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Studies in feline pre-transfusion testing: Evaluating a novel blood typing device and serial cross-matching in transfusion patients

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To
Friedolin,
Krikri, Molly,
Spotty
and the old gibbon couple at Sababurg Zoo

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List of abbreviations

ABCD	(European) Advisory Board on Cat Diseases
ACD	Acid citrate dextrose
BT	Blood transfusion
BSAVA	British Small Animal Veterinary Association
cDNA	Complementary DNA
CM	Cross-match
CMAH	Cytidine monophospho-N-acetylneuraminic acid hydroxylase
CPDA-1	Citrate phosphate dextrose adenine
DEA	Dog erythrocyte antigen
DIC	Disseminated intravascular coagulation
EDTA	Ethylenediamine tetra-acetic acid
FeLV	Feline leukaemia virus
FIV	Feline immunodeficiency virus
GEL	ID-Gel Test Feline Anti A + B Typing
Hct	Haematocrit
IC	RapidVet-H IC Feline immunochromatographic blood typing kit
Ig	Immunoglobulin
IMHA	Immune-mediated haemolytic anaemia
NI	Neonatal isoerythrolysis
ISTARE	International Surveillance of Transfusion-Associated Reactions and Events
MCM	Major cross-match
NeuAc	N-acetylneuraminic acid
NeuGc	N-glycolylneuraminic acid
PBS	Phosphate-buffered saline solution
PCV	Packed cell volume
PRCA	Pure red cell aplasia
RBC	Red blood cell
SHOT	Serious Hazards of Transfusion
TACO	Transfusion-associated circulatory overload
TRALI	Transfusion-related acute lung injury
TUBE	Pennsylvania tube haemagglutination test for the feline AB blood group system
WBC	White blood cell
WBT	Whole-blood transfusion

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1 Introduction

While transfusion therapy in both human and veterinary medicine has become a safe and effective form of contemporary emergency care, efforts to systematically identify risk factors and find ways to control them are ongoing. Such aspects as pre-transfusion testing, blood-banking safety and donor health monitoring have been studied for companion animals, and the community of veterinary clinicians and researchers has undertaken a concerted effort to standardise practices in order to ensure patient safety for companion animals in general and feline patients in particular (Brown 2012; Davidow 2013; Day & Kohn, 2012; Feldman & Kristensen, 1995; Tocci & Ewing, 2009; Vap et al., 2012).

As a practical matter, while devices for point-of-care pre-transfusion blood typing have helped to reduce problems of incompatible donor-patient blood type matching in the clinical setting, there is a constant need to improve the efficacy and reduce the cost of such devices to improve outcomes. On the other hand, post-transfusion alloantibody formation is a common and often unavoidable complication in veterinary transfusion medicine. Blood typing alone cannot anticipate such reactions, therefore cross-matching is considered the method of choice for the detection of serological incompatibilities between recipient and donor.

The evaluation of a novel device for point-of-care blood typing for cats is presented in the first part of this dissertation. To date, no studies have been undertaken in veterinary medicine that determine the status of alloantibody presence in either previously transfused or not previously transfused feline patients, neither has a point been determined, at which such patients can potentially develop alloantibodies after their first transfusion. The second part of this dissertation, therefore, reports on a series of cross-match-testing on hospitalised feline patients that received one or more transfusions due to various types of anaemias. It was designed to document the occurrence of a positive cross-match (CM) in feline transfusion patients presented at the Small Animal Clinic of Freie Universität Berlin.

1.1 History of transfusion medicine and transfusion safety

Blood has been recognised as critical to life from the beginning of recorded human history. The first recorded attempts to transfuse blood between animals of the same species were performed in a series of failed experiments by Lower in 1665, and more successfully a year later by the same physician (Starr 1998). Animal to human transfusions quickly followed, in the year 1667, when Denis transfused sheep's blood into a 16-year-old boy in a desperate attempt to heal him of a persistent fever (Cotter 1991; Davidow 2013; Duffin 2010; Hosgood 1990; Starr 1998).

Transfusion reactions were observed, yet not understood, from early on in transfusion medicine's history, which ultimately led to a hiatus in its use as a treatment option in human medicine that lasted until the 19th century (Starr 1998).

As an early measure towards transfusion safety, anticoagulants were introduced in the late 18th century, but their use was risky because the therapeutic range of the substances tested at the time was narrow and concentrations that were perceived as necessary were found to be unsafe (Cotter 1991; Hosgood 1990).

It wasn't until the discovery of blood types in the early 20th century (by Landsteiner) and the determination of a safe acid-citrate-dextrose concentration in the mid-20th century (by Lewisohn) which allowed for routine administration of blood components, along with the identification and reduction of risk factors (Duffin 2010; Starr 1998) that blood transfusions became a routine part of patient care.

In human medicine, various organisations were eventually founded to manage and regulate the business of making blood available to patients worldwide. The Blood Transfusion Betterment Association was founded in the 1920s by physicians such as Landsteiner and Ottenberg in New York City, serving both to organise the donation process and improve transfusion safety. Oliver founded the first reputable donor organisation in London at around the same time. Ijima's Nippon Blood Education Society in Japan and Tzanck's L'Oeuvre Transfusion Sanguine d'Urgence in France followed (Starr 1998).

Blood types for companion animals were first studied in the mid-20th century, which included the discovery and further genetic analysis of the feline AB blood group system by the end of the century (Auer & Bell, 1981; Eyquem et al., 1962; Griot-Wenk et al., 1993; Holmes 1950).

Historical reviews in the 1990s regarded veterinary transfusion medicine to be on par with the practice in human medicine, considering veterinary medicine useful in delivering animal models for human medicine (Cotter 1991; Hosgood 1990). Today, although most current

literature views transfusion medicine as an integral part of critical care and fluid therapy in veterinary medicine (Brown 2012; Giger 2009; Hohenhaus 2012; Kohn & Weingart, 2012), economic and logistical constraints have slowed progress as compared to human medicine.

1.2 Patient safety in transfusion medicine

Risks associated with transfusion procedures are kept to a minimum in human medicine through various organisations that oversee the blood donation and blood-banking processes in addition to establishing protocols and standards for transfusion.

National haemovigilance networks use various types of reporting systems regarding the incidence and possible sources of transfusion reactions and issue recommendations on how to avoid such instances. These include the Serious Hazards of Transfusion (SHOT) UK confidential haemovigilance reporting system in Great Britain (Bolton-Maggs & Cohen, 2013), the French haemovigilance network overseen by the French Blood Agency (Andreu et al., 2002) and the American Red Cross Hemovigilance Program (Eder et al., 2009). At the time of writing, the International Haemovigilance Network lists a total of 29 countries with systems in place that participate in the International Surveillance of Transfusion-Associated Reactions and Events (ISTARE) (‘National & International Haemovigilance Systems’ 2017). Such oversight is not as consistent in veterinary medicine, though recommendations and guidelines abound in the literature (Pennisi et al., 2015; Tocci & Ewing, 2009b; Vap et al., 2012; Wardrop et al., 2016).

1.2.1 Transfusion reactions

The term ‘transfusion reaction’ describes any of a number of complications associated with blood transfusions. These complications are generally divided into two types: first, acute transfusion reactions, which occur within 24 h of administering the blood or blood component and most frequently involve haemolytic or allergic incidences. Second, delayed transfusion reactions, in which a response does not manifest until 24 h post transfusion, such as delayed haemolytic or serologic reactions or the effects of infections. Another method of classification is in terms of the causative agent, that is, an immunological reaction to an antigen, such as those found on red blood cells (RBCs), white blood cells (WBCs) or platelets, or a reaction to an infectious (bacteria, viruses, etc.), physical (air) or chemical (citrate) agent. All blood components have the potential to cause such reactions (Strobel 2004). Human medicine also differentiates between delayed haemolytic and delayed serologic transfusion reactions. The former occurs with altered laboratory findings, while the latter may be detected by routine pre-transfusion antibody screening, but occurs without clinical sings at the time of testing (Centers for Disease Control and Prevention 2017). The incidence of delayed haemolytic transfusion

reactions in human medicine has steadily declined, while delayed serologic transfusion reactions have increased, the reasons having been identified as shorter hospital stays, better testing (in this case, antibody screening) and better differentiation between the two types of delayed transfusion reaction (Ness et al., 1990).

The most serious risks in human transfusion medicine – those which carry the highest mortality rates – are still acute transfusion reactions such as transfusion-associated circulatory overload and transfusion-related acute lung injury (Vamvakas & Blajchman, 2009), as well as haemolytic transfusion reactions (Gilliss et al., 2011). The probability of contracting infectious disease has been significantly lowered in human medicine, while bacterially contaminated units and unknown pathogens that are not screened for still pose a risk. Clerical errors (that lead to mismatched blood) have been reduced through improved labelling, but not all clinically relevant antibodies are detected in routine testing even in human medicine (Vamvakas & Blajchman, 2009).

Transfusion reactions in companion animals have likewise been studied, noting both acute and delayed immunological (haemolytic, non-haemolytic, febrile) and non-immunological (infectious, circulatory, mishandling) adverse events (Weinstein 2010). Table 1 provides an overview of transfusion reactions in dogs and cats based on the current research (Kohn & Weingart, 2014; Kohn 2011; Yagi & Holowaychuk, 2016).

An early study on transfusions in dogs reported on haemolytic transfusion reactions in 13% of dogs (8% acute and 5% delayed, determined by clinical signs as shown in Table 1), but notes that most of those could have been avoided, had cross-matching been part of routine pre-transfusion testing (Kerl & Hohenhaus, 1993). A later study on dogs reports a reduced rate of acute transfusion reactions of 3.9%, despite blood typing and cross-matching (Reitemeyer et al., 2000). While symptoms like vomiting, fever, facial oedema and acute haemolytic transfusion reactions have been reported in the past (Reitemeyer et al., 2000), the most common adverse events today in dogs appear to be transfusion associated circulatory overload and non-haemolytic febrile reactions (Davidow 2013). In a recent study on canine transfusion reactions, however, the rate of transfusion reactions was reported to be as high as 38% of dogs, possibly owing to an increase in the use of blood products other than packed RBCs, as well as better, more differentiated patient monitoring (the study reports 85% of all transfusion reactions having been febrile non-haemolytic, while 8.3% were acute haemolytic, 71.7% were non-specified delayed and 1.7% were anaphylactic) (Holowaychuk et al., 2014).

