for any sample turbidity [108]. The free plasma haemoglobin concentration can then be used to calculate a haemolysis percentage for the sample using the aforementioned formula. The HemoCue® system is calibrated against the International Council for Standardisation in Haematology's

international reference method for haemoglobin determination which utilizes the cyanmethemoglobin method [108].

Excessive haemolysis limits the benefit of the transfusion for the recipient, representing a reduction in RBC oxygen carrying capacity [23, 109]. Cell-free haemoglobin induces oxidative stress, with potentially detrimental effects to renal, myocardial and vascular systems [23, 110-112]. It may also reduce oxygen delivery through nitric oxide scavenging leading to vasoconstriction and endothelial injury [39, 113]. Haemolysis due to microvesicle shedding from the RBC membrane is associated with proinflammatory and procoagulant activity in aging, stored human RBCs [99, 114]. In vitro haemolysis has also been linked to an increased risk of adverse transfusion events, namely nonimmune mediated haemolytic reactions [23, 57, 109]. In the absence of bacterial contamination or excessive free haemoglobin, nonimmune mediated haemolytic reactions are generally of little clinical consequence other than reducing transfusion effectiveness [101, 115]. However, in an experimental dog sepsis model, high concentrations of free haemoglobin were hypothesised to exacerbate vascular and pulmonary injury and was associated with increased mortality [111]. A case series of four dogs documented severe to fatal acute haemolytic transfusion reactions believed to be caused by the transfusion of significantly haemolysed PRBCs units [116]. In vitro RBC haemolysis secondary to improper storage was suspected to be the cause, based on a high haemolysis percentage in samples of unused blood units stored in the same refrigerator. The blood units involved in the transfusion reactions were not tested for haemolysis pre-transfusion, unit expiration dates were unknown and while unlikely, bacterial contamination was also unable to be ruled out [116]. While this case series demonstrates potentially catastrophic consequences secondary to the transfusion of haemolysed RBCS, individual variability in illness severity pre-transfusion and propensity for in vivo haemolysis post-transfusion were also likely significant factors in the reactions observed [116]. There are no studies or published cases documenting the clinical significance of transfusing blood with a high haemolysis percentage in cats.

Increases in RBC haemolysis during storage are well characterised in the human literature [99]. Identification of the harmful effects of transfusing haemolysed blood has led to regulatory groups involved in human blood banking establishing haemolysis thresholds for

stored blood. The Council of Europe and US FDA specify maximum accepted end storage haemolysis percentages of 0.8% and 1% respectively [109]. Due to a lack of established haemolysis thresholds in veterinary blood banking, guidelines are generally adapted from human recommendations. The recently published AVTHM TRACS recommends RBC units be checked for haemolysis by sampling the main unit rather than a unit segment and that a maximum threshold of 1% be used to establish suitability of the unit for transfusion [14].

Haemolysis percentage at different time points of blood storage has been investigated in both stored cat [23, 25, 26] and dog blood [73, 109]. A recent study evaluating haemolysis in 180 units of canine PRBCs demonstrated >1% haemolysis in over half the units at days 36-42 of storage [109]. Increased haemolysis has also been documented during storage of feline blood products [23, 26]. Blasi Brugue and colleagues (2018) reported 13.8% of 180 units of feline PRBCS tested at day 29-35 of storage exceeded the recommended human blood banking haemolysis threshold of 1% [23]. Another recent study of CPDA-1 anticoagulated feline WB collected via an open system and stored for 35 days found mean haemolysis percentage exceeded 1% by 21 days of storage [26]. In contrast, a study of eight CPDA-1 anticoagulated feline WB units collected using a novel closed system retained a haemolysis percentage <1% at the end of 35 days storage [25]. The differences in blood collection methods may explain the disparity in observed haemolysis percentages [25, 26]. The effect RBC component processing, storage and transfusion technique has on feline RBC membrane fragility must be considered.

### 1.4.5 The storage lesion

During storage, RBCs undergo a number of biochemical and biomechanical changes collectively termed the ‘storage lesion’ [117]. A consequence of ex vivo hypothermic storage in a closed acidic environment, the storage lesion has implications for RBC survival and viability post-transfusion [117, 118]. During storage, RBCs are exposed to oxidative stress with a concurrent loss of normal in vivo biochemical and metabolic processing [118]. Initiated by haemoglobin iron oxidation, storage induced alterations to RBC metabolism lead to enhanced reactive oxygen species (ROS) formation [118]. Subsequent reversible and irreversible enzyme, lipid and protein oxidation further impair RBC metabolic functions, resulting in changes to RBC morphology and reduced deformability [118, 119]. During

storage metabolic impairments are compounded by RBC nutrient substrate depletion and waste product accumulation [118].

The human literature documents well defined storage induced biochemical changes including depletion of ATP and 2,3-diphosphoglycerate (2,3-DPG), a progressive decrease in pH, increased supernatant potassium and increased ammonia [19, 110, 118, 119]. Summarised in Table 1, there are an increasing number of studies that evaluate the in vitro features of storage lesion in either feline WB or PRBCs [17, 19, 20, 23, 24, 26, 94, 120]. To date there are no prospective or experimental veterinary studies investigating the clinical implications and effects of transfusion technique on storage lesion affected feline WB or PRBCs.

Biochemical evaluation of the feline storage lesion highlights species-specific RBC differences with implications for storage length, transfusion efficacy and safety. Both ATP and 2,3-DPG have been shown to decrease during feline blood storage, along with decreases in pH and glucose and increases in lactate, ammonia and sodium [17, 19, 20, 25, 94]. Biochemical derangements in stored feline blood are not uniformly reported, with conflicting reports of decreased then unchanged [120] and increased chloride concentration [19] and both significant decreases [19] and increases in potassium [25, 94]. Despite similar storage times, direct study comparison is difficult due to the low numbers of blood units analysed and the differences in RBC processing (WB versus PRBCs) and anticoagulant and/or preservative solutions used.

Ammonia accumulates during blood storage as a product of the deamination of amino acid derived from adenine, plasma and RBC associated proteins [17, 19, 20]. Both linear [17] or near linear [19] and non-linear [20] increases in ammonia have been described in feline WB and feline PRBCs stored for 28, 35 or 42 days. In a study of five dogs transfused with PRBCs stored between 18 and 30 days that contained increased ammonia concentrations, recipient blood plasma ammonia concentrations remained normal post-transfusion [121]. Notably, none of the dogs in this study had primary liver disease. The clinical relevance of ammonia accumulation during blood storage is unknown but considered potentially significant for transfusion recipients with hepatic disorders or receiving massive transfusions [17, 20, 121].

While acknowledging the lack of evidence base to make a recommendation, the AVHTM TRACS suggests that when available, dogs and cats at an increased risk of hyperammonemia receive fresh (< 24 hour) WB or PRBCS stored no longer than 7 days [14].

The significance of ATP and 2,3-DPG depletion during storage of feline blood is unclear. Unlike human and dog RBCs, cat RBCs have low concentrations of 2,3-DPG and it is chloride instead of 2,3-DPG that acts as a key haemoglobin oxygen affinity modifier [120]. Compared to other species, the P50 of feline haemoglobin also decreases little during storage [120]. ATP is an important energy substrate that helps maintain RBC deformability through maintenance of membrane elasticity, ideal cell geometry (surface area to volume ratio) and intracellular fluid viscosity [25, 117, 122]. Baseline ATP concentration is much lower in stored feline blood compared to canine and the clinical significance of ATP depletion during feline blood storage is unknown [25].

Progressive changes to RBC membrane integrity and shape during storage lead to reduced cell deformability and oxygen carrying capacity [117]. Human studies report irreversible storage induced morphological alterations in 6-9% of RBCs in PRBC units [118]. Depletion of ATP and 2,3-DPG and contact with anticoagulant are proposed mechanisms for progressive RBC membrane alteration, leading to loss of the deformable, biconcave shape [19, 25, 117]. Reactive oxygen species accumulation and oxidation of membrane lipids and proteins cause structural damage to the membrane cytoskeleton and promote shedding of membrane microparticles (MPs) leading to irreversible membrane transformation [118]. Initial, reversible change to poorly deformable echinocytes progresses to the irreversible formation of nondeformable spheroechinocytosis or spherocytes as storage length increases [117]. RBC shape change and the reduction in RBC surface to volume ratio through MP membrane loss may impair RBC ability to traverse narrow capillary beds [118]. Irreversible morphological phenotypes are also associated with increased RBC osmotic fragility (OF) and cell lysis [118, 123].

The osmotic fragility test measures the relative fragility or resistance of RBCs to in vitro haemolysis when exposed to solutions of decreasing osmolarity ranging from isotonic to hypotonic [124]. Whole blood is incubated in incrementally more hypotonic sodium chloride

solutions causing progressive increases in RBC water influx, swelling, leakage and finally cell rupture. The supernatant free haemoglobin concentration is spectrophotometrically determined and used to calculate a haemolysis percentage for each dilution based on the distilled water sample representing complete RBC lysis. When the haemolysis percentage for each sodium chloride dilution is plotted on a graph, a sigmoid curve is generated. From this curve the mean OF, which is the sodium chloride concentration at which 50% of the RBC are lysed, can be determined [124, 125].

Increased osmotic fragility has implications for RBC survival during the mechanical stressors of transfusion and in the recipient circulation post-transfusion. Human studies document reduced RBC deformability as a consequence of storage, resulting in increased osmotic and mechanical fragility [126]. Both Spada et al. (2018) and Crestani et al. (2018) report limited RBC morphological change in feline WB stored for 35 days, consisting of increased numbers of echinocytes [24, 25]. Crestani et al. (2018) also failed to demonstrate any significant change in feline RBC osmotic fragility, supported by a lack of observed spheroechinocytes on morphological analysis [25]. Conversely, a significant increase in osmotic fragility was documented in feline PRBCs units collected via vascular access ports and stored for 25 days [94]. Corpuscular changes and the associated rheological effects may lead to recipient microcirculatory flow impairment, reduced oxygen delivery and increased red cell haemolysis [39, 117]. The clinical consequence of morphological change however has not been well established, with a recent human study assessing perfusion of stored RBCs of varying age through a microcirculation-mimicking microfluidic system finding no change to RBC deformability capacity [127]. There are no studies investigating the clinical significance of transfusing storage lesion affected feline blood on either RBC viability during transfusion or within a recipient's circulation.

**Table 1 Summary of literature that has investigated storage lesion in feline blood**

| Reference | Units of blood | Collection system | Storage medium | Storage time (days) | Time points tested (day)[#] | Storage lesion features and significant changes[*] | | | Blood cultures: aerobic and anaerobic[**] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Decrease | Change not significant | Increased | |
| Wong and Haskins 2007 [120] | 4 WB units | Commercial blood bank | CPDA-1 | 35 | 0, 2, 4, 7, 14, 21, 28, 35 | $P50^{++}$ | Chloride | | Not performed |
| Aubert et al. 2011 [94] | Vascular access port collected = 8 PRBC units | Open | CPDA-1 | 25 | 0, 25 | Glucose<br>pH | | Potassium<br>MOF<br>LDH activity | Negative |
| | Conventionally collected = 8 PRBC units | Open | CPDA-1 | 25 | 0, 25 | pH | Glucose<br>MOF | Potassium<br>LDH activity | Negative |
| Spada et al. 2014 [17] | Experiment 1:<br>15 WB units<br>2 PRBC units<br><br>Experiment 2:<br>5 WB units<br>4 PRBC | Open | CPDA-1 Plus SAGM for PRBC units | 42 | Experiment 1:<br>0, 35, 42<br><br>Experiment 2:<br>0, 1, 2, 3, 7, 14, 21, 28, 35, 42 (PRBCS only) | | | Ammonia | Negative |
| Cummings et al. 2016 [20] | 13 PRBC units<br>n = 5 ammonia<br>n = 8 cytokine concentration | Semi-closed | CPD | 28 | 0, 7,14,21,28 | | IL-6 and IL-10 not detected<br>CXCL-8 detected in 4/8 unit, all time points | Ammonia | Not performed |
| Heinz et al. 2016 [19] | 10 units PRBC | Commercial blood bank | ACD-A plus AS-3 | 35 | 0, 1, 4, 7, 14, 21, 28, 35 | Potassium<br>Glucose | pH (to day 7 only) | Sodium<br>Chloride<br>Lactate<br>Ammonia | Negative |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Spada et al. 2018 [24] | 40 WB units | Open | CPDA | 35 | | WBC<br>Platelet count<br>Morphology: normal RBCs, schistocytes, RBCs with Heinz bodies, % recognisable WBCs (n=20) | RBC count, Hb, HCT, MCV, MCH, MCHC, RDW | Morphology: macrocytes, echinocytes, spherocytes, lysed RBCs (n=20) | Not performed |
| Crestani et al. 2018 [25] | 8 WB units | Commercial closed | CPDA-1 | 35 | 0, 7, 14, 21, 28, 35 | Glucose<br>Fibrinogen<br>2,3-DPG<br>ATP | RBC count, Hb, HCT, MCV, MCH, MCHC, RDW<br>MOF | Lactate<br>Potassium<br>Haemolysis %<br>PT, aPTT<br>Morphology: echinocytes | 1/8 units positive (*Serratia marcescens*) |
| Blasi Brugue et al. 2018 [23] | 489 PRBC units | Semi-closed | CPD plus SAGM | 35-42 | 0, 29-35 (n=180)<br>0, 36-42 (n=118) | PCV | | Haemolysis % | Negative (tested at 24 hours only) |
| Spada et al. 2019 [26] | 12 WB units | Open | CPDA | 35 | 0, 7, 14, 21, 28, 35<br>7, 14 (n=7)<br>21, 28 (n=9) | Glucose | RBC count, Hb, HCT, MCV, MCH, MCHC, RDW | Morphological index[++]<br>Haemolysis %<br>Sodium (day 28 only)<br>Potassium | Negative |

[#] Where day 0 represents the day of blood collection

[*] Unless otherwise stated comparisons performed between all-time points tested and day of blood collection and the significant findings listed refer to the maximal storage time tested only

[**] all cultures performed at the end of the storage period unless otherwise noted

[+] PO2 at which haemoglobin is 50% saturated

[++] Scoring system describes echinocyte transformation based on cell shape (discocyte, flat, ovoid) and protrusion/spicule number with increased index score representing progressive morphological change

2,3-DPG 2,3-diphosphoglycerate; ATP adenosine triphosphate; ACD-A anticoagulant citrate dextrose solution A; AS-3 additive solution formula 3 preservative; CPD tri-sodium citrate sodium phosphate dextrose; CPDA citrate phosphate dextrose adenine; CXCL chemokine ligand; IL interleukin; Hb haemoglobin; LDH lactate dehydrogenase; MOF mean osmotic fragility, PRBC packed red blood cells; SAGM saline adenine glucose mannitol; WBC white blood cell; WB whole blood

## 1.5 Transfusion medicine

### 1.5.1 Transfusion reactions

Adverse immunologic and nonimmunologic reactions can occur following the transfusion of any blood product [57, 82, 89]. Sources of reaction include factors relating to the donor, recipient and/or blood components [128]. The risk of adverse events may be reduced through diligent donor screening, blood collection technique, processing and storage practices [57]. Published in 2021, the AVHTM TRACS consist of a three-part systematic review of the current veterinary literature that seeks to standardise terminology and propose guidelines for the definitions, clinical signs, prevention, monitoring, diagnosis and treatment of transfusion reactions [14-16].

Transfusion reactions can be classified as either nonimmunologic or immunologic. Nonimmunologic reactions are defined as adverse reactions arising from physical, chemical, or microbiological properties, or volume of the blood product being transfused [16]. Nonimmunologic reactions encompass transfusion transmitted infections, acute or delayed non-immune mediated haemolysis (mechanical, thermal, chemical or osmotic), transfusion associated circulatory overload (TACO), air embolism, citrate toxicity/hypocalcaemia, hyperammonemia and hypotensive transfusions reactions [16, 57, 89]. Blood collection, processing, storage, and delivery methods can greatly influence the incidence of many nonimmunologic adverse reactions and provide a means to minimise occurrence through improved blood banking and transfusion protocols.

Immunological adverse reactions result from the recipient's immune system response to the blood product being transfused [16]. Immunologic transfusion reactions include immune mediated acute or delayed haemolytic transfusion reactions (HTR), type I hypersensitivity reactions, TRALI, post-transfusion purpura, transfusion associated graft versus host disease and delayed serologic transfusion reactions [16, 57]. Immunologic acute HTRs are type II hypersensitivity reactions that result in RBC destruction secondary to the transfusion of incompatible blood [16]. Delayed HTRs occur between 24 hours and 28 days after blood product transfusion and have been reported in 64% of cats that received a xenotransfusion of dog blood in one study [64].