Regarding cats, an early study had established that mismatched transfusions may lead to transfusion reactions in cats, particularly in type B cats, in which high anti-A antibody titres lead to stronger transfusion reactions (Auer & Bell, 1983). The first recommendation of pre-

transfusion testing as an integral part of feline transfusion medicine, however, was published as late as 1990 in a report of a transfusion reaction subsequent to the administration of unmatched blood to a type B cat (Giger & Akol, 1990). In a recent review, transfusion reactions in cats that had received typed blood were reported at 3% (Barfield & Adamantos, 2011). Indeed, transfusion reactions in cats remain rare: 4% as reported in one study, where reactions were acute febrile (n = 2) and acute fatal (1) due to mismatching (Castellanos et al., 2004). Another study recorded acute transfusion reactions in 1.2% of the cases (Weingart et al., 2004). Finally, the highest number of acute transfusion reactions in cats was recorded at 9% (11/126), 9 cases of which were self-limiting (Klaser et al., 2005). Types of acute transfusion reactions in cats include nausea, fever, oedema and tachypnoea and have been reported to occur despite prior pre-transfusion testing (including cross-matching) (Weingart 2003).

The occurrence of delayed haemolytic transfusion reactions in cats is discussed in the literature, and it has been postulated that a reduction in Hct subsequent to a transfusion might have been misinterpreted as being part of the underlying disease (Marion & Smith, 1983; Weingart 2003). Delayed transfusion reactions may also be underreported due to the retrospective nature of such studies (Klaser et al., 2005). Delayed haemolytic transfusion reactions in cats should be suspected in cases where the Hct does not increase as expected. Formulae for predicting increases in Hct based on patient weight and transfused volume have been studied in human paediatrics (Davies et al., 2007; Glatstein et al., 2005) and adapted for veterinary medicine, also serving as a way to assess transfusion efficacy (Griot-Wenk & Giger, 1995; Reed et al., 2014; Weingart et al., 2004).

Table 1 Overview of transfusion reactions for cats and dogs with corresponding clinical signs and possible causes. Adapted from: Kohn & Weingart, 2014; Kohn 2011; Yagi & Holowaychuk, 2016.

Type of reaction	Transfusion reaction/ clinical signs	Causes
Acute immunologic	Haemoglobinaemia, haemoglobinuria due to antigen-antibody reaction Fever, vomiting, tachycardia, tachypnoea, shock, DIC, sudden death	Haemolysis due to prior sensitisation, or due to AB mismatched blood in cats
	Oedema, urticaria, pruritus, fever, anaphylaxis	Immunoglobulin E-mediated immunologic reaction to white blood cells, platelets cytokines and other blood components
Delayed immunologic	Post-transfusion purpura	Development of antibodies against thrombocytes
	Icterus, positive Coombs test, haemolysis	Post-transfusion alloimmunisation
	None	Serologic transfusion reaction against alloantibodies detected through cross-matching only
Acute non-immunologic	Bacteraemia, sepsis, fever	Bacterial contamination of blood
	Heart failure, dyspnoea	Circulatory overload
	Haemolysis	Improper collection, storage or administration
	Vomiting	Feeding while administering a transfusion, improperly high drip rate
	Hypocalcaemia	Citrate overload from massive transfusions
	Hypothermia	Administration of inadequately warmed massive transfusions
	Hyperammonaemia	Accumulation in packed RBC units or whole blood during storage
	Hyperkalaemia	RBC damage during storage
	Air embolism	Improper administration technique
Delayed non-immunologic	Infectious disease such as FeLV, FIV, haemotropic bacteria and *Bartonella* sp. for cats and *A. phagocytophylym*, Babesia spp. and *Leishmania* spp. for dogs	Contaminated donor blood

1.2.2 Alloimmunisation

Humans (Strobel 2004), horses (Brown 2012) and cats (Bücheler & Giger, 1993) possess clinically relevant preformed antibodies against blood group antigens which are not their own, also called iso- or alloantibodies. This makes pre-transfusion blood typing mandatory for those species. Alloimmunisation, that is, the development of acquired alloantibodies, on the other hand, occurs when an individual is sensitised by antigens from another individual of the same species, most often occurring as a result of iatrogenic intervention (Zimring et al., 2011).

Alloimmunisation in humans has been studied extensively and the risk of developing such post transfusion alloantibodies has been reported at 2–30% (Abou-Elella et al., 1995; Ameen et al., 2009; Tormey et al., 2008) and it is assumed that alloantibodies are formed approximately 7–10 days after a prior transfusion in humans (Strobel 2004). Such antibodies may also be pregnancy-induced, as it has been described in humans (Zimring et al., 2011) and is an important factor for blood donor screening (Evanovitch 2012). It has also been reported that the recipient's sex, as well as the transfusion protocol used, may have an effect on alloimmunisation in humans (Ameen et al., 2009).

The development of alloantibodies remains a cause of potentially fatal haemolytic transfusion reactions in humans (Tormey et al., 2008; Vamvakas & Blajchman, 2009). A primary concern in transfusion medicine is therefore that of avoiding alloimmunisation and working towards finding new ways of avoiding the presence of acquired alloantibodies (Zimring et al., 2011).

Post-transfusion alloimmunisation has been documented in horses, where half of the study population developed transfusion-associated RBC antibodies after only one transfusion (Wong et al., 1986). Even though dogs do not have pre-formed isoantibodies (matching the clinically relevant blood types), antibody formation upon transfusion has been reported for dogs (Callan et al., 1995; Giger et al., 1995), therefore it has become common practice to perform pre-transfusion testing as a means to match transfusions, thereby avoiding premature sensitisation (Giger 2009). Gestation-induced alloimmunisation has been problematised in veterinary medicine, but no evidence has been found that confirms any such sensitisation in a study on dogs (Blais et al., 2009).

Neonatal isoerythrolysis (NI) on the other hand, a serious condition in which maternally-derived alloantibodies lead to RBC-destruction in neonates, is a concern in kittens and foals (Becht et al., 1983; Jonsson et al., 1990; Stormont 1975). Various studies have supported the wide use of blood typing in cats just because of this phenomenon (Griot-Wenk et al., 1993; Silvestre-Ferreira & Pastor, 2010; Streicher 2009).

Autoantibody-formation, that is, the development or existence of antibodies against the body's own RBCs is described in infections such as haemobartonellosis, as well as autoimmune disease such as systemic lupus erythematosus and immune-mediated haemolytic anaemia (IMHA), and it may also happen subsequent to transfusions (Hohenhaus 2004; Zumberg et al., 2001). It can complicate pre-transfusion testing, interfering with some tests' ability to produce correct results, as auto-agglutination may suggest a positive result where there is none (Brown 2012; Hohenhaus 2004).

Post-transfusion alloimmunisation is assumed in cats (Weingart et al., 2004) but has so far not been studied systematically.

1.2.3 Strategies to reduce the risks associated with transfusions

Transfusion reactions of various aetiologies contribute to morbidity and therefore constitute the main risk factor in transfusion medicine. Maintaining safety for patients in transfusion medicine has become a multi-level process that includes sound blood-banking practices, donor health screening, pre-transfusion testing of donors and recipients, selection of appropriate blood components and volumes, monitoring of patients and recording of adverse events (Feldman & Kristensen, 1995; Davidow 2013). Standards in veterinary medicine and veterinary clinical pathology are followed according to peer-reviewed quality control and quality assurance guidelines (Tocci & Ewing, 2009; Tocci 2010). The British Small Animal Veterinary Association (BSAVA) manuals series includes the volume Manual of Canine and Feline Haematology and Transfusion Medicine (Day 2012) and the most recent contribution is the comprehensive Manual of Veterinary Transfusion Medicine and Blood Banking (Yagi & Holowaychuk, 2016). A consensus statement was also issued in 2005 and revised in 2016 by the American College of Veterinary Internal Medicine and the Association of Veterinary Hematology and Transfusion Medicine on recommended donor screening protocols (Wardrop et al., 2005; 2016), in addition to the guidelines published by the European Advisory Board on Cat Diseases (ABCD) (Pennisi et al., 2015). Finally, information on pre-analytical factors such as collecting blood samples, as well as handling them and storing them has been published by the American Society for Veterinary Clinical Pathology in a set of detailed recommendations (Vap et al., 2012).

1.2.3.1 Pre-transfusion testing

Pre-transfusion testing in the early days included the, now obsolete, so-called biological test (injecting small amounts of the donor blood into the recipient) or a simple, yet time-consuming CM-test (Cotter 1991; Griot-Wenk & Giger, 1995). In human medicine today, pre-transfusion testing is treated as part of a complex including the upholding of standards and regulations, correct patient identification and history, paying stringent attention to sample labelling and

making the right choices regarding screening and follow-up laboratory testing. It is thus ensured that patients are properly identified, all medical records are taken into account and the best possible match is found for the patient to be transfused. It includes AB0 and Rh, as well as antibody-screening with additional testing such as computerised cross-matching, when initial screening suggests further tests (Evanovitch 2012).

Pre-transfusion testing in veterinary medicine is limited to manual laboratory methods and a few point-of-care devices. Routine pre-transfusion testing in small animal care has therefore been greatly facilitated in recent years thanks to the emergence of such point-of care devices. This has had a positive effect on both time concerns in emergency situations, as well as ease-of-use and cost (Kessler et al., 2010; Kohn, Niggemeier et al., 1997; Proverbio et al., 2011; Seth et al., 2011; Stieger et al., 2005). While in dogs a first non-compatible transfusion was known to be possible but discouraged out of concern for sensitisation or alloimmunisation, pre-transfusion testing for patient-donor compatibility is always necessary in cats and in horses because of their preformed antibodies (Griot-Wenk & Giger, 1995; Hohenhaus 2004; Owens et al., 2008).

1.2.3.2 Blood typing

The ability of reagents to bind to red blood cell surface antigens is the basis for the various blood typing methods developed for use in companion animals. They range from laboratory methods that require special materials, equipment and technical skill, to a number of point-of-care devices developed for use in general practice. The point-of-care tests for companion animals include blood typing cards (DMS Laboratories) and a gel column assay (Diamed). However, the former has some reliability issues (Brown 2012; Kohn et al., 1997), and the latter is no longer marketed, presumably due to the high cost of purchasing the required specialised centrifuge (Brown 2012). Finally, immunochromatographic point-of-care testing kits that use monoclonal antibodies have been recently developed and evaluated for both dogs and cats (Alvedia and DMS Laboratories), making blood typing in most settings fast, easy and reliable (Kohn et al., 2012; Seth et al., 2011; Spada et al., 2015). The various blood typing systems available for cats are presented in section 1.3.3, below.

1.2.3.3 Cross-matching

Cross-matching tests for incompatibilities based on naturally occurring or induced antibodies in donor and recipient blood. The major cross-match (MCM) checks the recipient’s plasma for the existence of antibodies against donor RBCs, while the minor CM uses donor plasma to check for antibodies against the recipient’s RBCs (Giger 2009). Traditionally, the so-called serologic CM involved manually mixing RBC suspensions with plasma or serum (Sandler &

Abedalthagafi, 2009). In human medicine, both cold and warm-phases are used and the indirect Coombs test is also part of standard cross-matching protocols. However, in human medicine cross-matching has been largely supplanted by the more efficient antibody screening, for which commercial kits have been available since the 1960s. Computerised cross-matching may also soon eliminate manual laboratory methods, thus enabling fast routine pre-transfusion testing for blood group and serologic incompatibilities. The current consensus in human medicine is that only if a recipient displays a positive antibody screen does it become necessary to resort to the more expensive and time consuming full manual CM (Evanovitch 2012; Sandler & Abedalthagafi, 2009).