Some transfusion reactions have causes that can be classified as either immunologic or nonimmunologic. Commonly reported, febrile non-haemolytic transfusion reactions (FNHTRs) can be classified as either immunologic or non-immunologic given that an acute increase in transfusion recipient body temperature may be due to either donor blood WBC or platelet antibody-antigen reactions or the transfusion of proinflammatory mediators within a stored blood product [16].

In cats, transfusion associated complication rates between 1.2% and 22.9% have been reported [6, 9, 31-33, 35]. Febrile non-haemolytic transfusion reactions are generally the most frequently reported [6, 9, 31-33, 35], with HTRs [6, 31], TACO [34], transfusion transmitted infections and hypersensitivity reactions also documented [16, 32, 35]. A recent retrospective feline study reported an association between transfusion reactions and increased risk of non-survival amongst 450 cases of RBC transfusion [33]. The AVHTM consensus statement authors strongly recommend administration of AB typed matched blood and suggest major crossmatching should also be performed prior to any feline transfusion to minimise the risk of a transfusion reaction in the recipient [14]. The incidence of FNHTRs in cats may be increased when pre transfusion crossmatching and administration of crossmatch compatible blood is not performed [6, 7].

Based on the lack of demonstrable benefit in the human literature, the AVHTM Consensus Statement authors do not recommend use of pre-transfusion treatment with antihistamines or antipyretics in either dogs or cats [14]. Over half the respondents in a survey of Australian general practice (GP), 24 hour, after hours only and specialist referral hospitals reported pre-medicating both dogs and cats prior to blood transfusions [8]. Respondents who did not blood type cats prior to transfusion were more likely to administer a pre-medication. As the authors note, pre-medication will not prevent potentially fatal acute HTRs such as those arising from the administration of type incompatible blood to cats. A lack of access, poor familiarity with, or cost associated with blood typing or cross matching methodologies and limited donor availability were all suggested as potential reasons for an absence of pre-transfusion compatibility testing in the aforementioned study [8].

### 1.5.2 Stored blood transfusion consequences and controversies

Transfusion of older, stored blood may have clinical consequences secondary to proinflammatory and immunomodulatory aspects of the storage lesion and reduced RBC viability and oxygen carrying capacity [39, 118]. Adverse physiological effects arising from the transfusion of blood with storage lesion induced changes have been documented in animal models and human in vitro studies [112, 118]. Human studies report proinflammatory sequelae of transfusing stored blood such as complement activation [100], hypercoagulability [129, 130] and endothelial injury, cell adhesion and capillary leakage [117, 118]. Older blood may also confer reduced transfusion benefit as indicated by reduced post-transfusion RBC recovery and lower haematocrit increases [131].

In humans, transfusion of autologous, older stored blood has been shown to increase markers of extravascular haemolysis such as bilirubin and non-transferrin bound iron (NTBI) in recipient circulation [131, 132]. Increased NTBI and the transfusion of cell-free haemoglobin and cytokines in stored blood can stimulate a proinflammatory response [118]. Increased NTBI may augment bacterial proliferation and is implicated in the increased risk of infection and sepsis documented in some transfusion recipients [132]. Post transfusion in vivo haemolysis has also been found to increase with the transfusion of older stored canine RBC products versus fresher blood [111, 133-135]. A study of ten healthy dogs transfused with autologous blood found increased free haemoglobin and iron in the circulation of dogs receiving PRBCs stored for 35 days versus 3 days. Consistent with increased post-transfusion intravascular haemolysis, the authors hypothesised early loss of transfused RBCs may have been secondary to storage induced increases in RBC osmotic fragility [135]. Callan et al. (2013) demonstrated an inflammatory response characterised by increased neutrophil counts, decreased platelet counts and increased serum monocyte chemoattractant protein-1 concentration in healthy dogs transfused with autologous 28-day old PRBC units [136]. This inflammatory response was not observed in the autologous 7-day old PRBC transfusion group [136]. The clinical implications of transfusing older stored blood with increased circulating levels of potentially proinflammatory free iron and haemoglobin and the potential to induce inflammatory changes in transfusion recipients requires further investigation in clinically unwell dogs.

The transfusion of biologic response modifiers (BRMs) such as cytokines, chemokines and oxidised lipids that accumulate during blood storage are linked to the pathogenesis of TRALI in people [118, 137]. Despite evidence in experimental animal models [137-139], there is no clear consensus regarding age of stored blood and the development of TRALI in susceptible human populations [140-142]. The prevalence of TRALI is also poorly characterised in veterinary medicine, with a lack of consensus regarding its occurrence or reported incidence in dogs and cats receiving transfusions [82, 143]. Based on the consensus definition of VetALI [144], Thomovsky et al. (2014) documented a 3.7% incidence of ALI in dogs receiving blood product transfusions. As noted by the authors however, this was not significantly different to the reported incidence of acute respiratory distress syndrome (ARDS) in critically ill dogs not receiving blood transfusions [143]. The incidence of TRALI is ill defined in cats with a recent retrospective study of 450 transfusion events in 267 cats failing to identify a single incident [33].

In addition to proinflammatory effects, components of storage lesion affected human blood such as NTBI may have immunomodulatory properties [118]. Shed MPs interact with and prime neutrophils and contribute to increased circulating free haemoglobin, heme and iron when lysed [145]. Together with ROS formation, these components of stored blood may trigger the recipient innate immune response [145]. Conversely, RBC transfusion linked immunosuppression, termed transfusion associated immunomodulation (TRIM) is also recognised in people [118]. Down regulation of cellular immunity results from modulation of T cell responses and decreased macrophage function [39]. Evidence of immune suppression following transfusion of stored, 21-day old LR RBC units has been demonstrated in critically ill human paediatric patients [146].

The immunomodulatory effects of storage lesion have not been well characterised in veterinary medicine. Implicated in immunologic reactions, cytokine and chemokine accumulation has been documented in both stored human [147] and canine RBC containing products [89]. Leukocytes synthesise and secrete cytokines and chemokines such as interleukin (IL) 6, IL-1$\beta$, chemokine ligand (CXCL) 8 (formerly IL-8) and tumour necrosis factor alpha (TNF-alpha) during blood storage [20, 147]. Corsi et al. (2014) evaluated cytokine concentration in leukoreduced (LR) and non leukoreduced (NLR) canine PRBCs over

a 5-week storage period. While the concentrations of IL-1B and TNF alpha did not significantly change over the storage period or between LR and NLR units, CXCL-8 was found to markedly increase with storage in the NLR units only [89]. Cummings et al. (2016) were unable to consistently quantify the inflammatory marker IL-6 or anti-inflammatory IL-10 during weekly testing of feline WB units stored for 28 days. Interestingly CXCL-8 was detected in half the units tested at all time points [20]. The clinical significance of cytokine and chemokine accumulation in stored blood has not been established in veterinary transfusion medicine.

The human literature contains a multitude of opposing observational, randomised controlled trials (RCTs), systematic reviews and meta-analyses investigating the effect of stored blood age on patient mortality [142, 148-151]. A recent series of large RCTs failed to demonstrate a benefit associated with administration of 'fresher' blood transfusions (average of 6 to 12 days old) compared to moderately aged RBC products (average 3 weeks of age) [148, 149, 152-155]. However, the safety of transfusing RBC products approaching maximal storage time in specific, more at-risk human patient populations is yet to be established [118]. A 2018 Cochrane review of the available high quality RCTs concluded that for adults, there is no evidence that length of RBC product storage has an effect on patient mortality [156]. The RCTs analysed included adult populations across the settings of intensive care, hospitalised inpatients, cardiac and major elective surgery patients, and haematology outpatients. While evidence for certain patient populations such as neonates and children are still lacking, the authors determined continued adherence to the blood banking practice of using the oldest available RBC units remains safe [156].

There is a lack of veterinary studies reporting adverse reactions associated with storage lesion affected RBC products. Most studies in cats reporting transfusion associated adverse events and transfusion efficacy as estimated by post-transfusion PCV increases do not identify or analyse the effect of transfused blood products storage duration [10, 31, 32, 34]. In studies where age of blood product is accounted for, it is analysed in relation to its effect on the primary outcome of cross match compatibility and not transfusion reactions or efficacy [7, 9]. A single, recent retrospective study documented an association between transfusion of older stored PRBC units and non-survival and transfusion related

complications in cats [33]. There are few clinical [133, 135, 136, 157, 158] and experimental dog models [111, 134, 159] examining age of blood product and the potential adverse effects of transfusing older blood products. Retrospective canine studies have associated duration of RBC unit storage with increased morbidity but not mortality in dogs receiving blood products [133, 157]. Hann et al. (2014) found age of PRBCs to be a negative risk factor for 30-day survival in dogs with haemolysis, the majority of which had immune mediated haemolytic anaemia. The transfusion of older blood units was also associated with the post-transfusion development of new or worsening coagulation failure and thromboembolic disease [157]. In dogs, transfusion of PRBCS and WB stored for more than 14 days may also be less effective at increasing recipient haematocrit compared to fresher blood. The aetiology of the anaemia in this study, which may also significantly impact transfusion efficacy, varied widely between transfusion recipients [160]. Common to all the clinical veterinary studies [133, 157, 158, 160], a lack of illness severity scoring and variation in study patient populations hinder the ability to extrapolate clear clinical recommendations regarding use of blood stored for shorter or longer periods.

The consequences of transfusing older stored blood products in specific disease states have been investigated in experimental animal models including dogs [111, 134, 159], mice [118, 132] and sheep [161]. Increased lung injury, degree of shock and mortality associated with the massive transfusion of maximally stored versus week old LR PRBC units has been demonstrated in an experimentally induced canine septic pneumonia disease model. Increased cell-free haemoglobin in the blood of dogs receiving the 42-day old LR PRBCs was suggested to represent ongoing in vivo haemolysis and reflect increased erythrocyte fragility secondary to the storage lesion. Of note, the 24-hour post-transfusion survival of RBCs used in the study as determined by radio-chromium labelling techniques was only greater or equal to a 60% threshold [111]. This is below the current Council of Europe and US FDA post-transfusion survival thresholds for human blood banking [79, 91]. In contrast, in a canine model of haemorrhagic shock the transfusion of 42-day old PRBCs resulted in no worse clinical outcomes compared to resuscitation with 7-day old PRBC units [134]. The relevance of experimental animal disease models to clinical practice requires further elucidation and exploration through prospective clinical research.

A 2015 study comparing two University teaching hospitals observed that the hospital with a blood bank protocol dictating transfusion of the oldest PRBC blood units first resulted in significantly older blood units being administered and less blood wastage due to unit expiry. During the one-year study period, the hospital that lacked a protocol and permitted clinician preference to determine blood usage discarded 96 units of expired PRBCs [162]. The disparity in how blood usage is determined reflects a current lack of high-quality evidence from prospective RCTs needed to help standardise veterinary blood banking protocols to minimise blood unit wastage and maximise transfusion clinical efficacy and safety. Though viewed through the lens of transfusion reaction prevention and not transfusion efficacy, the AVTHM TRACS provide peer-reviewed recommendations for blood usage based on storage age. Based on the available research in dogs, the AVHTM TRACS suggest transfusion of fresher RBC products to dogs with sepsis or anaemia with a haemolytic aetiology. Due to a lack of evidence, no recommendation could be made regarding age of blood products and influence on transfusion reactions in cats [14].

### 1.5.3 Method of transfusion delivery in cats

Feline blood transfusions are commonly administered using a syringe pump and 18 µm microaggregate in-line filter, ensuring removal of cellular or fibrin aggregates from the transfused blood components and enabling controlled delivery of smaller volumes of blood. This is in contrast to human and dog blood component transfusions which are delivered through standard human 170 - 260 µm in-line blood administration sets designed to filter out larger clots or cellular aggregates [14, 163]. Due to the priming volumes required and incompatibility with a syringe driver system, standard human administration sets are considered impractical for feline transfusions. In people, microaggregate filters with a mesh pore diameter of 20 to 40 µm are reserved for the autotransfusion of perioperative autologous haemorrhage [163]. In human neonatal or paediatric medicine where smaller volume transfusions are required, standard blood units may be pre-partitioned into smaller aliquots via satellite bags or a sterile connection device into a neonatal syringe system with in-built 150 µm filter, ready for direct patient transfusion via a syringe pump [164]. While veterinary 150 µm in-line blood filters that require a small priming volume are commercially available in some countries (SA150 Blood Filter; https://innovativeanimalsupplies.com/products), there is a lack of peer-reviewed evidence

assessing and validating transfusion technique in the cat. Most clinical cat studies assessing RBC survival using change in post-transfusion PCV [31, 32, 34], some of which primarily assess effect of crossmatch compatibility [6, 7, 10] fail to specify the transfusion administration techniques used. The two clinical studies that do reference transfusion technique include use of an 18 µm in line filter and syringe pump [9] and 200 µm in line filter and gravity administration set [35] however neither study was designed to evaluate the impact of transfusion methodology on transfusion efficacy or outcomes.

The use of a syringe, 18 µm microaggregate filter and syringe driver or pump for feline blood transfusion is recommended by some authors including the recent ISFM Consensus Guidelines on the Collection and Administration of Blood and Blood Products in Cats [28, 30, 82]. Based on the limited available evidence, AVHTM TRACS also suggests this to be an acceptable method for cat blood transfusion [14]. There is a single peer-reviewed study in cats using biotinylated RBCs to assess in vivo post-transfusion RBC survival of blood transfused using either gravity flow and a standard blood giving set or 18 µm in-line filter and syringe pump [18]. There was no significant difference in RBC survival between the transfusion methods within both the first 12 hours and six weeks post-transfusion. The marginally reduced overall RBC half-life when compared to other feline RBC survival studies was proposed by the authors to reflect cell biotin labelling injury rather than transfusion induced RBC damage. As only WB, stored for a maximum of 12 hours was evaluated [18], results may not represent the post-transfusion RBC survival of component PRBCs and/or stored blood transfused using a syringe pump and 18 µm filter. While the filter used in this study is capable of bidirectional flow, it was utilised as an in-line filter in contrast to the manufacturer recommendation that cellular fluids be aspirated through it prior to infusion [165].

In-line 18 µm microaggregate filters are utilized in the emergency setting to facilitate autotransfusion in both dogs and cats [166, 167]. Due to compatibility of human blood banking equipment for canine transfusion medicine, use of in-line microaggregate filters and syringes are generally reserved for emergency autotransfusions. In contrast to the cat, significant in vivo RBC loss within 24 hours of transfusion via syringe pump and 18 µm microaggregate filter has been reported in a canine post-transfusion RBC survival study

[168]. Only 1/7 dogs transfused with this technique had the transfused, biotin labelled RBCs detected in circulation at 24 hours with none detected at day seven. The mechanism for this unacceptably high and early in vivo RBC loss was unclear with ex vivo markers of haemolysis (RBC counts, osmotic fragility, plasma free haemoglobin) not significantly different between the gravity and syringe or volumetric peristaltic pump administration groups [168]. Cooley-Lock et al (2019) also failed to demonstrate any significant change to RBC morphology or osmotic fragility in fresh canine WB after simulated syringe pump transfusion at three different rates through an 18 µm microaggregate filter [169]. While post-transfusion RBC survival cannot be inferred from these results, a case series of 25 dog emergency autotransfusions using either a 210 µm or 18 µm filters also found no association between patient survival and filter pore size [166].

Differences in RBC survival post microaggregate filter passage may reflect significant species haemorheological differences and species-specific transfusion factors such as blood flow rate [18, 170]. The smaller feline RBC diameter of between 5.5 to 6.3 µm compared to the average 7 µm diameter of canine RBCs may help explain difference in species transfusion mechanics and filter passage [170]. Studies examining feline RBC deformability under hypoxic conditions reveal contrasting results depending on methodology. Hakim et al. (1988) reported a significant increase in cat blood nuclepore polycarbonate (4.7 µm pore) filtration rate and hence reduced RBC deformability under hypoxic conditions compared to unchanged dog blood filtration time [122]. In contrast, assessment by ektacytometry has revealed no significant changes to either canine or feline RBC deformability under hypoxic conditions [171].

Microaggregate filters were designed for pre-transfusion human blood filtration to remove potentially harmful microaggregates of cellular debris, platelets, white blood cells and fibrin that accumulate during blood storage [102, 172]. Despite the 18 µm filter commonly used in feline transfusion medicine being a human medical product, there is a paucity of information in the human literature regarding its use [102, 172, 173]. Passage of two or five day old human WB or PRBCs through an 18 µm filter via manual negative pressure was demonstrated to cause excessive RBC haemolysis and filter occlusion [102]. Similar results have been reported in older human PRBCs or WB transfused through an 18 µm filter using a

syringe pump. While up to 40 mL of fresh WB could be passed through a single filter at 200 mL/hr with no measurable haemolysis, filter occlusion occurred after the passage of 20 mL of 14-day old WB with marked haemolysis in any subsequently filtered blood. In 10 day old blood, slower infusion rates below 20 mL/hr still resulted in increased haemolysis [172].