In veterinary medicine, by contrast, manual or point-of-care cross-matching are the only other laboratory methods, along with blood typing, sound blood-banking techniques and donor screening, with which patient safety can be ensured prior to a transfusion. Both the simple slide and tube method of cross-matching are in use. In its full variant, the tube-based cross-matching technique used in veterinary medicine is a multi-step method using a 37°C-phase of detecting serologic donor-recipient incompatibilities that requires specialised laboratory personnel and can take more than 30 mins (Giger 2009; Stieger et al., 2005; Tocci 2010). Point-of-care kits are also on the market (by both Alvedia and DMS Laboratories) and Diamed also used to offer a gel-cartridge method for its proprietary transfusion-testing centrifuge (Tocci 2010). The Coombs test is currently not part of standardised protocols in veterinary medicine, neither is a phase that accounts for cold (4°C)-reacting antibodies, as is the case in human medicine (Brown 2012; Tocci & Ewing, 2009).

Veterinary medicine, more so than human medicine, must contend with the existence of unknown blood types or blood types for which there are no testing methods, therefore cross-matching has the clear advantage of accounting for donor-recipient incompatibilities that lie outside of the known blood typing systems (Brown 2012; Davidow 2013; Griot-Wenk & Giger, 1995).

The various species for which cross-matching is performed, possess varying titres and classes of antibodies. Therefore, the type of cross-matching chosen in veterinary medicine also depends on the species to be tested. Agglutinating and haemolysing techniques are available, as well as those taking advantage of complement. While methods that rely on agglutinating antibodies are good for feline and canine testing, they would not suffice for use in horses, a species in which both agglutinating and haemolysing antibodies are present. Testing with complement is, further, necessary for ruminants, since they hardly possess any agglutinating antibodies (Brown 2012).

While it is the case that pregnancy or a transfusion in the previous 6 months mandates cross-matching before transfusions in human medicine (Evanovitch 2012; Strobel 2004), the recommendations in veterinary medicine are less restrictive. Cross-matching for dogs is recommended in the most recent literature to be performed if no transfusion history is available, if there is a history of transfusion reactions and if the last transfusion was more than 4–7 days ago. It is also recommended if the donor's DEA 7 blood type is not known. Pregnancy is not considered a precondition for cross-matching, as no evidence for pregnancy-induced alloantibodies has been found (Blais et al., 2009; Brown 2012; Davidow 2013).

Factors that can, in rare cases, influence cross-matching results are infectious status and degree of anaemia (Giger 2009; Griot-Wenk & Giger, 1995). Persistent auto-agglutination will prevent correct readings, and for best results, samples should be neither lipaemic nor strongly haemolytic (Brown 2012; Giger 2009; Kohn & Weingart, 2009). Cross-matching results may also be affected by the age of samples used, as this has been reported for such testing in horses (Harris et al., 2012). In general, samples for cross-matching should not be older than 24 h, but exceptions are made for segments from blood units (Vap et al., 2012).

The current consensus in veterinary medicine appears to be that cross-matching will become the norm as standardised testing becomes more widely available (Giger 2009). The availability of point-of-care tests would also mitigate the financial and time costs of cross-matching that have been raised in the past as a concern for veterinary practice (Kerl & Hohenhaus, 1993).

Finally, cross-matching should not be expected to completely eliminate the risk of transfusion reactions, as they are reported in previously cross-matched recipient-donor combinations both in veterinary and human medicine (Hurcombe et al., 2007; Strobel 2004; Weingart et al., 2004; Weinstein 2010)

1.3 Considerations specific to feline transfusion medicine

Feline medicine has a lower frequency of blood transfusions as compared to canine medicine, which can be attributed, in part, to the slightly higher risk for donors (who must be sedated for the procedure), its limitation by small volumes and the lack of availability of commercial closed systems for blood unit collection (Hohenhaus 2012). Basic standards of transfusion medicine have nevertheless been described for cats, and feline transfusions have increased significantly since their establishment in veterinary practice, as one earlier study reports (Griot-Wenk & Giger, 1995). Studies and review articles have followed, establishing transfusion medicine as an important part of feline medicine (Barfield & Adamantos, 2011; Kisielewicz & Self, 2014; Kohn & Weingart, 2009; Pennisi et al., 2015; Weingart et al., 2004).

1.3.1 Feline blood types

After initial descriptions of (clinically relevant) isoagglutinins found in cats, but not in dogs, early foundational studies on feline blood groups recognised at least three different RBC antigens by cross-agglutinating RBCs and serum from three groups of cats (Eyquem et al., 1962; Holmes 1950, 1953). Such antigens were found on liver and spleen cells. At the time, similarities with certain human blood types were also proposed. Later reports consolidated the results from the previous decades and established the feline AB blood typing system as it is still recognised today (Auer & Bell, 1981). Having performed serological tests on cats, they determined that the feline blood typing system was, in fact, not serologically related to the human AB0 system and that the presence of subgroups could not be determined; they also discovered that a third phenotype existed, namely the AB blood type with both A and B antigens present on the RBCs (Auer & Bell, 1981).

About a decade later, the first reports on the biochemical basis for feline blood types were published, determining the specific membrane lipids, gangliosides with their sialic acid residues, that constitute feline AB blood type antigens. Blood type A is thus associated with N-glycolylneuraminic acid (NeuGc) and small amounts of N-acetylneuraminic acid (NeuAc), and blood type B only with NeuAc, while blood type AB possesses both antigens at roughly equal parts (Andrews et al., 1992; Butler et al., 1991; Griot-Wenk et al., 1993; Lehninger et al., 2005). The enzyme cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH), which converts NeuAc to NeuGc is responsible for controlling blood types in mammals, and its absence, malfunction or mutation is thought to cause the presence of blood type B and AB in cats (Andrews et al., 1992; Bighignoli et al., 2007; Gandolfi et al., 2016).

Serologically speaking, RBC antibodies in type A cats were found to be weak haemagglutinins of the IgM class and weak haemolysins of the IgG and IgM classes, both at equal proportions. The antibodies in B type cats are strong haemagglutinins and haemolysins of the IgM class (Bücheler & Giger, 1993). While RBC antigens can be detected in the feline foetus as early as 38 days (Auer & Bell, 1981), it is not until 6–8 weeks of age that isoantibodies can be detected in kittens and 12 weeks of age that they reach adult levels (Bücheler & Giger, 1993). However, isoantibodies, that is, naturally occurring maternal antibodies, are transmitted to kittens while ingesting colostrum even before they form them on their own, which explains the phenomenon of feline NI, when B queens are allowed to mate with A or AB toms (Casal & Giger, 1996; Giger & Casal, 1997).

Standard blood typing methods only detect phenotype, not genotype, which was understood early on to be an issue for breeders who need to avoid pairings that may lead to feline NI (Griot-Wenk et al., 1993). Genetically speaking, the three blood types in the AB blood group

system were assumed to correspond to at least two alleles. Early research determined that an A allele exists, which is dominant, while another allele corresponding to the type B phenotype is recessive; thus allowing for homozygous and heterozygous expressions of the type A phenotype and homozygous-only expressions of the type B phenotype (Giger et al., 1991). The allele for blood type AB was later found to be recessive towards the A allele and dominant over the B allele, allowing for homozygous and heterozygous expressions of the AB phenotype (Griot-Wenk et al., 1996). While it is still not entirely understood how the inheritance of the AB phenotype takes place, more recent studies using genomic and cDNA sequencing have proposed that three alleles are involved, with the following relationship in terms of hereditary dominance: A > a^{ab}> b. Genotype/ phenotype correspondences are thus proposed to be as follows: AA (type A), Aa^{ab} (type A) and Ab (type A); $a^{ab}b$ (type AB) and $a^{ab}a^{ab}$ (type AB); and bb (type B) (Bighignoli et al., 2007). Most importantly, this research has led to the development of commercial genetic testing for the b genotype, allowing breeders to make prudent mating decisions (Bighignoli et al., 2007; Tasker et al., 2014).

Distribution of feline AB blood types varies according to breed and geographic location. Therefore, studies have been and continue to be conducted in various locations to determine blood types among local cat populations (Table 2). Knowing the prevalence of blood types allows breeders and clinicians to assess the risk involved in mating particular animals with each other and in practicing transfusion medicine. Distribution of blood types within some pedigree breeds appears to be consistent regardless of region (Yagi & Holowaychuk, 2016), while variations in some populations do depend on geographic location, as well as the time at which a particular study was conducted (Day 2012). One study conducted a risk estimation for the occurrence of blood type B for various popular pedigree breeds. According to this estimation, the breed with the highest risk of having individuals with blood type B is British Shorthair at 0.77, followed by Devon Rex (0.66), Persian (0.49), Somali (0.47), Himalayan (0.45), Abyssinian (0.45), Birman (0.42) and Scottish Fold (0.39). According to this study, the overall estimate for domestic shorthair cats to test positive for blood type B is 0.05 (Giger et al., 1991). Other pedigree breeds, like Burmese, Siamese and Tonkinese appear to include only blood type A animals (Giger et al., 1991; Yagi & Holowaychuk, 2016). Studies on domestic non-pedigree cats report the prevalence of blood type A at a range of 100 to 62%, blood type B from 36 to 0% and type AB from 9.2 to 0% in various countries (Table 2).