Studies assessing haemolysis [174] and filter occlusion [169] in dog blood either aspirated or infused at a variety of rates respectively through an 18 µm filter failed to demonstrate significant increases in haemolysis or evidence of filter occlusion in fresh blood. In the former study both fresh PRBCs and PRBCs stored for less than 14 days were used with age of storage, rather than infusion rate or filter size associated with significant changes in haemolysis [174]. A mean volume of 50ml PRBC was either aspirated through the filter [174] or 60ml of WB infused through the filter [169] in either study. Aspirating blood through the filter prior to subsequent transfusion rather than using the filter for in-line infusion adheres to manufacturer recommendations for the method of filter use [165]. Blood volumes used in both studies also reflect manufacturer recommendations for a maximum volume of 50ml of WB to be aspirated through a single filter [165]. This recommendation references a human study that found no evidence of cellular injury based on plasma haemoglobin levels when less than 40ml of WB was passed through a single filter [173]. Notably the aforementioned studies only assessed in vitro markers of RBC injury such as haemolysis and may not be representative of subsequent in vivo RBC survival in a transfusion recipient's circulation. The disparity of results between canine and human studies also highlights fundamental species-specific differences in haemorheology, with conclusions drawn from studies conducted in other species unlikely to be applicable to feline blood transfusion medicine.

A survey of 73 private referral or veterinary teaching hospitals reported 82% of hospitals used syringe pumps for feline transfusions. Gravity flow was the next most commonly used technique (18%), followed by volumetric pumps (15%) [48]. Administration of blood products via gravity flow through a standard human blood administration set can present challenges related to the control of slower flow rates and delivery of smaller infusion volumes. This is of particular importance in feline transfusion medicine due to the comparatively smaller blood volume of cats and smaller transfusion volumes administered

compared to dogs. Certain patient populations at higher risk of volume overload such as cats with underlying cardiac or renal dysfunction or anaemia with a normal or increased intravascular volume status also benefit from precise infusion delivery. Use of an infusion pump that controls blood infusion rate and volume delivered may help attenuate this risk. Infusions pumps can be divided into peristaltic type pumps which can use either linear, rotary or shuttle mechanisms to dive blood flow or piston type pumps that push blood forward using a mechanism such as a syringe or diaphragm [175, 176]. Infusion pumps that are verified and approved for the use of blood component infusion are preferred over gravity delivery in human medicine [163].

Certain infusion pumps have been shown to increase the risk of RBC damage and subsequent haemolysis in both human [175-178] and dog blood [179]. Human studies have demonstrated increased free haemoglobin concentrations secondary to linear peristaltic pump RBC product infusion, a possible consequence of direct RBC compression within the pumps intravenous tubing. The clinical significance of the small absolute increases in free haemoglobin observed in either study is not clear [103, 180]. There is a single study evaluating the use of two types of linear peristaltic pump for the transfusion of feline blood. Transfusion of 35-to-42-day old stored PRBC units with the addition of SAGM via two types of peristaltic pump or gravity flow resulted in no significantly different increase in haemolysis. Blood was infused through a 180 µm in line filter at a standardised rate of 25 mL/hr to reflect what would commonly occur in clinical practice. While older stored blood subject to the effects of storage lesion was used, only in vitro markers of haemolysis were evaluated and may not be representative of subsequent in vivo RBC survival [29]. Reduced in vivo survival of dog RBCs transfused through a linear peristaltic pump has been demonstrated, with measured in vitro markers of haemolysis not significantly different to the gravity delivered control group [168]. While the mechanism for this finding is not clear, it suggests in vitro studies of infusion pump mediated RBC injury may not be representative of subsequent in vivo RBC survival.

Blood flow rate and storage time have been demonstrated to impact pump induced haemolysis in dog blood infused using linear or rotary style peristaltic pumps. Independent of pump type and infusion rate, stored WB was associated with a higher haemolysis

percentage. Storage lesion induced morphological changes with associated reduced RBC deformability and increased susceptibility to mechanical shear stress may explain this finding. While higher infusion rates also induced greater haemolysis, the linear peristaltic pumps tested were ultimately deemed by the authors as safe for use whereas the rotary peristaltic pump was not [179]. A recent paper evaluating a piston type pump found no significant increase in haemolysis with maximal simulated pump infusion rates using dog PRBC units [174]. Due to disparities even amongst similar pump types and differences in inter-species haemorheology, flow infusion rates, availability of blood component therapy and age of blood used, individual infusion pumps require species specific testing and validation.

Decreased osmotic fragility has been demonstrated in human studies after transfusion of variably aged RBCs through diverse infusion pumps and delivery systems [103, 177]. This observation has been suggested to reflect either haemolysis of damaged RBCs during the transfusion process [177] or biochemical changes related to transfusion associated potassium release causing cell shrinkage and therefore greater ability to withstand water influx [103]. Notably, blood component storage times varied markedly between the studies which used either two-to-three-day old WB or PRBCs [177] or a range of 9-to-51-day old LR PRBCs [103]. In contrast, no change to RBC osmotic fragility was detected in either fresh [169] or biotinylated [168] canine RBCs when transfused through an 18 µm filter using a syringe and syringe driver.

Based on recommendations originating from human research, blood transfusions are administered over a 4-hour period to minimise the risk of bacterial growth in blood that has reached room temperature [60]. Due to a lack of cat or dog studies examining the speed of initial transfusion rate it is also recommended that blood be transfused more slowly for the first 15 minutes based on human guidelines [14]. Evidence to inform recommendations for subsequent rates of transfusion delivery is also lacking, with individual patient factors such as causative disease, comorbidities and clinician experience ultimately dictating the rate of delivery [137]. The AVHTM consensus authors suggest slower rates of infusion for euvolaemic patients or those with significant renal or cardiac disease [14].

### 1.5.4 Donor characteristics

Blood donor characteristics may influence the longevity and quality of stored blood products. Human stored RBCs show marked inter-donor variability in susceptibility to storage lesion [99]. RBC deformability has also been shown to vary significantly between donors on both the day of donation and throughout storage [181]. Studies have explored the role that biological factors such as sex [182] and specific antibodies and RBC antigen selection have on RBC product quality [183]. Specific blood donor characteristics have been identified as risk factors for the development of TRALI including transfusion of plasma from female donors and blood from multiparous woman. The latter may be linked to higher levels of circulating human leukocyte antigen antibodies which are implicated in antibody mediated TRALI [137].

The Red Blood Cell-Omics (RED-Omics) study by the Recipient Epidemiology Donor Evaluation Study (REDS) – III program is a large data gathering initiative designed to investigate the role donor genetic and metabolic differences have in determining human RBC product quality. Future project aims include using the data gathered to investigate links between donor characteristics and blood recipient outcomes. Donor race/ethnicity, sex, age, donation history, behaviour, biochemical, haematological and DNA data have been collected [184]. Studies to date have explored susceptibility to vitro haemolysis, by spontaneous, osmotic, mechanical or oxidate mechanisms in defined donor subsets. Donor specific susceptibility to oxidative and osmotic haemolysis are reproducible intra-donor findings and may be attributable to donor genetics [185]. Donor age, ethnicity/racial background, and sex [186] and additive solution [187] have also been found to influence susceptibility to in vitro haemolysis.

The role of genetic and metabolic blood donor characteristics remains unexplored in veterinary transfusion medicine. The large diversity of cat breeds and influence of selective breeding pressures on genetic traits means inter-donor RBC heterogeneity likely exists and may influence RBC product quality and blood recipient outcomes. Anaemia secondary to increased RBC osmotic fragility has been reported in Abyssinian, Somali, Siamese, Ocicat and domestic short hairs with an underlying genetic RBC defect suspected [125]. While routine screening measures eliminate any outwardly unsuitable donor candidates they do

not account for the effect of osmotic, oxidative, mechanical, and thermal pressures within the blood storage environment. The influence that feline blood donor breed, genetics, sex, age and environment may have on quality of blood products presents an interesting area for future studies.

## 1.5 Conclusion

Advances to feline transfusion medicine have improved donation and transfusion safety and led to greater access to blood products. Feline blood is a precious resource. Despite ready access to commercial blood banks in some parts of the world, many clinicians still rely on in-house hospital run donor programs. The recent publication of peer-reviewed consensus statements and practical guidelines add to the growing body of literature characterising species specific features of blood transfusion unique to the cat. With many areas of feline transfusion medicine still largely unexplored, recommendations continue to be derived from the human and canine literature. Methods of feline blood collection and subsequent blood processing and storage practices have not been reported for Australian emergency and referral veterinary hospitals. The storage of donations collected with an open system also remains controversial. Recommendations for the method of transfusion delivery such as the routine use of an in-line 18 µm microaggregate filter for feline transfusions are derived from a limited evidence base. There are currently no studies investigating the impact that filter passage has on stored feline RBCs. While understanding of blood typing, donor screening and storage lesion have improved, the impact feline specific transfusion methodologies have on blood product quality requires further investigation.

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# Chapter 2: Objectives and Hypotheses

## 2.1 Objectives

*Survey*

- To describe how Australian emergency and specialty referral hospitals source feline blood products, approach collecting blood donations and to identify and characterise feline blood storage practices.

*Microaggregate filter passage and storage lesion*

- To determine whether passage through an 18 µm microaggregate filter damages feline RBCs as indicated by increased haemolysis percentage, MOF or haematological derangements and if these effects are compounded in blood that has been stored for 35 days.
- To determine if a commercially available open blood collection system carries risk of bacterial contamination.

## 2.2 Hypotheses

*Survey*

- The majority of Australian emergency and specialty referral hospitals would perform cat blood transfusions and use an open system for blood collection. Few hospitals would routinely store cat blood or perform blood component processing.

*Microaggregate filter passage and storage lesion*

- Fresh feline RBCs would exhibit no in vitro evidence of microaggregate filter induced damage compared to maximally stored blood.
- Blood collected with an open blood collection system would not be associated with an increased risk of bacterial contamination.

# Chapter 3: Transfusion practice: survey of feline blood transfusion and banking in Australia

## 3.1 Introduction

There are very few publications characterising feline transfusion medicine practices, including blood product sourcing and availability [1], and collection and storage methods [2, 3]. Survey studies originating primarily from North America [2] and the United Kingdom [1] provide some background data on cat transfusion practices and blood product availability within these regions respectively. The representation of transfusion practice revealed by these studies is significantly influenced by regional differences in commercial feline blood products and equipment accessibility and cannot be inferred to represent current Australian transfusion practice.

Half of all veterinary hospitals in the predominantly North American survey used a combination of commercial blood products and hospital run donor programs with 22% of hospitals relying on purchased blood products alone [2]. Only private referral and university teaching hospitals were surveyed in this study, likely explaining the almost uniform routine storage of feline packed red blood cells (PRBCs) and fresh frozen plasma (FFP) by respondents [2]. This is in contrast to the UK survey of referral and first opinion hospitals in which only 2.5% of respondents routinely stocked feline blood component products (PRBCs or plasma) [1]. When required, 31.1% of respondents in the UK study sourced blood via a pet blood bank and 28.6% used “blood donors from their records” [1].

A recently published Australian survey study provides the only peer-reviewed publication describing small animal transfusion practices in general practice (GP), 24-hour and after hours-only emergency, and referral hospitals in Australia. With no commercial feline blood products available within Australia, this survey study provides important preliminary data on the availability, sourcing, and storage of feline blood products by Australian veterinarians. However, feline versus canine blood donor sources were not clearly differentiated and feline blood collection methods and specifics regarding storage were not investigated [3].

Cat blood donations are generally collected using an open or modified semi-closed system due to the limited commercial availability of cat specific closed collection systems and the unsuitability of human equipment [4-8]. A system may be designated open or semi-closed based on the need for either complete or partial operator assembly of sterile system components such as needle, syringe/s and/or a collection bag and the need for the operator to add anticoagulant. Each additional step in collection system assembly represents a source of potential bacterial contamination during the collection of a blood donation. To the best of the author's knowledge commercial closed blood collection systems suitable for the cat are not available in Australia. Blood must be collected using an open system and the suitability for subsequent storage of this blood remains controversial [9].

The 2021 International Society of Feline Medicine (ISFM) consensus guidelines are the first published and officially endorsed, expert-led statement outlining practical protocols for cat blood collection and the administration of cat blood products for the general practitioner [9]. The guidelines reflect the current understanding of feline transfusion medicine 'best practice' based on the available scientific literature. The increased availability and use of commercial feline blood products in some regions is highlighted through the lens of the ethical considerations that must be given to donor sourcing and care [9]. Publication of the Association of Veterinary Hematology and Transfusion Medicine (AVHTM) Transfusion Reaction Small Animal Consensus Statement (TRACS) has provided further, evidence-based guidelines for cat blood donor screening, blood collection, processing and storage as a means to reduce adverse transfusion reactions [5]. It is unknown how well the recommendations within either consensus align with current feline blood banking and transfusion practices in Australia.

The objectives of this survey were therefore to describe how Australian emergency and specialty referral hospitals source feline blood products, approach collecting blood donations and to identify and characterise feline blood storage practices. We hypothesised that the majority of Australian emergency and specialty referral hospitals would perform cat blood transfusions and use an open system for blood collection with few hospitals routinely storing cat blood.

## 3.2 Materials and methods

On consultation with the author's institution human ethics committee, ethics review and approval were deemed unnecessary as questions were designed to investigate hospital practices rather than individual veterinary practice.

The survey was created using REDcap software (https://projectredcap.org/software). This software was used for all data collection and maintenance. The survey inclusion criterion comprised current employment in an Australian private or university small animal emergency and/or referral veterinary hospital. The survey was open to any employee of the hospital familiar with current hospital blood banking practices. All responses were anonymous. Where multiple responses from the same hospital were identified by the demographic information, only the initial response was included. The survey was distributed via an email invitation containing a web link to the survey. The email addresses of emergency, after-hours and/or referral hospitals in all Australian states and territories were obtained via internet search. The survey web link was also shared to a private Australian veterinary social media group. The survey was available between April 2021 and April 2022.

The total number of questions presented to respondents was dependent on whether they performed cat blood transfusions in hospital and/or stored cat blood (Appendix 1). Up to eight questions were presented if blood transfusions were not performed at the hospital, up to 15 questions if transfusions were performed but no blood was stored, and up to a total of 26 questions if hospital blood transfusions and blood storage both occurred. Question type included a mix of closed ended questions with options to select either one or one or more answer options. The last option for some questions included the ability to select a free text box to further specify or elaborate on an answer. The initial five questions were designed to obtain demographic information including classification of hospital type into 'emergency hospital only', 'private emergency and referral hospital' or 'university teaching hospital'.

*Statistical methods*

The dataset was exported from REDcap software into commercial spreadsheet software (Microsoft excel) for statistical analysis. Statistical analyses were performed in GenStat

(Version 22; VSN International, Hemel Hempstead, UK). Descriptive statistics with frequency (percentages) were reported for demographics, donor source, blood collection systems and anticoagulant type. Hospitals were classified as urban or rural using post code data referenced to the Australian Bureau of Statistics (ABS) Australian Statistical Geography Standard Section of State definitions. A chi-squared test was used to assess the association between hospital type and whether blood transfusions were performed or not. Ordinal logistic regressions were conducted when assessing associations between type of hospital and donor source, collection system and anticoagulant type. For all analyses, a p value of $< 0.05$ was considered significant.

## 3.3 Results

The survey was sent to 60 hospital email addresses with some addresses representing more than one individual hospital. A total of 33 responses were obtained, with exclusion of two respondents leaving a total of 31 survey responses included in the study. The exclusion of the two respondents was based on demographic information identifying them as duplicate hospital answers.

*Demographics and ability to perform blood transfusions (n = 31)*

Of the 31 responses, the majority were from private emergency and referral hospitals (15/31), followed by emergency-only hospitals (10/31) then university teaching hospitals (6/31). Most respondents were from Queensland (n = 10, 32.3%) followed by Victoria (n = 7, 22.6%), New South Wales (n = 6, 19.4%) then Western Australia (n = 4, 12.9%). There was one respondent each from the Australian Capital Territory, South Australia, and Tasmania. Based on ABS population classifications, nearly all respondents were located in urban (n = 29, 93.5%) compared to rural areas (n = 2, 6.5%).

*Performing cat blood transfusions*

A total of 29 (93.4%) hospitals identified that they performed cat blood transfusions. All university teaching hospital respondents (n = 6, 100%) performed cat blood transfusions, followed by private emergency referral hospitals (n = 14, 93.3%) and emergency-only hospitals (n = 9, 90%). The two respondents that did not perform cat transfusions included

one each of an emergency-only hospital and private emergency and referral hospital. Both hospitals were located in an urban area and selected “refer to another emergency and/or specialist referral centre that performs cat blood transfusions” as the procedure for cats that require a blood transfusion. There was no association between hospital type and whether cat blood transfusions were performed or not (p = 0.696).

Most hospitals performed between zero and one cat blood transfusion per month (n = 22, 75.9%), followed by two to three (n = 6, 20.7%) per month. One respondent indicated their hospital performed three to four (3.4%) transfusions a month.

*Blood donor sources (n = 29)*

The majority of respondents sourced blood from staff-owned cats (n = 22, 75.9%) or client-owned cats (n = 16, 55.2%). Of the hospitals sourcing blood from staff-owned donor cats, 13 used them in combination with client-owned donors, eight exclusively used staff cats and one respondent specified concurrent use of donor cats owned by friends or acquaintances of staff. Two hospitals sourced blood donors exclusively from either in-house donors or client-owned cats. There was one respondent each that only sourced donors from either another hospital or a combination of client-owned and in-house donors. There was a single response exclusively for ‘other’ with the comment that donor cats were owned by members of the public that were not always clients of the hospital as part of a community pet blood bank.

| **Blood donor source** | **Number** |
|---|---|
| Staff-owned and client-owned | 13 |
| Staff-owned and other | 1 |
| Staff-owned | 8 |
| Client-owned | 2 |
| Other | 1 |
| Client-owned and clinic in-house donor | 1 |
| Clinic in-house | 2 |
| Blood sourced from another clinic | 1 |

**Table 1:** Source of blood donors with responses collated to reflect selection of greater than one option. NB 'Other' specified by respondents to include cats owned by people other than staff or clients of the hospital such as members of the public or friends of staff.

| | **Blood donor source** | | | | |
|---|---|---|---|---|---|
| **Type of hospital** | Staff-owned | Client-owned | Clinic in-house donor | Another clinic | Other |
| Emergency | 6 | 4 | 1 | 0 | 0 |
| Private emergency and referral hospital | 12 | 9 | 1 | 1 | 1 |
| University teaching | 3 | 3 | 1 | 0 | 1 |

**Table 2:** Type of hospital and cat blood donor sources.

There was no significant association between type of hospital and donor source (p = 0.927).

*Blood collection systems and anticoagulant (n = 28)*

Of the 28 respondents that reported on blood collection methods, the majority used an open collection system not sterilised after assembly (n = 16, 57.1%). An open, partially pre-assembled system, not sterilised after assembly was the next most commonly used (n = 9, 32.1%), followed by a modified system (n = 2, 7.1%) then closed system (n = 1, 3.6%). One description of a modified system specified anticoagulant removal from a pre-assembled collection bag using a syringe and likely represented an open collection system, not sterilised after assembly. The other response to modified system referenced Animal Blood Resources International (ABRI), a company that sells a variety of small animal blood collection systems. No hospital reported using more than one type of collection system.

| | Collection system | | | |
|---|---|---|---|---|
| **Type of hospital** | 1) Open, non-sterilised | 2) Open, partially pre-assembled, non-sterilised | 3) Closed | 4) Modified |
| Emergency | 6 | 2 | 1 | 0 |
| Private emergency and referral hospital | 8 | 5 | 0 | 0 |
| University teaching | 2 | 2 | 0 | 2 |

**Table 3:** Type of hospital and collection systems used for cat blood collection. The full options for blood collection system appeared in the survey as: 1) Open (the entire collection system is assembled in-house, and anticoagulant is added by the operator. The system is not sterilised after assembly). 2) Open (the collection system is partially pre-assembled, partially assembled in-house. Anticoagulant is added by the operator. The system is not sterilised after assembly). 3) Closed (the system is fully pre-assembled, including anticoagulant and sterile). 4) Modified system (for example, sterilised after assembly or the addition of anticoagulant. Please specify below). An option for 'Other – please specify below' was also available.

There was no significant association between type of hospital and type of collection system used ($p = 0.468$).

The most commonly used anticoagulant was citrate phosphate dextrose adenine (CPDA-1) (n = 21, 70%), followed by citrate phosphate dextrose (CPD) (n = 5, 16.7%), then heparin (n = 2) and acid citrate dextrose (ACD) (n = 1, 3.3%). One respondent selected "other" and specified they were unsure of the type of anticoagulant used. Most hospitals (n = 26, 92.9%) only used one type of anticoagulant. Where hospitals used only one anticoagulant type, the most common anticoagulant was CPDA-1 (n = 20, 76.9%), followed by CPD (n = 4, 15.4%) and then heparin (n = 1, 3.8%). Two hospitals (7.1%) reported using more than one

anticoagulant for blood collection. One hospital used both CPD and CPD-1 and the other used both ACD and heparin.

There was no significant association between the type of anticoagulant used and the type of hospital (p = 0.242).

*Blood storage vs on demand collection (n = 28)*

The majority of respondents indicated blood was collected "on demand only" (n = 25, 89.3%). Two respondents indicated blood was collected both on demand and for storage and a single respondent indicated blood was only collected for storage. Two of the hospitals that store blood were identified as university teaching hospitals and the other a private emergency and referral hospital.

*Blood storage (n = 3)*

Of the three respondents that indicated they store cat blood, two reported storage of type A blood only and one reported storage of both type A and type B blood. No hospital stored AB blood. Two hospitals stored only either whole blood or blood components (PRBCs, FFP and frozen plasma). One respondent also reported the addition of saline adenine glucose mannitol (SAGM) to PRBC units as an additive preservative. All respondents storing blood reported using CPDA-1 as the anticoagulant for blood collection. Two respondents indicated use of a non-sterilised, open, partially pre-assembled, partially assembled in-house collection system with anticoagulant added by the operator after assembly. The other respondent selected the modified option and referred to the commercial company ABRI that sells blood collection systems.

Maximum blood storage times were reported as 28, 35 and 42 days and minimum storage times as 0, 1 and 2 days. Hospitals reported storing a maximum of 1, 2 or 3 bags of blood at any one time. Where more than one bag was reported to be stored, order of usage was reported to be determined by either selection of the "most compatible" bag or the "date of blood collection" or rarely, "size of bag". All respondents that stored blood reported some form of blood banking quality control for stored units prior to use. All respondents performed visual inspection of units for colour change. Additionally, one respondent also

specified that if required, haemolysis percentage could be checked on certain units prior to use, such as those that had reached the expiry date.

### 3.4 Discussion and conclusion

Data from this survey aids to further characterise features of feline blood sourcing, collection methods and storage in Australian emergency, private and university referral hospitals. Almost all hospitals (93.4%) performed cat blood transfusions, however only three hospitals (10%) stored cat blood. The high availability of cat blood transfusions in this study compared to the only other Australian transfusion survey in which 30.8% of respondents indicated no access to any cat blood products likely reflects differences in the surveyed populations [3]. Over 66.8% of respondents in the aforementioned survey identified as working in GP hospitals. Creation of hospital feline donor programmes including screening of recipients and collection of blood can be labour intensive and, prior to publication of the 2021 ISFM consensus guidelines, lacked clear, expert-led, evidenced-based protocolisation [9]. Poh and colleagues' 2021 survey also highlights current discrepancies between Australian small animal transfusion medicine practice such as use of blood component therapy, dedicated blood storage units, feline pre-compatibility testing and alignment with what is considered 'best practice'.

Not unexpectedly, an absence of commercial feline blood products in Australia is reflected in the high percentage of community-sourced blood donors in the present study. Cats owned by staff and their friends and acquaintances, members of the public, and hospital clients constituted the major sources (89.7%) of blood donors in this study. This is in line with results from the Australian survey of small animal transfusion practice where 91.3% of respondents sourced blood from staff, friend and acquaintance-owned dogs and cats [3]. Access to feline blood component therapy and stored cat blood was also reported in the Australian survey with twelve respondents indicating access to stored whole blood, 21 with access to FFP and nine with access to PRBCs [3]. The means of sourcing feline stored whole blood and methods of blood collection and preparation for storage were not described [3]. Only three hospital respondents in the present survey indicated routine storage of cat blood. While not explicitly stated within the question, it is assumed in the present study all

‘on demand’ cat blood transfusions comprised fresh whole blood. This represented the majority (89.3%) of blood collected and transfused, with only one respondent indicating transfusion of stored blood only, all of which was blood component therapy (PRBCs, FFP, frozen plasma). There are a number of possible reasons for the discrepancy in the numbers of respondents indicating access to stored WB and feline component therapy. One likely explanation is the higher response rate of 66 individuals in the other Australian survey study versus the 31 hospitals represented in the present study when comparing similar hospital categories. This indicates that some hospitals that store feline blood or use component therapy are not included in the present study. It is also possible Poh and colleagues’ (2021) survey may be overrepresenting access to stored feline WB and component therapy as they acknowledge only removing survey responses from the same IP address, making it possible that more than one response represents the transfusion practices and blood product access from a single hospital.

Most published studies quote a 35-day maximum storage period for feline WB anticoagulated in CPDA-1 regardless of collection via an open or closed system [7, 10, 11]. Notably these studies only evaluate in vitro markers of RBC viability such as haemolysis percentage, which may not translate to post-transfusion RBC in vivo survival. Both the respondents in this survey that collected WB for storage indicated use of CPDA-1 however maximum storage times differed; 28 vs 35 days. As a limited resource with a potentially costly and time-consuming donation process, establishing safe, maximally effective blood storage times is critical to optimise blood usage and minimise wastage. The longest storage time of 42 days was for PRBCs with the additive SAGM by the third hospital that routinely stored feline blood products. This time frame is in line with published maximum storage times for feline PRBCs with the SAGM additive in studies investigating feline PRBC quality controls [6] and infusion pump-mediated haemolysis [12]. The aforementioned study investigating quality controls found 19.5% of units between 26 and 42 days of storage exceeded the recommended 1% haemolysis threshold commonly used by blood banks [6]. Haemolysis threshold as a quality control marker was also specified by the survey respondent in this study as a means of checking blood prior to administration as it approached 42 days of storage and subsequent expiry. This is in line with recent recommendations made by the AVTHM consensus statement to check PRBC units for

haemolysis prior to administration using a maximum threshold of 1% to determine suitability for transfusion [5].

Of the three hospitals that stored feline blood products, two indicated that the order of blood usage was determined by unit age and the other based on compatibility. Administration of only the most compatible blood based on both blood type and cross-matching is viewed by some as essential to upholding feline transfusion 'best practice' [4, 5, 13]. Given the small number of units stored at any one time by respondents that stored blood in this survey and the reliance on staff, client and other community owned cat donors, adherence to this recommendation may not always be practical. The ability to call upon multiple potentially suitable blood donors to ensure crossmatch compatibility is reliant on donor availability, which may be further limited in after hours or time sensitive situations such as an emergency.

Commonly used and recommended anticoagulants for feline blood donations include CPDA-1, CPD and ACD [9]. This mirrors results from the present study where CPDA-1 and CPD accounted for 86.7% of the anticoagulants reportedly used. Similar results were obtained by the largely North American survey in which 65% of respondents used CPDA for cat blood collection and 6% used CPD. A much higher proportion (29%) used ACD compared to the single respondent in the present study, indicating possible differences in regional access, anticoagulant familiarity, or training. Two respondents in the present study indicated use of the anticoagulant heparin, which is not recommended by the recent consensus guidelines for the anticoagulation of cat blood intended for transfusion [9]. This recommendation likely arises from the risk of recipient systemic heparinization and subsequent clinically significant bleeding and lack of preservative properties needed for blood storage [14]. Citrate containing anticoagulants work by chelating the calcium in collected blood and are metabolised predominantly by the recipient's liver to bicarbonate with toxicity generally only occurring in cases of massive transfusion and/or hepatic dysfunction [14]. This divergence from expert led, evidence-based recommendations for cat transfusion medicine adds to similar findings of the Poh and colleagues (2021) Australian survey that highlighted other inconsistencies between 'best practice' and current transfusion practice [3].

Nearly all respondents in this study indicated use of a form of open blood collection system, including for blood intended for storage. While the recent ISFM Consensus Guidelines recommend blood collected using an open system should be used within 24 hours, the panel also highlights studies that have demonstrated a lack of microbial contamination in blood collected this way and stored for 35 days [7, 11]. While acknowledging a lack of strong evidence, the AVHTM consensus statement also suggests that with aseptic collection, processing, and appropriate storage, open or 'semi-closed' systems can be used for feline blood collection [5]. Differences in regional resource availability are highlighted by the contrasting results of the mostly North American survey which found a commercially available closed blood collection system was used in 56% of hospitals performing cat blood collections with the remaining 44% using an open system [2]. The proportion of these in-house blood collections designated for storage versus immediate use however was not presented [2].

A potential source of error in the present study arises from misunderstanding of the wording or meaning of some questions which is suspected based on outlier responses. It is possible that the single 'closed' collection system response is a misinterpretation of the question. No respondent storing blood indicated use of a closed system and to the best of the author's knowledge a commercial closed system for blood collection is not available for purchase in Australia. This may also reflect a lack of clarity surrounding collection system description which also likely influenced the selection of 'modified system' by some respondents. The systems subsequently described when prompted by selection of 'modified' could possibly fit into one of the other pre-specified categories for that question with further clarification.

Another limitation of this study was the overall small number of respondents. Sixty hospitals were contacted via email and despite the addition of social media posting, only 31 unique hospital responses were received. Results may therefore not be wholly representative of feline transfusion practice within these Australian hospital settings. Some hospitals classified as emergency and/or referral hospitals may also not have been emailed at all if not identified during initial internet searching and hospital list compilation. The small number of respondents hampered the identification of statistical significance in

comparisons such as differences between hospital type and blood transfusion practices. While the very low number of responses indicating hospital storage of cat blood likely reflects reality in Australia, this limited data analysis to descriptive statistics.

**Conclusion**

This survey study presents additional data that helps to characterise current feline transfusion practice and blood storage in Australian emergency, private referral, and university teaching hospitals. While most respondents performed cat blood transfusions, very few stored feline blood and the means of blood collection, storage times and subsequent processing differed. This lack of uniformity may reflect variations in resource access and the current dearth of high-quality evidence-based recommendations and protocols for feline blood banking in the veterinary literature.

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# Chapter 4: Effect of microaggregate filter passage on feline whole blood stored for 35 days

## 4.1. Abstract

Objectives: The aim of this study was to compare characteristics of fresh and stored feline red blood cells (RBC) after passage through an 18 µm microaggregate filter.

Methods: Nine cats were recruited for a single blood donation using an open collection system. A simulated transfusion using a syringe pump and microaggregate filter was performed over two hours with half the blood on the day of donation and after 35 days storage. Differences in haematological parameters, haemolysis percentage and osmotic fragility (OF) were compared on the day of donation pre-filter passage (D0-) versus day of donation post-filter (D0+) or day 35 storage pre-filter (D35-) and post-filter (D35+). Blood was cultured at D0+ and D35+.

Results: There were no differences in the D0- versus D0+ comparisons. In comparison to, D0-, greater haemolysis percentage, red cell distribution width (RDW) % and mean osmotic fragility were detected at D35-. For the same comparison, packed cell volume (PCV), RBC count, haemoglobin and haematocrit were lower at D35- ($p<0.05$). The same was found for D0- versus D35+, with the addition of a higher mean corpuscular haemoglobin (MCH) at D35+. For D35- versus D35+ only MCH was higher at D35+. At day 35, 6/9 units had haemolysis percentages that exceeded 1%. This increased to 8/9 of stored units post-filter passage. All blood units cultured negative.

Conclusions and relevance: Fresh RBCs exhibited no in vitro evidence of injury following passage through an 18 µm microaggregate filter. Increased MCH was observed in the stored blood and may represent haemolysis induced by the filter. All other changes can be explained by storage lesion rather than filter passage. The findings highlight the importance of blood banking quality controls and need for further research to assess the effects of transfusion technique, specifically filter passage, on storage lesion affected feline blood.

## 4.2 Introduction

Blood transfusions are essential to feline emergency and critical care medicine. While a few commercial blood banks exist, many practitioners worldwide collect blood in-house [1]. Accessibility to suitable donors can be challenging, and the storage of feline blood is controversial [2, 3]. Consequently, feline blood donations are often performed on demand for immediate transfusion to the recipient.

Storage of blood is advantageous, allowing collection from donors in a controlled environment and immediate access to blood products for recipients. There are few studies reporting on the ideal storage time of feline blood [2-7]. Official feline blood banking standards have not been established, with current recommendations to determine blood 'shelf life' extrapolated from human guidelines [3-5]. The United States Food and Drug administration (FDA) human blood banking regulations stipulate a minimum of 75% 24-hour post-transfusion red blood cell (RBC) survival in the recipient's circulation [8] and a maximum of 1% haemolysis after 42 days storage [9]. Using biotinylating cell labelling techniques, 85% RBC survival 24 hours post-transfusion has been demonstrated for feline whole blood (FWB) stored in citrate dextrose adenosine phosphate 1 (CPDA-1) for 35 days [6].