Table 2 Overview of studies on the distribution of feline blood types in domestic shorthair cats by geographic location

Region or Country	Author, year of publication	Distribution of blood types (%)		
		A	B	AB
Australia (Brisbane)	(Auer & Bell, 1981)	73.3	26.3	0.4
Australia (Sydney)	(Malik et al., 2005)	62.0	36.0	1.6
Brazil (Rio de Janeiro)	(Medeiros et al., 2008)	94.8	2.9	2.3
Canada	(Fosset & Blais, 2014)	94.4	5.0	0.6
China (Beijing)	(Zheng et al., 2011)	88.2	11.4	0.4
Denmark (Copenhagen)	(Jensen et al., 1994)	98.1	1.9	0
France	(Eyquem et al., 1962)	85.0	15.0	0
Germany	(Haarer & Grünbaum, 1993)	94.1	5.9	0
Germany (Berlin/ Brandenburg)	(Weingart et al., 2006)	98.7	1.1	0.2
Great Britain	(Knottenbelt et al., 1999)	83.7	14	2.3
Greece	(Mylonakis et al., 2001)	78.3	20.3	1.4
Hungary	(Bagdi et al., 2001)	100	0	0
India (Mumbai)	(Dahanukar et al., 2001)	88.0	12.0	0
Israel	(Merbl et al., 2011)	69.5	16.0	14.5
Japan	(Ikemoto et al., 1981)	90.3	9.7	0
Japan (Tokyo)	(Ejima et al., 1986)	90.0	0.8	9.2
New Zealand	(Cattin 2016)	85.3	13.9	0.8
Portugal (Lisbon)	(Marques et al., 2011)	97.5	2.1	0.4
Portugal (North)	(Silvestre-Ferreira et al., 2004)	89.3	4.4	6.3
Spain (Barcelona)	(Ruiz de Gopegui et al., 2004)	94.0	5.0	1.0
Spain (Gran Canaria)	(Silvestre-Ferreira et al., 2004)	88.7	7.2	4.1
Sweden	(Sköld 2013)	98.1	0	1.9
Switzerland	(Hubler et al., 1993)	99.6	0.4	0
Turkey	(Arikan et al., 2006)	73.1	24.6	2.3
United Kingdom (South East)	(Forcada et al., 2007)	67.6	30.5	1.9
United States	(Giger et al., 1991)	99.7	0.3	0
United States (New York City)	(Klaser et al., 2005)	94	6	0

An important study confirmed prior suspicions and pointed to the existence of clinically relevant blood types outside of the AB system by describing the *Mik* RBC antigen and the corresponding naturally occurring anti-*Mik* antibodies in *Mik*-negative individuals. The study reported on a renal transplant patient who had been given a transfusion that turned out to be

Mik-positive and elicited an acute haemolytic transfusion reaction because the patient was *Mik*-negative (Weinstein et al., 2007). A recent British study, on the other hand, failed to find evidence of non-AB incompatibilities and the authors question the necessity for routine cross-matching (Tasker et al., 2014).

1.3.2 Feline blood donors

Candidates for blood donations should be clinically healthy without being on any medications; they should have a weight (ideally above 5 kg) that allows for the collection of adequate amounts of blood with few side effects from the donation process and a routine blood panel is recommended prior to donations, particularly in infrequent donors (Barfield & Adamantos, 2011; Kohn & Weingart, 2012). Species-specific infectious disease screening is an important aspect of transfusion safety (Pennisi et al., 2015; Wardrop et al., 2016). Infectious disease distribution is, in part, contingent on location, so cost-risk assessment for the prevention of transfusion-transmitted diseases must be adapted and blood donors vaccinated and assessed accordingly (Barfield & Adamantos, 2011). Therefore, the recommendations regarding optimal and minimum donor screening may vary, depending on the committee or panel through which they were issued. For example, the European ABCD guidelines may be consulted for assessing the status of *Bordetella bronchiseptica* (Egberink et al., 2009), *Chlamydophila felis* (Gruffydd-Jones et al., 2009), feline calicivirus (Radford et al., 2009), feline herpesvirus (Thiry et al., 2009), FIV (Hosie et al., 2009), FIP (Addie et al., 2009), FeLV (Lutz et al., 2009), feline panleukopenia virus (Truyen et al., 2009) and rabies (Frymus et al., 2009). The most current ABCD guidelines recommend core screening for FeLV, FIV, *Bartonella* species and feline haemoplasma (Pennisi et al., 2015).

The most recent consensus statement by the American College of Veterinary Internal Medicine and the Association of Veterinary Hematology and Transfusion Medicine includes optimal and minimal standards of screening for feline donors, the latter being a concession to geographic variation, as well as cost and logistical considerations. According to the minimal standards issued there, PCR-testing for *Anaplasma phagocytophilum, Bartonella henselae* and *Mycoplasma haemofelis*, as well as rapid immunoassay tests for FeLV (antigen) and FIV (antibody) are advised (Wardrop et al., 2016). Because no screening program can provide absolute safety, it is best ensured that feline donors are indoor-only pets, in addition to using routine ectoparasite treatment to reduce exposure to blood-borne and vector-borne pathogens (Gary et al., 2006; Kohn & Weingart, 2012; Pennisi et al., 2015)

1.3.3 Blood typing in cats

Type A cats have a low titre of weakly agglutinating anti-B antibodies, as well as a low titre of haemolysing antibodies. Type AB cat serum has been reported to have neither haemolysins nor agglutinins. Type B cats, on the other hand, have high titres of both, which makes them considerably more susceptible to acute transfusion reactions (Bücheler & Giger, 1993)

Pre-transfusion testing was thus understood to be important early on in feline transfusion medicine. However, until the late 1990s only specialised laboratory methods were in use to help veterinarians identify a cat's blood type before transfusions or to prevent feline isoerythrolysis. The reagents used in feline blood typing were pre-treated and filtered antisera from type A and B cats until the early 1990s. Since type A serum contains low anti-B titres, it was supplanted by a more potent plant-derived reagent. Studies in human medicine had already discovered that certain plant-extracts, coined lectins (later expanded to include other substances) acted as hemagglutinins. As a result, a study examined a variety of such lectins as stand-ins for the anti-B reagent native to cats. A lectin derived from *Triticum vulgaris* was determined to be the most consistent of the reagents tested since it agglutinated best with the N-acetylneuraminic acid found on type B RBCs (Butler et al., 1991). The first study to report the use of alternative reagents was published in 1993 (Griot-Wenk et al., 1993). *Triticum vulgaris* lectin has been in use as a reagent at a concentration of 64 µg/ml in laboratory testing (Stieger et al., 2005).

The laboratory methods in use today are a slide method, in which standard anti-A and anti-B reagents (usually prepared type B serum and lectin, respectively) are mixed with patient blood on separate slides, then mixed and immediately analysed for agglutination. Additionally, a 'back-typing' test is recommended, so that blood type may be confirmed: in this test, RBCs from a cat where the blood type is known are mixed with the patient's plasma, agglutination with the opposite blood type confirms the findings (Griot-Wenk & Giger, 1995; Kohn et al., 1997; Stieger et al., 2005). In practice, this test is most reliable for confirming blood type B with the RBCs of a known type A cat, owing to the low antibody titres in type B cats (Kohn et al., 1997). The tube method is also based on the same principles and reagents but requires washing the RBC-suspension, a room-temperature incubation phase and microscopic evaluation, in addition to 'back-typing' (Kohn et al., 1997; Stieger et al., 2005).

The early card-based point-of-care method in feline pre-transfusion testing employed lyophilised anti-A antibodies (from feline serum) and lyophilised anti-B solution (lectin). It showed good agreement with laboratory methods with A and B blood types (Knottenbelt et al., 1999) but had some difficulty typing AB samples, as well as restrictions for patients with certain diseases, issues with the prozone effect (false negative results due to antibody-interference,

(Brown 2012)) and difficulty assessing agglutination in severely anaemic patients (Kohn et al., 1997; Stieger et al., 2005). Gel-based tube assays adapted from human medicine were developed for cats and showed promising agreement rates and ease of use, but were not widely successful, for cost reasons noted above (Brown 2012; Giger 2009; Kohn & Weingart, 2009; Proverbio et al., 2011; Stieger et al., 2005). More recently, immunochromatographic devices have also been developed for cats and have been evaluated in a few studies (Seth et al., 2011; Spada et al., 2015). Table 3 provides an overview of point-of-care devices available for feline blood typing.

Table 3 Overview of point-of-care test kits for the feline AB blood group system, with corresponding photographs and list of testing methods, reagents and additional comments regarding practical use. Photographs 2, 3 and 5 (numbered in descending order) taken by author, 1 by lab technician A. Mittag, 5 by Dr. C. Weingart.

Name	Photograph	Testing method	Reagents	Comments	Source
Alvedia Lab Test A + B		Immunochromatographic cell-capture strips in bulk	Monoclonal antibodies on test strip; diluent	Requires simple additional laboratory equipment Test results are read by operator	Alvedia Quick Test A + B, 2016 [package insert]
Alvedia Quick Test A + B		Immunochromatographic cell-capture strip in open single-use cartridge	Monoclonal antibodies on test strip; diluent	All-in-one point-of-care test Test results are read by operator	Alvedia Lab Test A + B, 2013 [package insert]
DiaMed ID-Gel Test Feline Anti A + B Typing		Single-use gel matrix immuno-chromatographic tube assay	Monoclonal antibodies suspended in gel; diluent	No longer on the market; required proprietary centrifuge to perform test Test results are read by operator	ID-Gel Test Feline Anti A + B Typing [package insert]
RapidVet-H Feline, DMS Laboratories		Single-use paper cards with test wells	Lyophilised monoclonal antibodies and *T. vulgaris* lectin; diluent	All-in-one point-of-care test Test results are read by operator	QuickVet/ RapidVet Feline Blood Typing Test, 2015 [package insert]
RapidVet-H IC Feline, DMS Laboratories		Immunochromatographic cell-capture strips in closed single-use cartridge	Monoclonal antibodies on test strip; diluent	All-in-one point-of-care test Test results are read by operator	RapidVet-H Feline, 2012 [package insert]
Quickvet Feline Blood Typing		Capillary driven micro fluidic technology within closed single-use cartridge	Monoclonal antibodies in cartridge; diluent	All-in-one point-of-care test Requires separate multipurpose computerised analyser to read test	RapidVet-H IC Feline, 2012 [package insert]

1.3.4 Cross-matching in cats

The mechanism of cross-matching for cats does not differ from that for dogs. No special solutions are needed. The point-of-care kit available for cross-matching may be used for both species (Tocci & Ewing, 2009). The general recommendations for cats regarding pre-transfusion cross-matching are slightly stricter than for dogs: because of the discovery of the new blood type *Mik,* some authors postulate that, barring the existence of blood typing methods for the *Mik* antigen and other, unknown antigens, cross-matching ought to be performed prior to every feline transfusion (Davidow 2013; Giger 2009; Weinstein et al., 2007).

Whereas the simple slide CM method is a procedure that can be used in most day-to-day practices in feline transfusion medicine, recommendations vary as to its value. On the one hand, it is generally recommended for cats because it shows good results for the AB blood group system (Brown 2012; Giger 2009; Griot-Wenk & Giger, 1995; Kohn & Weingart, 2009). On the other hand, the tube method is considered preferable because the slide method may not reliably detect unknown RBC-antigens such as the *Mik*-antigen (Kohn & Weingart, 2012). Cross-matching should, further, not be used to indirectly determine blood type, since the existence of unknown RBC-antigens like *Mik* might falsify such tests (Weinstein et al., 2007). The simple slide CM may still be a useful test in cases where the more reliable tube method is unavailable (Yagi & Holowaychuk, 2016). Finally, FeLV may be implicated in incorrect CM results in cats and should be considered when cross-matching affected patients (Griot-Wenk & Giger, 1995).

1.4 Significance of the work described here

The work described in this dissertation examines two facets of the overall complex of transfusion medicine for feline patients: namely, the efficacy of a novel and potentially economic immunochromatographic device for point-of-care blood-typing of feline patients, and, second, the window in which alloimmunity develops post transfusion, the determination of which can help in developing guidelines for the inclusion of cross-matching in pre-transfusion screening tests.