Collectively termed the 'storage lesion', the progressive biochemical and biomechanical RBC changes that occur during storage may have implications for RBC survival and viability post-transfusion [10, 11]. Alterations to RBC membrane integrity and shape lead to reduced cell deformability and oxygen carrying capacity and increased haemolysis [4, 10, 12]. Biochemical, haematological and morphological features of storage lesion have been documented in feline blood [3-5, 11, 13-17]. Both unchanged [3] and significantly increased [17] mean osmotic fragility (MOF) and varying levels of haemolysis have been reported [3-5].

Cat transfusions are typically delivered via syringe driver [1] and 18 µm microaggregate filter [18, 19]. This technique has been demonstrated to cause no significant in vivo loss of RBCs

compared to traditional gravity dependent delivery methods in cats. Notably, in this study blood was stored for less than 12 hours prior to transfusion [20].

To date, there are no studies assessing the effects of filter passage on stored feline RBCs. The objective of this study was to determine whether passage through a microaggregate filter damages feline RBCs as indicated by increased haemolysis percentage, MOF or haematological derangements and if these effects are compounded in blood that has been stored for 35 days. We hypothesised that feline RBCs would exhibit no in vitro evidence of microaggregate filter induced damage compared to maximally stored blood and blood collected with an open blood collection system would not be associated with an increased risk of bacterial contamination.

## 4.4 Materials and Methods

*Blood donors and sampling protocol*

Nine domestic cats between one and nine years of age with a minimum lean body weight of 4.5 kg were recruited. Cats were in good health based on history, physical examination and results of screening tests (Appendix 2) including complete blood count (CBC), serum biochemistry, feline immunodeficiency virus and feline leukemia virus tests (Witness FIV/FeLV; Zoetis Animal Health) and echocardiogram. Informed consent was obtained from each owner and the study protocol was approved by the University of Sydney Animal Ethics Committee (project number: 2018/1358).

Prior to donation, 6/9 cats received between 50-100 mg of gabapentin administered per os. For blood donation, cats were sedated with intramuscular and/or intravenous (IV) injection/s of 0.19-0.25 mg/kg midazolam, 0.15-0.2 mg/kg butorphanol and 3.75-8.3 mg/kg ketamine. An intravenous catheter was placed, and mask oxygen provided. Additional sedation with alfaxalone (0.4 – 1.6 mg/kg IV) was used for 3/9 cats. Cats were monitored with electrocardiography, pulse oximetry and oscillometric blood pressure. Vital parameters and anaesthetic depth were assessed every five minutes until cats regained consciousness (Appendix 3). Cats were placed in lateral recumbency and the neck clipped of fur followed by surgical skin preparation. An open collection system consisting of a 21 G butterfly needle

(Surflow winged infusion set; Terumo) attached to a three-way stopcock (Discofix 3-way stopcock; B Braun), 50 mL syringe (50ml syringe luer lock; Terumo) containing CPDA-1 in a 1:7 ratio to the intended blood volume, and 100 mL blood storage bag (Animal Blood Banking International) was used to collect blood via jugular venepuncture. Using aseptic technique, CPDA-1 was aspirated from a blood donation bag (Single blood bag 450ml CPDA-1; Terumo) and added to the collection set via the 50ml syringe just prior to each donation using aseptic technique. A maximum of 10 mL/kg blood was collected per cat. Half the blood was partitioned into the storage bag via the three-way stopcock. The extension line was stripped of blood and sealed with metal clips. Blood units were stored upright in a dedicated refrigerator at + 4°C for 35 days and not mixed during storage. A maximum of three cats donated blood on any one day.

*Simulated transfusion and blood culture*

Blood sampling was performed at four time points; the day of donation, pre-filter passage (D0-); the day of donation, post-filter passage (D0+); after 35 days of storage, pre-filter passage (D35-); after 35 days of storage, post-filter passage (D35+). On the day of donation, half the blood was reserved in the collection syringe for immediate sampling and simulated transfusion. The syringe was disconnected from the three-way stopcock and 4 mL of blood transferred to EDTA and plain tubes. The syringe was then connected to a micro-bore extension set and 18 µm microaggregate filter (Hemo-nate; Utah Medical Products Inc.) and loaded into a syringe driver (Perfusor Space Syringe Infusion Pump; B. Braun Vet Care). Blood was 'transfused' over a 2-hour period. The initial 10 mL was inoculated into a blood culture bottle (Oxoid Signal Blood Culture bottle; Thermo Fisher Scientific) for anaerobic and aerobic culture with the final 4 mL reserved post filter passage for analysis. Blood culture bottles were incubated at 37°C +/- 1°C in both aerobic and anaerobic conditions with daily inspection for 5 days for signs of bacterial growth. After 35 days of storage, blood reserved in the storage bags was aseptically drawn into a syringe via a spike (Swan-lock take set; Codan US Corp.). Blood collection for analysis and simulated transfusion as described above was performed (D35- and D35+).

*Haematology and haemolysis*

Haematological analysis was performed at each time point within 6 hours of sample collection using an automated haematology machine (Sysmex XN 1000RF Vet Analyser; Roche Diagnostics). Parameters measured included RBC count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC) and red blood cell distribution width (RDW). A manual packed cell volume (PCV) was performed and used to calculate MCH and MCHC.

As previously described in cats, Hb concentration was determined using a specific analyser (Hb 201+ System; HemoCue Inc.) with daily calibration using a 120 g/L standard [21]. Plasma was obtained by centrifuging (Orbital 360; Clements) 1 mL of anticoagulated blood at 1200 g for 15 minutes. Supernatant Hb concentration was determined by a spectrophotometer (Plasma/Low Hb; HemoCue Inc.) for determination of low Hb concentration. This analyser was calibrated each day of use with 1.0 g/L and 5.0 g/L controls. Percentage haemolysis was calculated using the formula:

Haemolysis (%) = Supernatant Hb (g/L) x (100-PCV) / Total Hb (g/L) [5].

*Osmotic fragility (OF) testing*

Osmotic fragility testing was performed using 360 µL of blood reserved from each time point based on methods previously described [3, 22, 23]. A stock solution of 10% phosphate buffered saline (PBS) with pH 7.4 was diluted with distilled water to create a 1% NaCl solution. Using this solution and distilled water, 18 test tubes were prepared with NaCl concentrations ranging from 0.9-0.0% at 0.05% increments. To each tube, 20 µL of blood was added and mixed by inversion. Samples were incubated at 22°C for 45 mins then centrifuged (Centrifuge 5810R; Eppendorf AG) at 2000 g for 10 mins. The optical density of each supernatant was determined spectrophotometrically (Tecan M1000 Pro plate reader; Tecan Austria GmbH) at a wavelength of 540 mn. Distilled water was used as a blank. Haemolysis percentage for each dilution was calculated based on the distilled water sample representing complete RBC lysis. Osmotic fragility curves were plotted, and the MOF values were determined from the curves as the concentration of NaCl at which 50% haemolysis occurred.

*Statistical analysis*

Data were grouped according to storage time and filter passage. All data were assessed for normality using a Shapiro-Wilk test and statistical analysis performed using commercially available software (IBM SPSS Statistics, Version 24). The Wilcoxon signed ranks test was used to compare differences in the ranks of haematological parameters, percent haemolysis and OF for D0- *versus* D0+, D0- *versus* D35-, D0- *versus* D35+ and D35- *versus* D35+. Statistical significance was determined using a z-statistic applied to sum of ranks, and differences were considered statistically significant when $P<0.05$.

## 4.5 Results

*Haematological parameters, haemolysis percentage and MOF*

Results are reported in Table 1.

**Table 1 Effect of storage and microaggregate filter passage on haematological parameters including mean osmotic fragility and haemolysis percentage in nine feline whole blood units**

| | | | | Range | |
|---|---|---|---|---|---|
| **Parameter** | **Day** | **Filter passage** | **Median** | **Min** | **Max** |
| PCV (%) | 0 | Pre filter | 27[a] | 20 | 31 |
| | | Post filter | 24[a] | 19 | 32 |
| | 35 | Pre filter | 22[b] | 15 | 27 |
| | | Post filter | 22[b] | 13 | 25 |
| HCT (%) | 0 | Pre filter | 0.27[a] | 0.2 | 0.36 |
| | | Post filter | 0.24[a] | 0.18 | 0.33 |
| | 35 | Pre filter | 0.23[b] | 0.17 | 0.29 |
| | | Post filter | 0.22[b] | 0.14 | 0.28 |
| RBC count (x $10^{12}$/L) | 0 | Pre filter | 6.45[a] | 4.29 | 8.51 |
| | | Post filter | 6.08[a] | 4.56 | 8.78 |
| | 35 | Pre filter | 5.95[b] | 3.67 | 7.31 |
| | | Post filter | 6.01[b] | 3.4 | 6.84 |
| Hb (g/L) Benchtop analyser | 0 | Pre filter | 93[a] | 66 | 114 |
| | | Post filter | 90[a] | 68 | 114 |
| | 35 | Pre filter | 86[b] | 55 | 96 |
| | | Post filter | 71[b] | 62 | 107 |

| | | | | | |
|---|---|---|---|---|---|
| Hb (g/L) Diagnostic laboratory | 0 | Pre filter | 95[a] | 66 | 120 |
| | | Post filter | 87[a] | 62 | 115 |
| | 35 | Pre filter | 87[b] | 57 | 98 |
| | | Post filter | 86[b] | 52 | 95 |
| MCV (fl) | 0 | Pre filter | 40.5[a] | 35.9 | 45.9 |
| | | Post filter | 40.2[a] | 35.5 | 42.7 |
| | 35 | Pre filter | 39.2[a] | 33.2 | 42.5 |
| | | Post filter | 38.2[a] | 33.6 | 45.5 |
| MCH (pg) | 0 | Pre filter | 14.1[a] | 12.3 | 15.4 |
| | | Post filter | 14[a] | 12.2 | 15.6 |
| | 35 | Pre filter | 14.2[a] | 12.4 | 15.5 |
| | | Post filter | 14.4[b] | 12.5 | 15.7 |
| MCHC (g/L) | 0 | Pre filter | 354[a] | 335 | 387 |
| | | Post filter | 351[a] | 326 | 376 |
| | 35 | Pre filter | 364[a] | 348 | 414 |
| | | Post filter | 376[a] | 337 | 413 |
| RDW (%) | 0 | Pre filter | 15.5[a] | 13.2 | 21.5 |
| | | Post filter | 15.7[a] | 13.2 | 21.2 |
| | 35 | Pre filter | 20.4[b] | 17.6 | 22 |
| | | Post filter | 19.9[b] | 17.8 | 22.4 |
| Haemolysis (%) | 0 | Pre filter | 0.23[a] | 0.12 | 0.29 |
| | | Post filter | 0.17[a] | 0.08 | 0.36 |
| | 35 | Pre filter | 1.31[b] | 0.81 | 1.85 |
| | | Post filter | 1.48[b] | 0.84 | 2.07 |
| MOF (NaCl %) | 0 | Pre filter | 0.54[a] | 0.48 | 0.55 |
| | | Post filter | 0.53[a] | 0.47 | 0.55 |
| | 35 | Pre filter | 0.58[b] | 0.53 | 0.63 |
| | | Post filter | 0.59[b] | 0.53 | 0.64 |

Only comparisons involving day 0 pre-filter samples are represented in the table. Superscript letters that are different when compared to day 0 pre-filter within each category indicate significant differences ($P<0.05$) using the Wilcoxon signed ranks test.

MCV and MCHC are calculated from manual PCV and reference laboratory haemoglobin and not the HCT value.

Haemolysis % was calculated from the equation found in the methods using the Hb 201 total haemoglobin measurement.

PCV = packed cell volume; HCT = haematocrit; RBC = red blood cell; Hb = haemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; MOF = mean osmotic fragility

There were no significant differences (P>0.05) in any parameter pre (D0-) and post filter (D0+) passage on the day of blood collection. Comparing D0- and D35- there was a greater percentage haemolysis (Z = -2.803, p = 0.005), RDW (Z = -2.67, p = 0.008) and MOF (Z = -2.701, p = 0.007) and lower PCV (Z = -2.536, p = 0.011), RBC count (Z = -2.547, p = 0.011), haemoglobin concentration (benchtop analyser) (Z = -2.49, p = 0.013), haemoglobin concentration (diagnostic laboratory) (Z = -2.552, p = 0.011) and haematocrit (Z = -2.49, p = 0.013) at D35-. The same significant increases and decreases were found when comparing D0- and D35+ with the addition of a higher MCH (Z = -1.481, p = 0.016). Comparing D35- and D35+, the only significant change was a higher MCH at D35+ (Z = -1.994, p = 0.046).

*Microbiologic analysis*

Bacteria were not cultured aerobically or anaerobically from any of the units at either D0+ or D35+.

## 4.6 Discussion

This study is the first to examine in vitro markers of quality in fresh and stored FWB that has been passed through a microaggregate filter. Presumably, stored RBCs affected by the storage lesion are less deformable and more at risk of damage when travelling through micropores. However, the results of this study indicated that most changes could be attributed to the storage lesion. Increased MCH was the only statistically significant change that could be ascribed to an effect of filter passage. The haematological changes and notably significant increase in haemolysis percentage observed at day 35 of storage may have clinical implications for blood recipients and blood banking quality control programs.

Designed to remove microaggregates of cell debris, platelets, white blood cells and fibrin that accumulate during blood storage,[24, 25] the specific filter used in this study is recommended for feline blood transfusions [18, 19]. While clinical use has been validated in a feline RBC survival study [20], a similar study in dogs identified significant in vivo loss of RBCs within 24 hours of transfusion via syringe pump and microaggregate filter. Shearing stress and mechanical trauma from filter passage were suggested as mechanisms for early RBC clearance by the recipients' reticuloendothelial system. Interestingly, the authors found

no significant changes to RBC count, plasma Hb or OF in fresh blood transfused via gravity, pump or syringe pump with microaggregate filter [26]. Similarly, no significant changes to CBC variables, OF or RBC morphology were reported in a recent study evaluating effect of syringe driver and microaggregate filter passage on fresh canine whole blood (WB) [27]. While the species and study methodologies differ, the results are in line with the present study, demonstrating a lack of microaggregate filter induced in vitro damage to fresh blood.

Packed cell volume, RBC count, Hb and HCT all decreased significantly during storage. While not reported in stored FWB [3, 4, 16], a decrease in PCV during storage of feline packed red blood cells (PRBCs) has been described [5]. The PCV of canine and human stored PRBCs generally increases with storage due to increased RBC membrane permeability and subsequent intracellular water influx and cell swelling [28-30]. It has been theorised that feline RBCs are resistant to swelling due to their specific surface area to volume ratios and possibly reduced OF compared with canine RBCs [5]. This is supported by the lack of significant end-storage MCV change in this study.

Studies of canine PRBCs [31-33] report significant PCV decreases with storage, ascribed to RBC sedimentation in the primary collection bag prior to transfer to satellite bags for storage. The authors believe this may have played a role in the changes to PCV, RBC count, Hb, and HCT noted in this study. Settling of the RBCs dependant to the plasma was noted in the syringes during the simulated infusion. Decreased RBC count and HCT or PCV and an increase in MCH and MCHC are also well described secondary to in vitro haemolysis in human [34, 35] and canine [36] blood. While statistical significance was not reached, the observed increase in MCHC during storage supports this theory. It is likely a combination of species-specific RBC factors including blood collection and handling technique contributed to the observed changes.

Mean corpuscular haemoglobin only significantly changed in comparisons involving the filter passage of stored blood. In both the D0- versus D35+ and D35- versus D35+ comparisons, MCH increased. Mean corpuscular haemoglobin is the average mass of Hb per RBC and is calculated from Hb and RBC count or (as used in this study) PCV [35]. Total Hb measurements were performed by methodologies that lyse RBCs, therefore not

discriminating intracellular from free haemoglobin. Consequently, increased MCH may occur due to in vitro haemolysis where PCV is decreased and Hb concentration remains stable [35] or as is theorised in this case, decreased comparatively less than the PCV. The significant decreases in RBC and PCV and increase in haemolysis percentage in the D0- versus D35+ comparison supports this theory. Mean corpuscular haemoglobin was the only parameter to significantly change in the stored blood pre and post filter comparison. Filter passage-enhanced in vitro haemolysis where the threshold of change for the other indicators of haemolysis did not reach statistical significance may also explain this finding.

Mean osmotic fragility significantly increased by the end of storage, a finding replicated with or without filter passage. Storage related increases in MOF have been reported in feline PRBCs collected via vascular access ports (VAP) with the increase in RBC fragility attributed to VAP-induced membrane damage [17]. Increased MOF has also been documented in stored human WB [37] and PRBCs [12, 38] and stored canine PRBCs [31]. Changes to MOF are not a uniformly reported feature of storage lesion, with some human blood storage studies [39-41] and a recent study of stored FWB collected by a novel closed collection system [3] demonstrating no statistically significant MOF increase.

Stored human RBCs shed membrane microvesicles, leading to irreversible alterations to their shape and the formation of spheroechinocytes and spherocytes that have less ability to withstand increased osmotic stress [12]. Increases in echinocyte number [3, 4, 16], spherocytes and lysed RBCs [16] have been identified in FWB stored for 35 days. Red blood cell membrane alteration during storage likely caused the increase in MOF observed in this study, with the significant end storage RDW increase supporting RBC anisocytosis and morphological variation [42]. While increased MOF and haemolysis percentage failed to reach statistical significance in the pre and post filter comparison at day 35, the overall increase in haemolysis suggests filter passage-induced injury may be amplified in storage lesion affected RBCs. The clinical implications of transfusing stored feline blood with increased OF is unknown and may impact longevity of transfused RBCs in the recipient.

Haemolysis percentage significantly increased from the day of collection compared to

D35. In line with recently published studies investigating haemolysis percentage in stored FWB, no sample had a haemolysis percentage that exceeded 1% at the point of collection (D0) [3-5] or after filter passage on D0. By D35, two thirds (6/9) of the blood units had a haemolysis percentage exceeding the FDA limit of 1%. This increase was significant for both D0 versus D35 pre and post filter comparisons. This finding is similar to a recent study where mean haemolysis percentage in FWB units exceeded 1% by 21 days of storage [4]. Another study of feline PRBCs documented a mean haemolysis percentage of >1% in 13.8% of units stored for longer than 28 days. In contrast, a study of eight FWB units retained a haemolysis percentage <1% at the end of 35 days storage [3]. The comparatively lower mean haemolysis at all times points in that study may have been a consequence of reduced shear stress associated with using a novel closed system and smaller 20 mL aspiration syringes for blood collection [3]. Although not statistically significant when compared with D35 pre filter samples, 8/9 (89%) units had >1% haemolysis post filter passage at D35. A recent canine study found no significant increase in haemolysis percentage in PRBCs stored for less than 14 days after passage through an 18µmfilter at maximal manual infusion rates [43].

The clinical significance of transfusing blood with >1% haemolysis has not been established in cats. Free haemoglobin induces oxidative stress, with potentially detrimental effects to renal, myocardial, and vascular systems [44, 45]. Transfusion of significantly haemolysed blood carries an increased risk of adverse transfusion reactions and in dogs, has resulted in death secondary to the transfusion of what was suspected to be improperly stored blood [46]. The findings in the present study highlight the importance of blood banking quality controls and need for further research to clarify the consequences of syringe pump infusion and filter passage on storage lesion affected feline blood.

This study has a number of limitations. It is likely that the small number of blood units evaluated made some statistical comparisons underpowered. Red blood cell morphological analysis was not performed, limiting further interpretation of some haematological alterations. Five samples on the day of collection and three samples at D35 had a haemolysis percentage that decreased between pre and post filter passage analysis. Similar results have been found in humans [25] and dogs [43] and the reasons for this are unclear. Proposed mechanisms include settling of blood during the transfusion and/or partial filter

occlusion leading to inconsistencies in HCT/PCV and plasma Hb results [25]. Haemolysis percentage was derived from haemoglobin concentrations measured via automated analysers rather than gold standard reference methods. While designed for very low-level plasma haemoglobin concentrations, the Plasma/Low Hemoglobin system demonstrates non-linearity for haemoglobin concentrations between 0 and 0.3 g/L. The inability to distinguish between very low-level haemoglobin concentrations may have impacted accuracy of some haemolysis calculations.

## 4.7 Conclusions

Feline whole blood undergoes haematological changes, including increased haemolysis percentage and increased MOF with storage. While the significant MCH increases suggest enhanced in vitro haemolysis in stored blood due to microaggregate filter passage, the difference in haemolysis percentage was not significant in the comparison assessing isolated filter passage effect on stored blood. In vivo studies are required to understand whether haematological derangements associated with the storage lesion and potentially enhanced by transfusion and filter passage associated shear stress translate to reduced RBC viability within recipient circulation.

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# Chapter 5: General Discussion and Conclusion

This thesis describes the completion of a survey study to characterise how Australian emergency, referral and university veterinary hospitals source feline blood products, approach blood donation collections and identify and describe feline blood storage practices. The survey study provides a clinical context and local background information to help define feline transfusion practice within Australia. In an emergency situation, a blood bank that stores blood for immediate use is advantageous for both blood donor and recipient. However, stored blood is subject to progressive biochemical and haematological RBC changes termed the 'storage lesion' that may alter cell biomechanics during transfusion with implications for subsequent RBC survival. An experimental study was conducted to investigate the effect that the common feline transfusion delivery method of syringe driver and microaggregate filter passage has on fresh versus stored whole feline blood.

The survey study revealed that nearly all Australian emergency and referral hospital respondents performed feline blood transfusions. No associations were found between the type of hospital surveyed and blood donor source, type of collection system used or preferred anticoagulant. Most donor cats were community sourced from staff members, their friends and acquaintances, members of the public and/or hospital clients. Only three of the 31 hospitals surveyed indicated routine storage of feline blood. Storage of feline blood facilitates immediate access to blood products, removing potentially detrimental time delays when urgent blood transfusion is required. As well as benefiting the transfusion recipient, blood collection for storage may also be safer for the donor when performed as a planned donation in a pre-screened cat with adequate equipment, staffing levels and experience.

Australia lacks access to commercial feline blood products, instead relying on individual hospital-run blood banks that may be costly and time consuming to maintain. In addition, feline blood storage requires more specialised equipment and knowledge. The small volume of blood collected per donation necessitates use of either a feline specific closed collection system or a custom, in-house modified open system to ensure aseptic collection for blood intended to be stored longer than 24 hours. Storage of blood collected with an open system

is controversial. The majority of respondents indicated use of some form of open blood collection system. No hospital that stored blood indicated use of a closed blood collection system. The single closed system response from a hospital not storing blood is suspected to be an error related to question misinterpretation as to the best of the author's knowledge closed collection systems suitable for cat blood collection are not commercially available in Australia. The small number of hospitals identified in the survey as storing feline blood may also reflect the lack of feline specific evidence-based recommendations or expert-led consensus guidelines in the veterinary literature at the time of survey distribution. This is also highlighted by disparate component therapy processing, blood storage times and determination of the order of usage of stored blood units by the surveyed hospitals storing blood. Future studies are needed to investigate factors contributing to access to stored feline blood.

The results of the simulated transfusion study indicated that blood storage rather than microaggregate filter passage resulted in the development of markers of RBC injury as determined by in vitro testing. Fresh whole blood demonstrated no evidence of in vitro injury after syringe pump delivery and microaggregate filter passage, adding to the existing evidence [1] in favour of the suitability of this method for feline transfusion delivery. While in-line filter use diverged from manufacturer recommendations for cellular products like WB to be aspirated through the filter prior to patient transfusion, the maximal 25ml volume of WB passed through a single filter was well below the recommended 50ml maximum [2, 3]. The differing effects of negative pressure filtration versus syringe pump driven in-line filter passage on stored feline WB were not assessed as filter use was designed to replicate common clinical usage practices. Further studies are needed to characterise clinical blood filter usage for the delivery of feline blood. Fresh canine WB passed through an 18 µm microaggregate filter at rates of 12.5, 25 and 50ml/hr using volumes not exceeding 60ml was not associated with excessive filter occlusion or alterations to RBC fragility [4]. A canine study has also demonstrated that storage of PRBCs rather than a rapid infusion rate through an 18 µm microaggregate filter was associated with unacceptable (> 0.8%) RBC haemolysis in 15% of units [5]. Notably, this study aspirated blood through the filter from a donor bag as per manufacturer recommendations [5]. While extrapolating canine study results is inherently imperfect, in addition to the findings of the simulated transfusion study

presented in this thesis, feline transfusion medicine uses smaller volumes and slower infusion rates of WB compared to dogs. Different infusion rates and the impact that greater volumes of WB being passed through a single filter might have on stored feline RBCs still warrant further investigation.

In spite of the fact that blood was collected using an open system, aerobic and anaerobic bacterial culture of blood from the day of collection and at the end of the storage period, after a simulated two-hour transfusion, was negative. The storage of blood collected using an open system is controversial due to an increased risk of bacterial contamination. Even though the sample size of the experimental study was small, it adds to other published studies where use of an open system has not been associated with increased risk of bacterial growth [6-8]. Large scale microbiological surveillance studies are required to help quantify and further define the risk associated with open system blood collection designated for storage.

Nearly all changes in haematological parameters in feline blood collected and stored for the purpose of transfusion, including greater RBC osmotic fragility and increased haemolysis percentage, could be attributed to storage lesion. Compared to the day of blood collection, greater RDW percentage and decreased PCV, RBC count, haemoglobin and haematocrit were detected at the end of storage. In addition to these changes, MCH was greater in stored blood after filter passage compared to stored blood prior to filter passage. Despite the lack of other supporting haematological changes, the greater MCH in stored blood may reflect enhanced microaggregate filter induced in vitro haemolysis. By the end of storage, two thirds (6/9) of the samples had a haemolysis percentage exceeding the human blood banking FDA guideline threshold of 1% [9]. While not statistically significant, this increased to 8/9 after filter passage. The transfusion of free haemoglobin may have detrimental effects on recipient organ function [10-12] and be associated with an increased risk of adverse transfusion reactions [13]. While the extent of haemolysis required to induce clinically significant consequences in a recipient cat is unknown, these findings highlight the importance of blood banking quality controls. Additionally, the other in vitro RBC changes such as increased osmotic fragility may have clinically significant in vivo consequences for transfused RBC survival. The impact that shear stress secondary to transfusion delivery

method has on storage lesion affected blood also requires further investigation. Future studies, including in vivo post-transfusion survival studies are necessary to determine the clinical significance of the observed in vitro changes in the experimental study.

In conclusion, the survey of Australian veterinarians provides background information to aid the characterisation of current Australian feline blood transfusion practice in the emergency, referral, and university hospital settings. The findings highlight the impact geographical location and resource access have in determining local veterinary practice. The simulated feline transfusion study revealed no evidence of microaggregate filter induced in vitro injury to fresh feline whole blood. Storage lesion rather than the evaluated transfusion technique of syringe driver and microaggregate filter delivery is a significant contributor to in vitro evidence of RBC injury.

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# Appendix 1: Feline blood collection and blood banking survey for emergency, private emergency and referral and university teaching hospitals

| Question number | Question | Answer options if provided. If no options, answer is free text. |
|---|---|---|
| 1 | Type of clinic | 1 Emergency hospital only<br>2 Private emergency and referral hospital<br>3 University teaching hospital |
| 2 | Name of clinic | |
| 3 | Suburb | |
| 4 | Post code | |
| 5 | State | |
| 6 | Does your clinic perform cat blood transfusions? | 1 Yes<br>0 No |
| 7<br>If "No" selected | What do you recommend for cats that requires a blood transfusion? Select all that apply. | 1 Referral to another emergency and/or specialist referral centre that performs cat blood transfusions<br>2 Referral to a local partner or branch vet hospital that performs cat blood transfusions<br>3 Xenotransfusion with dog blood<br>4 Other - please specify |
| 8<br>If "Other – please specify" selected | Other - please specify | |
| 9 | On average, how many cat blood product transfusions does your hospital perform per month? | 1 0 - 1<br>2 2 - 3<br>3 3 - 4<br>4 > 4 |
| 10 | From where do you source your blood donors? Select all that apply. | 1 Staff-owned cats<br>2 Client-owned cats<br>3 Clinic in-house donors<br>4 Blood is sourced from another clinic<br>5 Other - please specify |
| 11<br>If "Other – please specify" selected | From where do you source your blood donors (other)? | |

| | | |
|---|---|---|
| 12 | Which system is used for blood collection? Select all that apply | 1 Open (the entire collection system is assembled in-house, and anticoagulant is added by the operator. The system is not sterilised after assembly).<br>2 Open (the collection system is partially pre-assembled, partially assembled in-house. Anticoagulant is added by the operator. The system is not sterilised after assembly).<br>3 Closed (the system is fully pre-assembled, including anticoagulant and sterile).<br>4 Modified system (for example, sterilised after assembly or the addition of anticoagulant. Please specify below).<br>5 Other - please specify below. |
| 13<br>If "Other – please specify below" selected | What collection system do you use? | |
| 14<br>If more than one collection system selected | In what circumstances are different collection systems being used? | |
| 15 | Which anticoagulant is used for blood collection? Select all the apply. | 1 ACD<br>2 CPD<br>3 CPDA-1<br>4 Heparin<br>5 Other - please specify |
| 16<br>If "Other – please specify" selected | Please specify what other anticoagulant/s you use for blood collection | |
| 17 | Do you store cat blood or collect it 'on demand'? | 1 Store<br>2 On demand collection only<br>3 Mix of both |
| 18<br>If 'Store' or 'Mix of both' selected | What blood type/s do you store? Select all that apply. | 1 A<br>2 B<br>3 AB |
| 19 | What cat blood components do you store? Select all the apply. | 1 Whole blood<br>2 Packed red blood cells<br>3 Liquid plasma<br>4 Fresh frozen plasma<br>5 Frozen plasma<br>6 Other - please specify below |
| 20 | Please specify any other cat blood components stored. | |
| 21 | Is an additive preservative solution used? | 1 Yes<br>0 No |

| | | |
|---|---|---|
| 22<br>If 'Yes' selected | If yes, which one? | |
| 23 | What is the maximum storage time of blood (in days)? | |
| 24 | What is the minimum number of red blood cell units stored at any one time? (all blood types) | |
| 25 | What is the maximum number of red blood cell units stored at any one time? (all blood types) | |
| 26 | How is order of stored blood usage determined? | |
| 27 | Are any blood banking quality controls conducted on stored units before use? Select all that apply. | 1 Visual inspection of units for colour change<br>2 Sample centrifuged for visual haemolysis check<br>3 Blood culture<br>4 None<br>5 Other - please specify |
| 28<br>If "Other – please specify" selected | Please specify any other blood quality controls | |

# Appendix 2: Screening check list for each potential donor cat

**Screening Test Check List**

Name of Cat: _____
Name of Owner: _____
Breed: _____ Age: _____
Sex: _____AIS number: _____
Check performed by: _____
Information statement provided and ethics consent form signed: □

**History**
Vaccination: _____
Flea and worming treatment: _____

**Physical Examination**
Weight: ____ kg
HR: _____
PULSE QUALITY: _____
RR: _____
TEMPERATURE: _____
APPEARANCE: _____
INTEGUMENT: _____
CIRCULATORY: _____
RESPIRATORY: _____
DIGESTIVE: _____
GENITOURINARY: _____
MUSCULOSKELETAL: _____
NEURAL: _____
LYMPH NODES: _____
EYES: _____
EARS: _____
BCS: _____

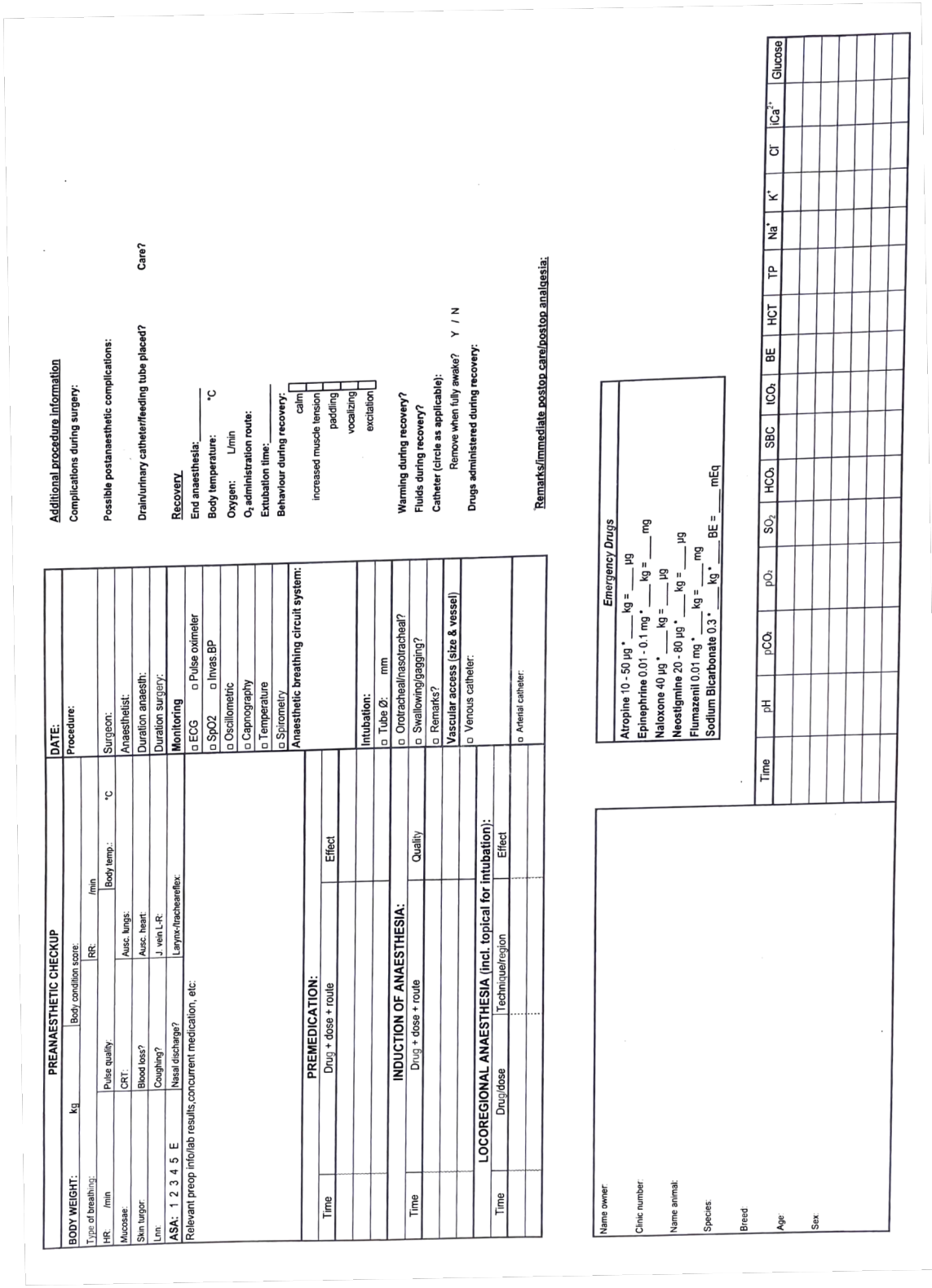
COMMENTS: _____

**Clinical pathology – tubes to collect**
EDTA
Lithium heparin
Plain serum clot tube

**Tests to perform**
FIV/FeLV Witness test POS/NEG

Echocardiogram NORMAL/ABNORMAL

# Appendix 3: Blank example of the anaesthetic monitoring form completed for each cat during blood donation



**PREANAESTHETIC CHECKUP** — **DATE:**

**BODY WEIGHT:** kg | Body condition score: | **Procedure:**

Type of breathing: | RR: /min

HR: /min | Pulse quality: | Body temp.: °C | Surgeon:

Mucosae: | CRT: | Ausc. lungs: | Anaesthetist:

Skin turgor: | Blood loss? | Ausc. heart: | Duration anaesth:

Lnn: | Coughing? | J. vein L-R: | Duration surgery:

**ASA:** 1 2 3 4 5 E | Nasal discharge? | Larynx-/tracheareflex: | **Monitoring**

Relevant preop info/lab results,concurrent medication, etc:

□ ECG □ Pulse oximeter

□ SpO2 □ Invas.BP

□ Oscillometric

□ Capnography

□ Temperature

□ Spirometry

**Anaesthetic breathing circuit system:**

**PREMEDICATION:**

| Time | Drug + dose + route | Effect |
|---|---|---|
| | | |
| | | |
| | | |

**INDUCTION OF ANAESTHESIA:**

| Time | Drug + dose + route | Quality |
|---|---|---|
| | | |
| | | |
| | | |

**LOCOREGIONAL ANAESTHESIA (incl. topical for intubation):**

| Time | Drug/dose | Technique/region | Effect |
|---|---|---|---|
| | | | |
| | | | |

**Intubation:**

□ Tube Ø: mm

□ Orotracheal/nasotracheal?

□ Swallowing/gagging?

□ Remarks?

**Vascular access (size & vessel)**

□ Venous catheter:

□ Arterial catheter:

**Additional procedure information**

**Complications during surgery:**

**Possible postanaesthetic complications:**

**Drain/urinary catheter/feeding tube placed?** **Care?**

**Recovery**

**End anaesthesia:**

**Body temperature:** °C

**Oxygen:** L/min

**$O_2$ administration route:**

**Extubation time:**

**Behaviour during recovery:**

calm

increased muscle tension

paddling

vocalizing

excitation

**Warming during recovery?**

**Fluids during recovery?**

**Catheter (circle as applicable):**

Remove when fully awake? Y / N

**Drugs administered during recovery:**

**Remarks/immediate postop care/postop analgesia:**

Name owner:

Clinic number:

Name animal:

Species:

Breed:

Age:

Sex:

*Emergency Drugs*

**Atropine** 10 - 50 µg • ____ kg = ____ µg

**Epinephrine** 0.01 - 0.1 mg • ____ kg = ____ mg

**Naloxone** 40 µg • ____ kg = ____ µg

**Neostigmine** 20 - 80 µg • ____ kg = ____ µg

**Flumazenil** 0.01 mg • ____ kg = ____ mg

**Sodium Bicarbonate** 0.3 • ____ kg • ____ BE = ____ mEq

| Time | pH | $pCO_2$ | $pO_2$ | $SO_2$ | $HCO_3$ | SBC | $tCO_2$ | BE | HCT | TP | $Na^+$ | $K^+$ | $Cl^-$ | $iCa^{2+}$ | Glucose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
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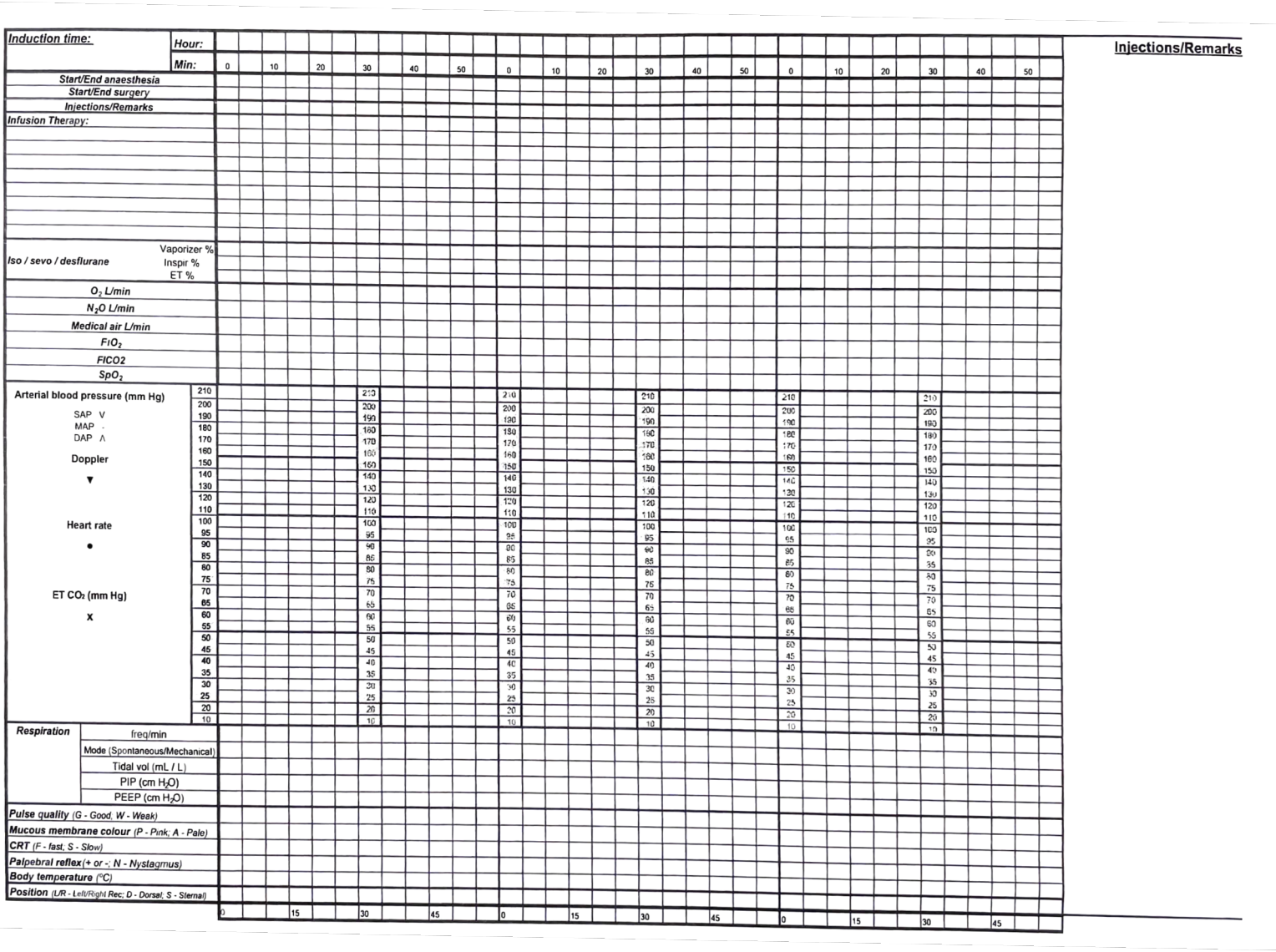
Induction time:
Hour:
Min:
0 10 20 30 40 50 0 10 20 30 40 50 0 10 20 30 40 50
Injections/Remarks
Start/End anaesthesia
Start/End surgery
Injections/Remarks
Infusion Therapy:
Iso / sevo / desflurane
Vaporizer %
Inspir %
ET %
O2 L/min
N2O L/min
Medical air L/min
FiO2
FICO2
SpO2
Arterial blood pressure (mm Hg)
SAP V
MAP -
DAP Λ
Doppler
▼
Heart rate
●
ET CO2 (mm Hg)
x
210 200 190 180 170 160 150 140 130 120 110 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 10
Respiration
freq/min
Mode (Spontaneous/Mechanical)
Tidal vol (mL / L)
PIP (cm H2O)
PEEP (cm H2O)
Pulse quality (G - Good, W - Weak)
Mucous membrane colour (P - Pink, A - Pale)
CRT (F - fast, S - Slow)
Palpebral reflex (+ or -; N - Nystagmus)
Body temperature (°C)
Position (L/R - Left/Right Rec; D - Dorsal; S - Sternal)
0 15 30 45 0 15 30 45 0 15 30 45

Appendix 4: Copy of published study: Effect of microaggregate filter passage on feline whole blood stored for 35 days

Original Article

jfms Journal of Feline Medicine and Surgery

*Journal of Feline Medicine and Surgery*
1–7

DOI: 10.1177/1098612X211009145
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SAGE

# Effect of microaggregate filter passage on feline whole blood stored for 35 days

**Sophia A Morse and Erin T Mooney**

## Abstract

*Objectives* The aim of this study was to compare the characteristics of fresh and stored feline red blood cells (RBCs) after passage through an 18 µm microaggregate filter.

*Methods* Nine cats were recruited for a single blood donation using an open collection system. A simulated transfusion using a syringe driver and microaggregate filter was performed over 2 h with half the blood on the day of donation and the other half after 35 days of storage. Differences in haematological parameters, haemolysis percentage and osmotic fragility (OF) were compared on the day of donation pre-filter passage (D0–) vs day of donation post-filter (D0+) or day 35 storage pre-filter (D35–) and post-filter (D35+). Blood was cultured at D0+ and D35+.

*Results* There were no statistically significant differences in the D0– vs D0+ comparisons. There were statistically significant ($P < 0.05$) increases in haemolysis percentage, red cell distribution width (RDW) percentage and mean OF, and decreases in packed cell volume (PCV), RBC count, haemoglobin and haematocrit for D0– vs D35–. The same was found for D0– vs D35+ with the addition of a significant increase in mean cell haemoglobin (MCH). For D35– vs D35+ only MCH significantly increased. At day 35, 6/9 units had haemolysis percentages that exceeded 1%. This increased to 8/9 of stored units post-filter passage. All blood units cultured negative.

*Conclusions and relevance* Fresh RBCs exhibited no in vitro evidence of injury following passage through an 18 µm microaggregate filter. Increased MCH was observed in the stored blood and may represent haemolysis induced by the filter. All other changes can be explained by storage lesion rather than filter passage. The findings highlight the importance of blood banking quality controls and the need for further research to assess the effects of transfusion technique, specifically filter passage, on storage lesion-affected feline blood.

**Keywords:** Erythrocytes; storage lesion; micropore filters; blood transfusion; erythrocyte indices; haemolysis; osmotic fragility; in vitro techniques; syringes

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## Introduction

Blood transfusions are essential to feline emergency and critical care medicine. While a few commercial blood banks exist, many practitioners worldwide collect blood in-house.[1] Accessibility to suitable donors can be challenging, and the storage of feline blood is controversial.[2,3] Consequently, feline blood donations are often performed on demand for immediate transfusion to the recipient.

Storage of blood is advantageous, allowing collection from donors in a controlled environment and immediate access to blood products for recipients. There are few studies reporting on the ideal storage time of feline blood.[2–7] Official feline blood banking standards have not been established, with current recommendations to determine blood 'shelf life' extrapolated from human guidelines.[3–5] The US Food and Drug Administration (FDA) human blood banking regulations stipulate a minimum of 75% 24 h post-transfusion red blood cell (RBC) survival in the recipient's circulation[8] and a maximum of 1% haemolysis after 42 days of storage.[9] Using biotinylating cell-labelling

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techniques, 85% RBC survival 24 h post-transfusion has been demonstrated for feline whole blood (FWB) stored in citrate dextrose adenosine phosphate 1 (CPDA-1) for 35 days.[6]

Collectively termed the 'storage lesion', the progressive biochemical and biomechanical RBC changes that occur during storage may have implications for RBC survival and viability post-transfusion.[10,11] Alterations to RBC membrane integrity and shape lead to reduced cell deformability and oxygen-carrying capacity and increased haemolysis.[4,10,12] Biochemical, haematological and morphological features of storage lesion have been documented in feline blood.[3–5,11,13–17] Both unchanged[3] and significantly increased[17] mean osmotic fragility (MOF) and varying levels of haemolysis have been reported.[3–5]

Cat transfusions are typically delivered via syringe driver[1] and 18 µm microaggregate filter.[18,19] This technique has been demonstrated to cause no significant in vivo loss of RBCs compared with the traditional gravity-dependent delivery methods in cats.[20] Notably, in this study blood was stored for <12 h prior to transfusion.[20]

To date, there have been no studies assessing the effects of filter passage on stored feline RBCs. The objective of this study was to determine whether passage through a microaggregate filter damages feline RBCs as indicated by increased haemolysis percentage, MOF or haematological derangements, and if these effects are compounded in blood that has been stored for 35 days.

## Materials and methods

### *Blood donors and sampling protocol*

Nine domestic cats between 1 and 9 years of age with a minimum lean body weight of 4.5 kg were recruited. Cats were in good health based on history, physical examination and results of screening tests including complete blood count (CBC), serum biochemistry, feline immunodeficiency virus and feline leukaemia virus tests (Witness FIV/FeLV; Zoetis Animal Health) and echocardiogram. Informed consent was obtained from each owner, and the study protocol was approved by the University of Sydney Animal Ethics Committee (project number: 2018/1358).

Prior to donation, 6/9 cats received between 50 and 100 mg of oral gabapentin. For blood donation, cats were sedated with intramuscular and/or intravenous injection(s) of 0.19–0.25 mg/kg midazolam, 0.15–0.2 mg/kg butorphanol and 3.75–8.3 mg/kg ketamine. An intravenous catheter was placed, and mask oxygen provided. Additional sedation with alfaxalone was used for 3/9 cats. Cats were monitored with electrocardiography, pulse oximetry and oscillometric blood pressure. Full vitals and anaesthetic depth were assessed every 5 mins until cats regained consciousness. Cats were placed in lateral recumbency and the neck clipped of fur followed by surgical skin preparation. An open collection system consisting of a 21 G butterfly needle attached to a three-way stopcock, 50 ml syringe containing CPDA-1 in a 1:7 ratio to the intended blood volume and 100 ml blood storage bag (Animal Blood Banking International) was used to collect blood via jugular venepuncture. A maximum of 10 ml/kg of blood was collected per cat. Half the blood was partitioned into the storage bag via the three-way stopcock. The extension line was stripped of blood and sealed with metal clips. Blood units were stored upright in a dedicated refrigerator at +4°C for 35 days. A maximum of three cats donated blood on any 1 day.

### *Simulated transfusion and blood culture*

Blood sampling was performed at four time points: the day of donation, pre-filter passage (D0–); the day of donation, post-filter passage (D0+); after 35 days of storage, pre-filter passage (D35–); and after 35 days of storage, post-filter passage (D35+). On the day of donation, half the blood was reserved in the collection syringe for immediate sampling and simulated transfusion. The syringe was disconnected from the three-way stopcock and 4 ml of blood transferred to EDTA and plain tubes. The syringe was then connected to a micro-bore extension set and 18 µm microaggregate filter (Hemo-nate; Utah Medical Products) and loaded into a syringe driver (Perfusor Space Syringe Infusion Pump; B Braun Vet Care). Blood was 'transfused' over a 2 h period. The initial 10 ml was inoculated into a blood culture bottle (Oxoid Signal Blood Culture bottle; Thermo Fisher Scientific) for anaerobic and aerobic culture with the final 4 ml reserved post-filter passage for analysis. Blood culture bottles were incubated at 37°C ± 1°C in both aerobic and anaerobic conditions with daily inspection for 5 days for signs of bacterial growth. After 35 days of storage, blood reserved in the storage bags was aseptically drawn into a syringe via a spike (Swan-lock take set; Codan US). Blood collection for analysis and simulated transfusion as described above was performed (D35– and D35+).