2 Publication I

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Original Article

Evaluation of a novel feline AB blood typing device

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2.1 Abstract

This prospective study evaluated a novel immunochromatographic (IC) blood typing test for the AB blood group system. Typing was conducted comparatively on ethylenediamine tetra-acetic acid-anticoagulated blood samples from 89 sick and 16 healthy cats with the IC test, as well as two tests as reference methods, a tube agglutination and a gel column test. The samples were between 0 and 10 days old (median 3 days) and were tested for haemolysis and agglutination; the packed cell volume ranged from 0.07 to 0.57 l/l (median 0.40 l/l). The reference methods agreed with each other in 100% of the test runs. Of the 85 samples tested as blood type A by the two reference methods, 80 were correctly identified by the IC test, four were misidentified as AB and one was rated inconclusive. All B samples were correctly typed. Two of the three AB samples were correctly identified by the IC test and one was rated inconclusive. The sample quality had no influence on test performance. Of 30 repeats, 28 were readable and showed agreement in 27 cases. The agreement of the IC test with the control methods was 96.1% for the 103 conclusive tests, and it showed high sensitivity and specificity for A and B antigen detection. It is suggested that AB results be reconfirmed with a laboratory method and that a 'back-typing' be performed with plasma from B samples to detect the presence of alloantibodies. Given its very good performance and ease of use, the IC test can be recommended for clinical settings.

2.2 Introduction

The feline AB blood group system was first described over three decades ago[1] and is associated with two erythrocyte antigens. Blood type A is linked to N-glycolylneuraminic acid and, to a much lesser extent, to N-acetylneuraminic acid. While the latter is linked to type B, cats with blood type AB carry both erythrocyte antigens.[2–4] Identifying these blood types has become the standard in feline transfusion medicine as it plays a significant role in reducing the incidence of transfusion reactions.[5–9] Blood typing also plays an important role in breeding programmes, as mating type B queens with type A or AB toms is linked to the rare, but potentially fatal, feline neonatal isoerythrolysis.[10–13] To this end, both laboratory and point-of-care testing methods for the AB blood group system are available to veterinary laboratories and practices.

The available blood typing methods for the feline AB blood group system have been evaluated in a number of studies.[14–18] The slide and tube agglutination methods are mainly used in specialised laboratories and clinics; the tube method is often relied on as the standard method.[15, 18] One commercially available point-of-care test is supplied in a card format with lyophilised anti-A and anti-B reagent wells in addition to a well that screens for auto-agglutination. A newer point-of-care test kit is based on immunochromatographic (IC) sample migration along a single membrane that contains bands with monoclonal antibodies for antigens A and B, as well as a control band. A multicolumn cartridge system with gel matrices (GEL, ID-Gel Test Feline Anti A + B Typing, Diamed) into which monoclonal anti-A and anti-B antibodies are embedded was a reliable method[15] but is no longer on the market at the time of writing.

This study was performed to assess the agreement with the reference methods and to evaluate the ease of use in everyday practice of a novel feline AB blood typing device, the RapidVet-H IC Feline test (DMS Laboratories). This point-of-care test was recently developed as an all-in-one kit and is based on IC sample migration technology. Here, the IC test is compared with a tube agglutination assay and the GEL test as reference methods.

2.3 Materials and methods

2.3.1 Blood samples

Ethylenediamine tetra-acetic acid (EDTA)-anticoagulated blood samples are routinely collected for diagnostic purposes at the Small Animal Clinic of Freie Universität Berlin from feline patients and blood donors. Unused remnants of these blood samples were included in this prospective study. Owner consent to use these blood samples for scientific purposes is routinely given at the intake examination every time a patient is first seen at the clinic, therefore

no further approval for this study was needed. Eighty-nine such samples were used in this study and 16 samples were provided by outside laboratories. In order to determine sample quality, each sample's packed cell volume (PCV) was measured using microcapillaries that were centrifuged for 5 mins at 14,926 g. The same microcapillary was used to test for haemolysis on the following scale: no haemolysis visible to the human eye (0) to haemolysis that does not allow for visual differentiation of red blood cells (RBC) from plasma (4+). A drop of the sample was placed on a slide to detect the presence of agglutination and was assessed on the following scale: no agglutinates (0) to 1–2 large agglutinates with clear plasma (4+). Both scales were adapted from a previous study.[15] The same investigator tested all of the samples, including quality assessment and typing, and another conducted a blind analysis of the IC test results. A total of 30 repeats were performed as part of the evaluation process, 22 and eight of which were same day and next day repeats, respectively.

2.3.2 Typing methods and equipment

Single-use, sterile laboratory equipment (tubes, slides, pipettes, etc) was used throughout. This study used 135 IC blood typing kits. The device was compared with tests that are or have been in regular use at the Small Animal Clinic of Freie Universität Berlin.

In a modified version of the Pennsylvania tube test (TUBE),[15] a specific amount of an EDTA whole blood sample is tested for an agglutination reaction when added to a standardised amount of both an anti-A serum and an anti-B solution containing *Triticum vulgaris* lectin. The whole blood samples are washed before being added to the reagents, and a standardised 3–5% RBC suspension is used for the test. The reactions take place in tubes during an incubation period of 15 mins at room temperature, after which the tubes are centrifuged. The supernatant is assessed macroscopically, as is the sediment in the process of gentle re-suspension. Microscopic evaluation of the re-suspended RBCs allows for more precise results in this method, when no agglutination is detected macroscopically. The TUBE method mandates 'back-typing' to confirm B or AB test results, that is, patient plasma is tested for the presence or absence of alloantibodies by incubating with a 3–5% type A RBC suspension. The GEL test used to be available to veterinary laboratories large enough to accommodate a special, single-use centrifuge required for the multicolumn gel cartridges. Two of the columns contain a gel matrix laced with monoclonal antibodies for A and B, the third serves as a negative control, containing only the gel matrix. The typing methods in this study were used and interpreted according to established in-house protocols and manufacturer's instructions, as well as according to the most recent published guidelines.[15, 18, 19]

The IC test is a cell-capture assay, the mechanism of action of which is based on sample migration along three intersecting membranes arranged as an inverted T in the cartridge: the

vertical control membrane contains a substance in a specific area that captures all cells; the horizontal membrane on the left-hand side contains monoclonal antibodies for type A blood and the horizontal membrane on the other side contains monoclonal antibodies for type B blood (Figure 1). The test is marketed as a kit containing a dropper bottle of diluent and optionally five or ten 92 x 57 x 6 mm semicircular cartridges (in sealed pouches) with a microtube pre-filled with 600 µl of a diluent, as well as two disposable pipettes for each cartridge. The cartridge has three viewing ports that are arranged in a circular fashion around the sample port, directly above the portion of each membrane that contains the cell-capturing substance and the monoclonal antibodies, respectively. This means there is a control port, as well as a port each for the A positive and the B positive readings (Figures 2–5). The manufacturer recommends storage at room temperature.

The cartridges were used exactly as prescribed by the manufacturer: they were first labelled, then one drop (30 µl) of a feline EDTA whole blood sample was added to the tube with the help of one of the included pipettes. The next three steps had to follow immediately: inverting the tube several times to mix properly, placing two drops (60 µl) of the now diluted blood into the cartridge sample port with the other pipette and adding to it two drops (80 µl) from the dropper bottle. A conclusive reading was defined in this study's protocol as the appearance of a clearly visible red vertical indicator line filling at least 25% of one (or both) of the A or B viewing ports within 10 mins of starting the test, along with the appearance of the horizontal indicator line in the control viewing port. If those criteria were not met, the result was rated inconclusive; if no line appeared at all, then it was deemed not readable (Figures 1–5). For the majority of test runs (59 tests in total), the IC assay was used before all other tests, in order to minimise bias. For the purposes of this study, detailed notes were taken in order to monitor any deviation from the device's expected performance. Times were noted for the following events: first time any line appears; time at which line appears at 25, 50, 75 and 100% of its full length from top to bottom of the A/B viewing ports. Photographs were taken at predetermined stages of the device's operational process.

2.3.3 Statistical analysis

Results were obtained and analysed according to the recommendations set forth elsewhere for method comparison studies.[20–22] Sensitivity for A and B antigen detection was calculated as the number of true positives for each antigen determined by the IC test divided by the number of positives determined by the reference methods. Conversely, specificity was calculated as the number of true negatives for each antigen determined by the IC test divided by the number of negatives determined by the reference methods. Overall agreement with the

reference methods was determined via contingency tabulation and the Cohen's kappa coefficient was used to assess the robustness of the test's performance results.[22]

2.4 Results

2.4.1 Blood samples

Clinical data were available for 89/105 of the sample population wherein the following breeds were represented: domestic shorthair (n = 70), Persian mix (n = 4), British Shorthair (n = 3), Maine Coon mix (n = 3), Norwegian Forest cat (n = 3), Siamese (n = 2), Birman (n = 1), Chartreux (n = 1), Persian (n = 1) and Siberian (n = 1). Disease distribution was as follows: gastrointestinal disorders (n = 16), wounds/ trauma (n = 15), disorders of the urogenital tract (n = 13), neoplasia (n = 10), respiratory (n = 8), neuromuscular/ orthopaedic (n = 8), endocrine (n = 4), ophthalmic (n = 4) or infectious disease (n = 3), immune-mediated haemolytic anaemia (n = 3), post-operative haemorrhage (n = 2), fever of unknown origin (n = 1), dental disease (n = 1) and routine surgery (n = 1). One of 15 tested cats was feline leukaemia virus (FeLV)-positive. The samples were between 0 and 10 days old (mean 3 days) and were stored between 2 and 4°C. The PCV ranged from 0.07 to 0.57 l/l (median 0.40 l/l), 16/105 samples had a PCV <0.30 l/l (median 0.26 l/l, range 0.07–0.29 l/l), six samples had a PCV <0.20 l/l. Very weak to weak slide agglutination occurred in 9/105 samples, and the plasma of 79/105 samples showed very weak to very strong haemolysis.

2.4.2 TUBE and GEL test

The TUBE and GEL tests, which yielded exactly the same testing results on all 105 samples (which corresponds to 100% agreement) were used as the reference methods against which the IC test's performance was evaluated. According to the reference methods, 85 samples were A positive, 17 samples B positive and three samples AB positive.

2.4.3 IC test

Eighty of the A samples were correctly identified by the IC method, four were misidentified as AB and one test was inconclusive (very weak indicator line). All of the 17 B samples and two of the three AB samples were correctly identified by the IC method (one AB test was inconclusive owing to a very weak indicator line in the A viewing port). The seven samples that were over six days old did not lead to any problematic test results. The same applied to the 16 anaemic samples. Hence, the misidentified A samples did not come from anaemic cats. The first of the four misidentified A samples had a high PCV of 0.52 l/l and showed very weak RBC agglutination, as well as very weak haemolysis. The second showed weak RBC agglutination and haemolysis. The third showed very weak haemolysis and the fourth, from a cat with previously diagnosed immune-mediated haemolytic anaemia, had a PCV of 0.33 l/l and

showed no quality divergence at all. The one sample that had tested positive for FeLV caused no problems with blood typing (blood type A). The 10 samples with intermediate to very strong haemolysis tested concordantly with the IC device. The IC test showed an overall agreement of 96.1% with the control methods for blood types A, B and AB in the 103 conclusive samples (Table 1). In addition, a statistical analysis of the results reported here using Cohen’s kappa shows a coefficient of 0.89 for the reported 96.1% agreement of the IC test results with the standards. Its sensitivity was 100% for both A and B antigen detection, and it showed a specificity of 100% for A antigen detection, as well as a 95% specificity for B antigen detection.

Of the 30 repeats, two that belonged to A samples were not readable (no indicator line appeared within 10 mins). One of those was a same day, the other a next day repeat. Twenty-eight were readable and showed agreement in 27/28 cases (96.4%); the one divergent sample was from a type A cat misidentified in the first run as AB and in the repeat run as B. The sample had a PCV of 0.42 l/l and showed weak haemolysis (1+) and weak agglutination (1+). This cat had chronic malaise after blunt trauma to the jaw (not tested for FeLV).

Of 131 readable test runs, 107 results were obtained in ≤5 mins (81.7%), the rest (24) were recorded in ≤10 mins (18.3%). In three of the 135 utilised cartridges the indicator line only reached 25% of the viewing port, in five cases only 50%, while in the remaining 127 cases the full indicator line was visible by the 10 min cut-off.

2.5 Discussion

The IC test examined in this study is designed to be a quick and reliable patient-side device for everyday use by the heterogeneous group of users in a veterinary practice. It is important that such devices are quick to use because of the often acute nature of cases in transfusion medicine; they must be reliable as a prerequisite for helping avoid haemolytic transfusion reactions; and veterinary staff of various training levels should be able to use them in order to participate in the care of patients in the area of transfusion medicine. The test was quick, as in most cases results were obtained in ≤5 mins and in most cases it could be used and interpreted easily with the enclosed step-by-step instructions. The manufacturer indicates that an incomplete indicator line in the viewing port is not a sign of a failed test. However, the occurrence of such incomplete lines in eight cases, along with that of a weak indicator line in two cases, particularly in AB results, presents the potential for difficulties in interpreting this test by inexperienced users and, we suggest, ought to be a consideration in revising the package insert.

The IC test's agreement of 96.1% with the reference methods compares well with other point-of-care tests described elsewhere: 91% and 95% for the card test and 95% for the other immunochromatographic test.[17, 18]

Of 135 performed tests, two were inconclusive and two were unreadable (corresponding to 1.5% each), which attests to a high reliability of the test kit. The IC test's high specificity and sensitivity for the individual blood types also demonstrates a test performance that is comparable to tests examined in other studies.[18] The 100% sensitivity for the B antigen is particularly important, as type B cats, with their high titre of anti-A alloantibodies, transfused with type A or AB blood, can exhibit especially severe transfusion reactions.[23] The Cohen's kappa coefficient of 0.89 corresponds to a score of 'very good' and is considered a more robust measure of agreement between two methods of clinical measurement than a simple percentage alone.[22]

Severe haemolysis or agglutination can cause problems both in determining blood type and in cross-matching.[8] Potential issues with sample quality, such as degrees of erythrocyte agglutination, anaemia and haemolysis, were determined in this study and the numbers indicate that, overall, the sample quality had no influence on test results. However, the agglutination in this study did not measure above weak, and true auto-agglutination did not occur; therefore it could not be determined as to which degree agglutination affects test performance, as it has been shown to do with the card test (see the manufacturer's recommendations).[14]

A patient's disease status has likewise been suspected in previous studies to cause discrepancies in blood testing results, as has the possibility of antigenic mimicry with RBC surface antigens or a change in the activity of the cytidine monophospho-N-acetylneuraminic acid hydroxylase, which is responsible for converting N-acetylneuraminic acid to N-glycolylneuraminic acid.[18] Anaemia caused by FeLV, in particular, has been named in the context of diseases that can impede routine blood testing.[6, 18] There was no conclusive evidence that the divergent results in this study can be explained by patient disease status, level of anaemia or other sample quality impediment.

Generally, in a clinical setting, when result interpretation is difficult, seeking a second opinion can lead to a more confident decision-making process. This is easiest, in turn, when results can be documented as part of the permanent patient record. Currently available laboratory blood testing methods, for example, the tube test do not all allow for easy documentation of test results without the availability of microscopic photography. Owing to the ability to photograph the results of the IC test, and because results can be read for hours following initial testing, these documentation needs are met.

As with other typing methods, it is suggested that rare blood type results, such as blood type AB in general and B in breeds where B is not usually seen, should be reconfirmed with a standard laboratory method. In general, it is recommended that for B samples 'back-typing' be performed to detect the presence of anti-A alloantibodies in their serum.[15, 18]

It must be pointed out, however, that no matter how high the agreement of a test with the reference methods it cannot afford complete protection against haemolytic transfusion reactions. The identification of the *Mik* antigen is a case in point.[24, 25] No testing methods other than cross-matching can be used to identify RBC incompatibilities as the possibility of there being more unknown antigens exists. Thus, any type of blood testing, including point-of-care testing, still caries this caveat, despite very good performance results.[7] In fact, it could be argued that no transfusion should be administered without performing a cross-match.[26] Transfusion reactions directed at blood components other than RBCs, such as white blood cells and plasma proteins, cannot be tested for, but tend to be self-limiting. Lastly, screening for infectious diseases in blood donors and proper handling of blood products are integral to safe transfusion protocols.[25] Therefore there still remains an incalculable risk to every transfusion.

2.6 Conclusions

Given its high agreement rate and ease of use, as well as its convenient storage requirements, the RapidVet-H IC Feline test can be recommended for clinical settings.

2.7 Funding

Dr. Kohn received research support in the form of test kits and financial reimbursement for laboratory materials from DMS Laboratories, USA.

2.8 Conflict of interest

The authors do not have any potential conflicts of interest to declare.

2.9 Tables and figures

Table 1 Blood typing results of the TUBE/ GEL and first run immunochromatographic (IC) tests. The numbers in parentheses are percentages

	TUBE/ GEL tests			
IC test	**A (n = 85)**	**B (n = 17)**	**AB (n = 3)**	**Total (n = 105)**
A	80	0	0	80
B	0	17	0	17
AB	4	0	2	6
Inconclusive	1	0	1	2
Total	**85 (80.9)**	**17 (16.2)**	**3 (2.9)**	**105 (100)**

Figure 1 RapidVet-H immunochromatographic feline blood typing cartridge, opened

Figure 4 RapidVet-H immunochromatographic feline blood typing cartridge showing an B result

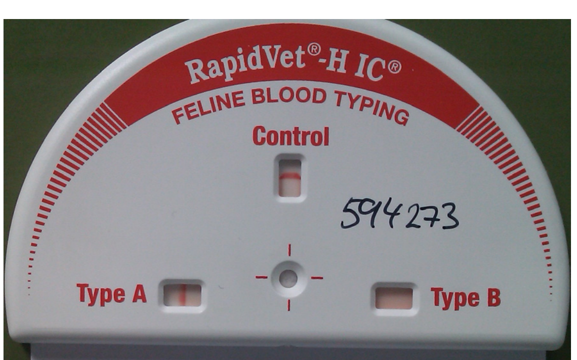

Figure 2 RapidVet-H immunochromatographic feline blood typing cartridge showing an A result

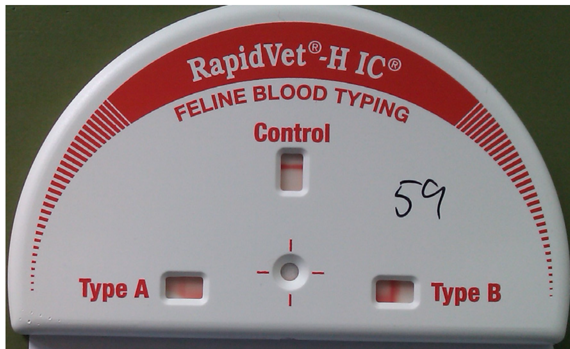

Figure 5 RapidVet-H immunochromatographic feline blood typing cartridge showing the test of an AB sample

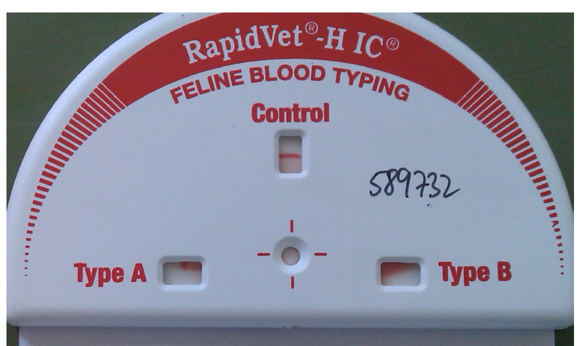

Figure 3 RapidVet-H immunochromatographic feline blood typing cartridge showing the test of an A sample with 25% of the indicator line visible

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3 Publication II

Hourani L, Weingart C, Kohn B.

Alloimmunisation in transfused patients: serial cross-matching in a population of hospitalised cats.

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Original Article

Alloimmunisation in transfused patients: serial cross-matching in a population of hospitalised cats

Hourani L, Weingart C, Kohn B

3.1 Abstract

Objectives Cross-matching is currently recommended as part of pre-transfusion testing for repeat transfusions in cats 4 days after having received an initial transfusion. This prospective study determined when and if cats developed positive cross-match (CM) results after having been transfused with AB-compatible blood.

Methods Donors were selected according to standard transfusion safety protocols. Twenty-one hospitalised anaemic recipients (blood type A n = 20, blood type B n = 1) received 1–4 (median 2) whole-blood transfusions (WBTs) over 1–6 days (median 2) in 33 transfusion instances. The tube CM method, including major, minor and recipient control, was employed. Macroscopic and microscopic agglutination reactions were evaluated according to a predetermined scale. CM tests with a positive recipient control could not be evaluated.

Results No signs of an acute transfusion reaction were observed. A total of 63 CMs were performed. In one cat with immune-mediated haemolytic anaemia (IMHA) the CM could not be evaluated (positive recipient control). The minor CM was negative in all cases. Fifteen of 20 cats had a negative major CM (MCM) 1–12 days (median 5) after their first transfusion. A positive MCM was observed in five cases after 2–10 days (median 5) post-first WBT. These five cats had received a total of 1–4 (median 2) WBTs. Cats with a negative MCM had received 1–3 (median 2) WBTs. In 51.5% (17/33) of transfusion instances, the cat's haematocrit increased as expected, with cats with a positive MCM at 40% (4/10) vs 56.5% (13/23) if MCM was negative.