### *Haematology and haemolysis*

Haematology was performed at each time point within 6 h of sample collection using an automated haematology machine (Sysmex XN 1000RF Vet Analyser; Roche Diagnostics). Parameters measured included RBC count, haemoglobin (Hb), haematocrit (HCT), mean cell volume (MCV), mean cell Hb (MCH), mean cell Hb concentration (MCHC) and red blood cell distribution width (RDW). A manual packed cell volume (PCV) was performed and used to calculate MCH and MCHC.

As previously described in cats, Hb was determined using a specific analyser (Hb 201 + System; HemoCue) with daily calibration using a 120 g/l standard.[21] Plasma was obtained by centrifuging (Orbital 360; Clements) 1 ml of anticoagulated blood at 1200 *g* for 15 mins. Supernatant Hb was determined by a spectrophotometer (Plasma/Low Hb; HemoCue) for determination of low Hb levels. This analyser was calibrated each day of use with 1.0 g/l

and 5.0 g/l controls. Percentage haemolysis was calculated using the formula:

$$\text{Haemolysis } (\%) = \text{Supernatant Hb } (\text{g/l}) \times (100 - \text{PCV}) \ / \ \text{Total Hb } (\text{g/l}).$$[5]

### Osmotic fragility testing

Osmotic fragility (OF) testing was performed using 360 µl of blood reserved from each time point based on methods previously described.[3,22,23] A stock solution of 10% phosphate buffered saline with pH 7.4 was diluted with distilled water to create a 1% NaCl solution. Using this solution and distilled water, 18 test tubes were prepared with NaCl concentrations ranging from 0.9% to 0.0% NaCl in 0.05% increments. To each tube, 20 µl of blood was added and mixed by inversion. Samples were incubated at 22°C for 45 mins then centrifuged (Centrifuge 5810R; Eppendorf AG) at 2000 *g* for 10 mins. The optical density of each supernatant was determined spectrophotometrically (Tecan M1000 Pro plate reader; Tecan Austria) at a wavelength of 540 nm. Distilled water was used as a blank. Percentage of haemolysis for each dilution was calculated based on the distilled water sample representing complete RBC lysis. OF curves were plotted, and the MOF values were determined from the curves as the concentration of NaCl at which 50% haemolysis occurred.

### Statistical analysis

Data were grouped according to storage time and filter passage. All data were assessed for normality using a Shapiro–Wilk test and statistical analysis performed using commercially available software (SPSS Statistics, Version 24; IBM). The Wilcoxon signed ranks test was used to compare differences in the ranks of haematological parameters, percentage haemolysis and OF for D0– vs D0+, D0– vs D35–, D0- vs D35+ and D35– vs D35+. Statistical significance was determined using a z-statistic applied to sum of ranks, and differences were considered statistically significant when $P < 0.05$.

## Results

### Haematological parameters, haemolysis percentage and MOF

Results are reported in Table 1.

There were no significant differences ($P > 0.05$) in any parameter pre- (D0–) and post-filter (D0+) passage on the day of blood collection. Comparing D0– and D35– there were statistically significant increases in percentage haemolysis ($Z = -2.803$, $P = 0.005$), RDW ($Z = -2.67$, $P = 0.008$) and MOF ($Z = -2.701$, $P = 0.007$), and decreases in PCV ($Z = -2.536$, $P = 0.011$), RBC count ($Z = -2.547$, $P = 0.011$), haemoglobin (benchtop analyser) ($Z = -2.49$, $P = 0.013$), haemoglobin (diagnostic laboratory) ($Z = -2.552$, $P = 0.011$) and haematocrit ($Z = -2.49$, $P = 0.013$). The same significant increases and decreases were found when comparing D0– and D35+, with the addition of a statistically significant increase in MCH ($Z = -1.481$, $P = 0.016$). Comparing D35– and D35+, the only significant change was an increase in MCH ($Z = -1.994$, $P = 0.046$).

### Microbiological analysis

Bacteria were not cultured aerobically or anaerobically from any of the units at either D0+ or D35+.

## Discussion

This study is the first to examine in vitro markers of quality in fresh and stored FWB that has been passed through a microaggregate filter. Presumably, stored RBCs affected by the storage lesion are less deformable and more at risk of damage when travelling through micropores. However, the results of this study indicated that most changes could be attributed to the storage lesion. Increased MCH was the only statistically significant change that could be ascribed to an effect of filter passage. The haematological changes and notably significant increase in haemolysis percentage observed at day 35 of storage may have clinical implications for blood recipients and blood banking quality control programmes.

Designed to remove microaggregates of cell debris, platelets, white blood cells and fibrin that accumulate during blood storage,[24,25] the specific filter used in this study is recommended for feline blood transfusions.[18,19] While clinical use has been validated in a feline RBC survival study,[20] a similar study in dogs identified significant in vivo loss of RBCs within 24 h of transfusion via syringe pump and microaggregate filter.[26] Shearing stress and mechanical trauma from filter passage were suggested as mechanisms for early RBC clearance by the recipients' reticuloendothelial system.[26] Interestingly, the authors found no significant changes to RBC count, plasma Hb or OF in fresh blood transfused via gravity, pump or syringe pump with microaggregate filter.[26] Similarly, no significant changes to CBC variables, OF or RBC morphology were reported in a recent study evaluating effect of syringe driver and microaggregate filter passage on fresh canine whole blood (WB).[27] While the species and study methodologies differ, the results are in line with the present study, demonstrating a lack of microaggregate filter-induced in vitro damage to fresh blood.

PCV, RBC count, Hb and HCT all decreased significantly during storage. While not reported in stored FWB,[3,4,16] a decrease in PCV during storage of feline packed RBCs (PRBCs) has been described.[5] The PCV of canine and human stored PRBCs generally increases with storage owing to increased RBC membrane permeability and subsequent intracellular water influx and cell swelling.[28–30] It has been theorised that feline RBCs are resistant to swelling owing to their specific surface area-to-volume

**Table 1** Effect of storage and microaggregate filter passage on haematological parameters, including mean osmotic fragility (MOF) and haemolysis percentage in nine feline whole blood units

| Parameter | Day | Filter passage | Median | Range | |
|---|---|---|---|---|---|
| | | | | Minimum | Maximum |
| PCV (%) | 0 | Pre-filter | 27[a] | 20 | 31 |
| | | Post-filter | 24[a] | 19 | 32 |
| | 35 | Pre-filter | 22[b] | 15 | 27 |
| | | Post-filter | 22[b] | 13 | 25 |
| HCT (%) | 0 | Pre-filter | 0.27[a] | 0.2 | 0.36 |
| | | Post-filter | 0.24[a] | 0.18 | 0.33 |
| | 35 | Pre-filter | 0.23[b] | 0.17 | 0.29 |
| | | Post-filter | 0.22[b] | 0.14 | 0.28 |
| RBC count ($\times 10^{12}$/l) | 0 | Pre-filter | 6.45[a] | 4.29 | 8.51 |
| | | Post-filter | 6.08[a] | 4.56 | 8.78 |
| | 35 | Pre-filter | 5.95[b] | 3.67 | 7.31 |
| | | Post-filter | 6.01[b] | 3.4 | 6.84 |
| Hb (g/l) Benchtop analyser | 0 | Pre-filter | 93[a] | 66 | 114 |
| | | Post-filter | 90[a] | 68 | 114 |
| | 35 | Pre-filter | 86[b] | 55 | 96 |
| | | Post-filter | 71[b] | 62 | 107 |
| Hb (g/l) Diagnostic laboratory | 0 | Pre-filter | 95[a] | 66 | 120 |
| | | Post-filter | 87[a] | 62 | 115 |
| | 35 | Pre-filter | 87[b] | 57 | 98 |
| | | Post-filter | 86[b] | 52 | 95 |
| MCV (fl) | 0 | Pre-filter | 40.5[a] | 35.9 | 45.9 |
| | | Post-filter | 40.2[a] | 35.5 | 42.7 |
| | 35 | Pre-filter | 39.2[a] | 33.2 | 42.5 |
| | | Post-filter | 38.2[a] | 33.6 | 45.5 |
| MCH (pg) | 0 | Pre-filter | 14.1[a] | 12.3 | 15.4 |
| | | Post-filter | 14[a] | 12.2 | 15.6 |
| | 35 | Pre-filter | 14.2[a] | 12.4 | 15.5 |
| | | Post-filter | 14.4[b] | 12.5 | 15.7 |
| MCHC (g/l) | 0 | Pre-filter | 354[a] | 335 | 387 |
| | | Post-filter | 351[a] | 326 | 376 |
| | 35 | Pre-filter | 364[a] | 348 | 414 |
| | | Post-filter | 376[a] | 337 | 413 |
| RDW (%) | 0 | Pre-filter | 15.5[a] | 13.2 | 21.5 |
| | | Post-filter | 15.7[a] | 13.2 | 21.2 |
| | 35 | Pre-filter | 20.4[b] | 17.6 | 22 |
| | | Post-filter | 19.9[b] | 17.8 | 22.4 |
| Haemolysis (%) | 0 | Pre-filter | 0.23[a] | 0.12 | 0.29 |
| | | Post-filter | 0.17[a] | 0.08 | 0.36 |
| | 35 | Pre-filter | 1.31[b] | 0.81 | 1.85 |
| | | Post-filter | 1.48[b] | 0.84 | 2.07 |
| MOF (NaCl %) | 0 | Pre-filter | 0.54[a] | 0.48 | 0.55 |
| | | Post-filter | 0.53[a] | 0.47 | 0.55 |
| | 35 | Pre-filter | 0.58[b] | 0.53 | 0.63 |
| | | Post-filter | 0.59[b] | 0.53 | 0.64 |

Only comparisons involving day 0 pre-filter samples are represented. Superscript letters that are different when compared with day 0 pre-filter samples within each category indicate significant differences ($P < 0.05$) using the Wilcoxon signed-rank test
Mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) are calculated from manual packed cell volume (PCV) and reference laboratory haemoglobin, and not the haematocrit (HCT) value
Haemolysis % was calculated from the equation found in the methods using the Hb 201 total haemoglobin measurement
RBC = red blood cell; Hb = haemoglobin; MCH = mean cell Hb; RDW = red cell distribution width

ratios and possibly reduced OF, compared with canine RBCs.[5] This is supported by the lack of significant end storage MCV change in this study.

Studies of canine PRBCs[31–33] report significant PCV decreases with storage, ascribed to RBC sedimentation in the primary collection bag prior to transfer to satellite bags for storage. The authors believe this may have played a role in the changes to PCV, RBC count, Hb and HCT noted in this study. Settling of the RBCs dependent to the plasma was noted in the syringes during the simulated transfusion. Decreased RBC count and HCT or PCV and an increase in MCH and MCHC are also well described secondary to in vitro haemolysis in human[34,35] and canine[36] blood. While statistical significance was not reached, the observed increase in MCHC during storage supports this theory. It is likely that a combination of species-specific RBC factors, including blood collection and handling technique, contributed to the observed changes.

MCH only significantly changed in comparisons involving the filter passage of stored blood. In both the D0– vs D35+ and D35– vs D35+ comparisons MCH increased. MCH is the average mass of Hb per RBC and is calculated from Hb and RBC count or (as used in this study) PCV.[35] Total Hb measurements were performed by methodologies that lyse RBCs, therefore not discriminating intracellular from free Hb. Consequently, increased MCH may occur as a result of in vitro haemolysis where PCV is decreased and Hb concentration remains stable,[35] or as is theorised in this case, decreased comparatively less than the PCV. The significant decreases in RBCs and PCV and increase in haemolysis percentage in the D0– vs D35+ comparison supports this theory. MCH was the only parameter to significantly change in the stored blood pre- and post-filter comparison. Filter passage-enhanced in vitro haemolysis where the threshold of change for the other indicators of haemolysis did not reach statistical significance may also explain this finding.

MOF significantly increased by the end of storage, a finding replicated with or without filter passage. Storage-related increases in MOF have been reported in feline PRBCs collected via vascular access ports (VAPs), with the increase in RBC fragility attributed to VAP-induced membrane damage.[17] Increased MOF has also been documented in stored human WB[37] and PRBCs,[12,38] and stored canine PRBCs.[31] Changes to MOF are not a uniformly reported feature of storage lesion, with some human blood storage studies[39–41] and a recent study of stored FWB collected by a novel closed collection system[3] demonstrating no statistically significant MOF increase.

Stored human RBCs shed membrane microvesicles, leading to irreversible alterations to their shape and the formation of sphero-echinocytes and spherocytes that have less ability to withstand increased osmotic stress.[12] Increases in echinocyte number,[3,4,16] spherocytes and lysed RBCs[16] have been identified in FWB stored for 35 days. RBC membrane alteration during storage likely caused the increase in MOF observed in this study, with the significant end storage RDW increase supporting RBC anisocytosis and morphological variation.[42] While increased MOF and haemolysis percentage failed to reach statistical significance in the pre- and post-filter comparison at day 35, the overall increase in haemolysis suggests that filter passage-induced injury may be amplified in storage lesion-affected RBCs. The clinical implications of transfusing stored feline blood with increased OF is unknown and may affect the longevity of transfused RBCs in the recipient.

Haemolysis percentage significantly increased from the day of collection compared with D35. In line with recently published studies investigating haemolysis percentage in stored FWB, no sample had a haemolysis percentage that exceeded 1% at the point of collection (D0)[3–5] or after filter passage on D0. By D35, two-thirds (n = 6/9) of the blood units' haemolysis percentage exceeded the FDA limit of 1%. This increase was statistically significant for both D0 vs D35 pre- and post-filter comparisons. This finding is similar to a recent study where mean haemolysis percentage in FWB units exceeded 1% by 21 days of storage.[4] Another study of feline PRBCs documented a mean haemolysis percentage of >1% in 13.8% of units stored for longer than 28 days. In contrast, a study of eight FWB units retained a haemolysis percentage <1% at the end of 35 days of storage.[3] The comparatively lower mean haemolysis at all times points in that study may have been a consequence of reduced shear stress associated with using a novel closed system and smaller 20 ml aspiration syringes for blood collection.[3] Although not statistically significant when compared with D35 pre-filter samples, 8/9 (89%) units had >1% haemolysis post-filter passage at D35. A recent canine study found no significant increase in haemolysis percentage in PRBCs stored for <14 days after passage through an 18 µm filter at maximal manual infusion rates.[43]

The clinical significance of transfusing blood with >1% haemolysis has not been established in cats. Free haemoglobin induces oxidative stress, with potentially detrimental effects to renal, myocardial and vascular systems.[44,45] Transfusion of haemolysed blood carries an increased risk of adverse transfusion reactions and in dogs, has resulted in death.[46] The findings in the present study highlight the importance of blood banking quality controls and the need for further research to clarify the consequences of syringe pump infusion and filter passage on storage lesion-affected feline blood.

This study had a number of limitations. It is likely the small number of blood units evaluated made some statistical comparisons underpowered. RBC morphological analysis was not performed, limiting further interpretation of some haematological alterations. Five samples on the day of collection and three samples at D35 had a

haemolysis percentage that decreased between pre- and post-filter passage analysis. Similar results have been found in humans[25] and dogs,[43] and the reasons for this are unclear. Proposed mechanisms include the settling of blood during the transfusion and/or partial filter occlusion, leading to inconsistencies in HCT/PCV and plasma Hb results.[25] Haemolysis percentage was derived from haemoglobin concentrations measured via automated analysers rather than gold-standard reference methods. While designed for very low-level plasma haemoglobin readings, the plasma/low haemoglobin system demonstrates non-linearity for haemoglobin readings between 0 and 0.3 g/l. The inability to distinguish between very low-level haemoglobin values may have affected the accuracy of some haemolysis calculations.

## Conclusions

FWB undergoes haematological changes, including increased haemolysis percentage and increased MOF, with storage. While the significant MCH increases suggest enhanced in vitro haemolysis in stored blood due to microaggregate filter passage, the difference in haemolysis percentage was ultimately not statistically significant in the comparison assessing isolated filter passage effect on stored blood. In vivo studies are required to understand if haematological derangements associated with the storage lesion and potentially enhanced by transfusion and filter passage-associated shear stress translate to reduced RBC viability within recipient circulation.

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**Informed consent** Informed consent (either verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (either experimental or non-experimental animals) for the procedure(s) undertaken (either prospective or retrospective studies). No animals or humans are identifiable within this publication, and therefore additional informed consent for publication was not required.

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