Conclusions and relevance Twenty-five percent (5/20) of the feline recipients likely developed alloantibodies against erythrocyte antigens outside of the AB system as early as 2 days post-first WBT. This adds data to the recommendation to include cross-matching in pre-transfusion screening tests.

3.2 Introduction

Transfusion medicine has been established as a safe and effective type of patient care. An increased focus on patient safety has helped identify and control various risk factors with which it is associated, limiting the incidence of acute and delayed immunological and non-immunological transfusion reactions. Modern haemovigilance and blood-banking guidelines have been in place and are continually improving in human medicine. Such efforts are being mirrored in veterinary medicine as permitted by economic and logistical considerations, allowing such therapy to play an important role in feline healthcare.[1–4]

One aspect of increased safety in transfusion medicine is the establishment of routine pre-transfusion testing.[5] A number of point-of-care blood typing systems have been developed and evaluated for use in feline patients.[6–10] However, because blood typing devices only detect specific known antigens on both recipient and donor red blood cells (RBCs), they can neither account for antigens outside of the AB system nor for alloantibodies present in the recipient.

Post-transfusion alloimmunisation is a common complication in human medicine. It is, to date, unavoidable and can pose a safety hazard for previously transfused human patients.[11] Such alloimmunisation has also been documented for dogs and horses in veterinary medicine.[12–14] Cross-matching is the standard method for the detection of such serologic incompatibilities between recipient and donor.[15]

The current indication for a cross-match (CM) in dogs and cats is when a patient has an unknown transfusion history, has shown a previous transfusion reaction and/ or if a prior blood transfusion was administered ≥4 days prior to a planned transfusion.[16–18] Some authors have expressed the notion that routine cross-matching ought to be introduced for all pre-transfusion testing in cats[15,19] owing to the clinically relevant naturally occurring antibodies in that species,[20] as well as the presence of potentially unknown blood groups, as evidenced by the discovery of the *Mik* erythrocyte antigen.[21]

To our knowledge, no studies with serially performed cross-matching have been undertaken that determine the status of alloantibody presence in both previously transfused or not previously transfused feline patients, and neither has a point been determined at which such patients may eventually develop alloantibodies after their first transfusion.

This paper reports on a study designed to document the occurrence of positive CM results, as well as the usefulness of routine cross-matching in feline transfusion patients presented at the Small Animal Clinic at Freie Universität Berlin.

3.3 Materials and methods

3.3.1 Study population

The subjects of this prospective clinical study were client-owned anaemic cats that received at least one AB-compatible whole blood transfusion (WBT) while hospitalised at the Small Animal Clinic. Owner consent was routinely given at patient intake for the use of surplus samples for research purposes; therefore, no further approval for this study was needed. Inclusion criteria were the availability of a minimum of 250 µl of whole blood for cross-matching from up to 2 days prior to the date of the first transfusion, the availability of donor blood for cross-matching and that none of the patients had received any intravenous blood products prior to their first transfusion at the clinic. Cross-matching took place across 23 months.

Following in-house transfusion safety standards, blood donors were healthy client-owned pets no more than 8 years of age that weighed no less than 4 kg and were kept indoors exclusively. Regular vaccinations and deworming were expected, as was a known medical history. Donor screening included a physical examination with an emphasis on auscultation, routine blood work, AB blood typing and testing for feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) (SNAP combo plus FeLV antigen/ FIV antibody test; IDEXX Laboratories). Donors were sedated with a combination of ketamine (5–6 mg/kg IM) and midazolam (0.1 mg/kg IM). Whole blood, 6–7 ml per kg body weight, was obtained from the donor by jugular venepuncture while in ventral recumbency. The blood was collected into an open system using antiseptic technique into syringes prefilled with citrate, phosphate, dextrose and adenine (CPDA-1) solution and transferred into 150 ml paediatric blood-collection bags (Fenwal). Blood units were stored for no more than 21 days in a designated refrigerator kept at a temperature of 4 ± 2°C. A 1–2 ml aliquot of the CPDA-1-preserved donor blood was set aside for later cross-matching.

3.3.2 Transfusions and pre-transfusion testing

Pre-transfusion blood typing was routinely performed by trained laboratory personnel on donor and recipient blood samples, using the slide method as described elsewhere.[2,6] An in-house modification of the University of Pennsylvania tube method for blood typing was used in cases where results were unclear or seemed to indicate blood type AB.[6,10]

Transfusions were performed on anaemic patients at their attending veterinarian's discretion rather than strict laboratory cut-offs; both clinical status and, among other laboratory results, haematocrit (Hct) were taken into account. Patients received WBTs through a peripheral venous catheter placed into the cephalic or medial femoral vein or through a central venous catheter placed into the jugular vein. The method of delivery was through a standard, gravity-

driven transfusion line with an integrated 200 µm filter (Sangofix; B. Braun). Recipients were monitored for signs of acute transfusion reactions by recording heart rate, respiratory rate, rectal temperature and mucous membrane status at regular intervals.[22]

3.3.3 Cross-matching

Routine pre-transfusion testing included cross-matching (major, minor and recipient control) when a patient had received a transfusion more than 3 days prior to a planned transfusion. In all other cases, cross-matching was part of a pilot study evaluating the usefulness of including cross-matching in routine pre-transfusion testing in anaemic patients, in whom monitoring required regular and yet volume-conscious sampling. Cross-matching was performed by the same investigator according to an established in-house protocol, as well as following the most recent published data for a simplified tube procedure common in veterinary medicine.[16–18,23] Whole blood from both donor (EDTA-anticoagulated sample) and recipient (CPDA-1-preserved sample) was washed prior to testing as follows: plasma was first extracted by centrifuging at 385 *g* for 2 mins. The remaining RBCs were then washed by mixing them with 500 µl of phosphate-buffered saline solution (PBS) and subsequently centrifuging the suspension at 96 *g* for 1 min. This process was repeated three times.

Both recipient and donor plasma was checked for degree of haemolysis according to the following scale: no haemolysis visible to the human eye (0) to haemolysis that does not allow for visual differentiation of RBCs from plasma (4+) (Table 1). Substrates for cross-matching were plasma and RBCs suspended at a 3–5% concentration in PBS. The major cross match (MCM) required 40 µl of recipient plasma gently mixed with 20 µl of the prepared 3–5% donor RBC solution. The minor CM uses 40 µl of donor plasma gently mixed with 20 µl of prepared 3–5% recipient RBC solution. In order to control for auto-agglutination, 40 µl of recipient plasma were likewise mixed with 3–5% recipient RBC solution. The substrates were then incubated at 37°C for 15 mins. After a 15-second burst of centrifugation at 865 *g*, the supernatant was examined for haemolysis and compared with that of the plasma originally used. The pellet was then resuspended through gentle agitation while checking for agglutination in the tube against a white background, as well as on a slide under a microscope, if macroscopic examination was negative. Macroscopic and microscopic agglutination were recorded according to the following scale: no agglutinates (0) to 1–2 large agglutinates with clear plasma (4+) (Table 2). Microscopic assessment required the examination of the suspended RBCs within 60 seconds of placing a drop of RBC solution on the slide and agitating the slide in order to view RBCs and possible RBC agglutinates in motion. The scales used here were adapted from a study assessing degree of agglutination in blood typing.[6]

Tests took place, when possible, prior to and serially every 2 days after a blood transfusion, depending on the availability of samples.

A CM was considered positive if haemolysis or an agglutination reaction was observed macroscopically. If no reaction was evident, a microscopic evaluation was always included. If the recipient control displayed auto-agglutination, the CM was considered invalid.

In all cases, the Hct before transfusion was compared with that measured routinely 10–72 h after transfusion and compared with the expected Hct based on a formula suggested elsewhere.[2,24,25]

3.3.4 Statistical analysis

Calculations for collected data were performed using commercial data analysis software (IBM SPSS Statistics version 23.0.0.2, Microsoft Excel version 15.20). Descriptive data analysis was performed on the parameters: patient signalment, underlying disease, indication for transfusions, number of transfusions per recipient, number of donors per recipient, storage time for blood units, volume of blood units, number of CMs and number of positive MCMs. A mixed linear regression analysis was used to determine relationships between the occurrence of a positive MCM, as a risk factor, and Hct development, a measure of transfusion success, as the outcome. The individual animal was included as a random effect in the model. A P value <0.05 was considered significant.

3.4 Results

Twenty-one feline patients aged 1–16 years (median 8 years) were included in the study. Their body weight at the time of transfusion ranged from 2.5–8.3 kg (median 4.5 kg) and various breeds were represented. The majority were domestic shorthairs (n = 15), in addition to the following pedigree or pedigree-mix cats: British Shorthair (n = 2), Maine Coon (n = 1), Maine Coon mix (n = 1), Selkirk Rex (n = 1) and Turkish Van (n = 1). The patients included 17 males (16 neutered and one intact) and four spayed females. Disease distribution was as follows: blood loss anaemia (regenerative, n = 4), kidney disease with non-regenerative anaemia (n = 4), anaemia of inflammatory disease (non-regenerative, n = 4), FeLV infection with non-regenerative anaemia (n = 3), immune-mediated haemolytic anaemia (IMHA) (non-regenerative, n = 2), neoplasia with non-regenerative anaemia (n = 2), diabetes mellitus with regenerative anaemia (n = 1) and pure red cell aplasia (PRCA) with non-regenerative anaemia (n = 1). Twenty cats belonged to blood type A and one domestic shorthair cat had blood type B.

Pre-transfusion Hct ranged from 6.5 to 23.5% (median 14.2%). The patients received 1–4 (median 2) AB-compatible WBTs from 1–5 donors (median 2) over 1–6 days (median 2 days). The volumes administered ranged from 10 to 30 ml (median 20 ml), which were used alone or in combination with other units, amounting to a transfusion volume per patient and day (henceforth labelled as transfusion instance) that ranged from 1.8 to 18 ml/kg body weight (median 5.6 ml/kg).

No clinical signs of an acute transfusion reaction, as evidenced by changes in heart rate, respiratory rate, rectal temperature and/ or mucous membrane status were recorded for the duration of the study.

Sixty-three CMs were performed in total, each CM including minor and major cross-matching, as well as a recipient control. One CM took about 30 mins to complete. The minor CM was negative in all 63 cases.

Fifteen of 21 cats had a negative MCM between 1 and 12 days (median 5 days) after initial transfusion. The CM in one cat with IMHA was not valid because it displayed a positive recipient control. Five of 20 (25%) cases had a positive MCM 2–10 days (median 5 days) after the initial blood transfusion given to transfusion naïve patients, with macroscopic agglutination ranging from 0 to 1+ and microscopic agglutination from (+) to 2+ (Table 3). Positive CMs were not found in transfusion-naïve patients. Cats with a positive MCM had anaemia of inflammatory disease (n = 2), blood loss anaemia (n = 1), FeLV infection with non-regenerative anaemia (n = 1), PRCA (n = 1); they received a total of 1–4 (median 2) blood transfusions from 1–4 donors (median 2) (Table 3). Patients with a negative MCM received 1–3 (median 2) blood transfusions (Table 4). Those with a positive MCM received 2.8–9 ml/kg (median 5.8 ml/kg) of whole blood per transfusion instance, while those with a negative MCM received 1.8–18 ml/kg (median 5.1 ml/kg) per transfusion instance. The storage age of units that had been given to positive MCM patients ranged from 0 to 21 days (median 7 days).

Patients' Hct increased as expected in 51.5% (17/33) of the transfusion instances. The Hct changed at an actual range from -3.8 to 15.2 percentage points (median 2.8 percentage points), as compared with the expected increase of 0.9 to 9 percentage points (median 2.8 percentage points). In patients with a positive MCM, the Hct increased as expected at a rate of 40% (4/10) vs 57% (13/23) for those with negative MCM results. The mixed model linear regression analysis did not identify a statistically significant association between inadequate Hct increase and the occurrence of a positive MCM in the course of testing (Table 5). However, patients with a positive MCM achieved an Hct that was, on average, 1.04 less than expected. The Hct measured in those with a negative MCM had an Hct that was, on average, 0.53 more than expected ($P = 0.237$; Table 5).

The bilirubin concentration was not available in enough cases to enable a consistent assessment of its development. The volume of intravenous fluids administered to patients was, depending on clinical status, at or below maintenance rate for all but one patient (19/20) with severe dehydration due to diabetic ketoacidosis.

3.5 Discussion

This study addressed the need for cross-matching as a measure to avoid potential transfusion reactions in cats. Based on the occurrence of positive MCMs reported here, 25% of patients may have developed alloantibodies against erythrocyte antigens. The earliest occurrence of such alloimmunisation was documented 2 days after the first AB-compatible blood transfusion was administered.

One limitation on cross-matching in the clinical setting is cautious blood sampling from anaemic cats; that is, clinicians responsible for hospitalised anaemic cats tend to sample as little as possible for monitoring Hct development and, as such, the amount left over for cross-matching is often quite small. In our experience, however, as little as 250 µl of blood sufficed in order to adequately perform a CM. It is therefore realistic to expect to be able to safely cross-match in anaemic cats, even when samples are taken for monitoring.

Pre-transfusion testing may be affected by the age of samples used. A study in equine medicine reported a higher occurrence of incompatible CMs with acid citrate dextrose preserved donor samples as early as 1 week after collecting; the authors of that study go so far as to recommend fresh donor samples for cross-matching, without, however, suggesting an acceptable time period between obtaining the sample and performing the CM.[23,26] The general recommendation in veterinary medicine regarding the age of specimens for cross-matching is that they should be under 24 h old, with the exception of unit donor segments that may be as old as the unit itself. In the current study, donor samples were taken at the time of blood donation and preserved with CPDA-1. The age of donor samples thus correlated with the age of the units, which ranged from 0 to 21 days (median 7 days) in our study. We cannot, therefore, exclude an influence of longer storage on our CM results.

Three of the patients in this study were diagnosed with FeLV, an infection that has been associated with false-positive MCM results.[24] Of the FeLV-positive patients cross-matched here, one showed a positive MCM in the course of testing. It is unlikely that this represents such a false positive, since the positive MCM did not occur until the seventh day after the initial transfusion.

Transfusion patients frequently receive glucocorticoid therapy in immunosuppressive doses when underlying diseases such as IMHA, PRCA, leukaemia and so on are present. It is unclear

whether such treatment suppresses the development of alloantibodies in transfused patients.[27] Seven of 20 patients received glucocorticoid treatment, two of which showed a positive MCM after having been on this treatment for 8 and 12 days respectively. More data are needed in order to be able to make claims regarding the clinical relevance of positive MCMs in immunosuppressed cats.

The efficacy of transfusions is determined by examining various factors, the simplest of which is whether or not an acute transfusion reaction has taken place. Acute transfusion reactions were not observed in the subjects studied here. RBC viability may also be measured by examining post-transfusion Hct development; a study on transfusions in cats reported that in 43.3% of the patients the Hct was close to the value calculated prior to transfusion.[2] In the present study, the number is slightly higher at 51.5% for the study population. The group of patients that showed a positive MCM had a lower response rate (40%), which may be an indication of the severity of disease in those patients; however, a delayed transfusion reaction cannot be ruled out. An increase of the bilirubin concentration in a patient's blood as a parameter that measures post-transfusion haemolysis would aid in determining whether a delayed transfusion reaction has taken place. Unfortunately, this parameter was not available frequently enough as it was not measured at the same intervals as the blood count, owing to volume constraints in anaemic patients. Fluids administered to our patients should not have had an effect on Hct development, as all patients received fluids at or below maintenance rate, except for one severely dehydrated patient that received a higher volume until rehydration was achieved. It should also be noted that strongly regenerative anaemia may have caused the Hct to increase more than expected and patients with continuing haemorrhage or haemolysis may have displayed a less than expected Hct increase, while the effectiveness of blood transfusions is best assessed in patients with ineffective erythropoiesis.

The small data set of this study precluded statistical significance; however, our numbers indicate that the Hct increases were less marked in those patients with positive MCMs (Table 5), despite our not having administered mismatched blood. This is a result that warrants further investigation.

The possibly deleterious effects of multiple red cell transfusions, because of their ability to increase the likelihood of allo-antibody formation, have been described for humans.[11] In the present study, both patients that received more than one transfusion and those that received blood from more than one donor also constitute a majority (3/5) in the group of patients with positive MCMs. Despite our low case number, such results ought to be taken into consideration when weighing the benefits of multiple transfusions for anaemic patients.

Transfusion reactions have been reported to occur in veterinary medicine despite compatible CMs. One study reports on this for feline patients with the occurrence of delayed haemolysis (n = 2) and acute haemolysis (n = 1),[28] and another demonstrates it for horses.[29] Despite such findings, cross-matching remains one of only two pre-transfusion testing methods available to veterinarians. The type of cross-matching used in veterinary medicine has been largely replaced in human medicine by computer cross-matching and the more sensitive antibody screening.[30] However, in feline transfusion medicine, it remains an effective, easy and relatively economical method for detecting profound incompatibilities in the clinical setting. Not only does it overcome the inability of blood typing to detect unknown or rare blood types such as that defined by the *Mik*-antigen, but it also helps find the best match for patients that are at risk of having formed alloantibodies.[15,16,18] Several authors, therefore, hint at the expediency of introducing cross-matching into routine pre-transfusion testing.[15,18,31]

A limitation of this study lies in its clinical design. Retesting our results was not part of the study design, given that only blood samples left over from patient monitoring were available for cross-matching. Also, monitoring patient Hct was not followed according to a schema predetermined in this study, but rather at the discretion of each patient's clinician; the same applied to the decision to perform transfusions. This is exemplified by the transfusion of one patient with an Hct of 23.5%. This patient had been diagnosed with anaemia of inflammatory disease and therefore was at risk of rapidly worsening,[32] as well as not having responded adequately to the first transfusions.

3.6 Conclusions

We have shown that the possibility of a positive MCM exists as early as 2 days after initial transfusion. We have no data supporting the use of CMs in pre-transfusion testing of transfusion-naïve cats, as none of our positive CMs occurred prior to the initial administration of blood transfusions. However, there is no direct way to assess the status of non-AB antibodies in cats, and there is no easy way to determine delayed transfusion reactions in hospitalised patients. This, in addition to a lack of evidence for the textbook rule of cross-matching 4 days post-initial transfusion, should encourage the development of more diligent pre-transfusion testing protocols, which could include performing CMs as part of routine pre-transfusion testing in cats. Experimental studies would be needed to provide a solid evidence base for routine pre-transfusion cross-matching.

Although cross-matching, just like blood typing, cannot avoid sensitisation and does not help increase the likelihood of a successful transfusion as evidenced by an increase in Hct, it is the only other means with which the overall risk of transfusions can be lowered and is therefore a tool towards increasing patient safety. Our recommendation is therefore to consider

introducing cross-matching into routine pre-transfusion testing protocols as part of a continued effort to increase patient safety, along with diligent documentation, sound blood-banking practices and an emphasis on the indication for individual transfusions.

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3.9 Conflict of interest

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3.10 Tables

Table 1 Scale for the visual assessment of the degree of plasma haemolysis

0	Plasma is nearly transparent
(+)	Plasma is slightly discoloured
1+	Plasma is light red
2+	Plasma is red
3+	Colour of plasma is close to colour of RBCs
4+	Colour of plasma cannot be visually differentiated from RBCs

Table 2 Scale for the microscopic assessment of the agitated RBC-solution in the CM tube method

0	No agglutinates visible upon agitation of RBCs
(+)	A few tiny agglutinates with most of the RBCs in suspension
1+	Many small agglutinates along with RBCs in suspension
2+	Some larger agglutinates with many small agglutinates
3+	Several large agglutinates with clear plasma
4+	1 -2 large agglutinates with clear plasma

Table 3 Cases with a positive major cross-match (MCM): diagnosis, number of blood transfusions instances (BT), number of donors, number of cross-matches (CMs), time to 1st positive MCM, grade of agglutination and post-transfusion haematocrit (Hct) development (upon final transfusion, where more than one transfusion was given)

Case	Underlying or concurrent disease	BT (n)	Donors (n)	CMs (n)	Time between first BT and first positive MCM (days)	Macroscopic agglutination (grade)	Microscopic agglutination (grade)	Hct increase as expected?
3	Haemorrhage	2	2	4	10	1+	2+	Yes
11	FeLV-infection	1	1	5	7	0	2+	Yes
13	Inflammatory disease	1	1	2	2	0	1+	No
20	Pure red cell aplasia	2	2	4	4	0	(+)	No
21	Inflammatory disease	4	4	4	5	0	2+	No

Table 4 Cases that did not display a positive major cross-match (MCM): diagnosis, number of blood transfusions (BT), number of donors, number of cross-matches (CM) and post-transfusion haematocrit (Hct) development (upon final transfusion, where more than one transfusion was given)

Case	Underlying or Concurrent Disease	BT (n)	Donors (n)	CMs (n)	Hct increase as expected?
1	Neoplasia	1	2	2	No
2	Kidney Disease	2	3	3	No
4	Kidney Disease	2	2	5	No
5	Kidney Disease	1	2	3	Yes
6	Inflammatory Disease	3	2	5	Yes
7	Immune mediated haemolytic anaemia	2	2	3	Yes
8	Haemorrhage	2	2	2	Yes
9	Haemorrhage	2	2	2	No
12	Haemorrhage	2	2	4	Yes
14	FeLV-infection	1	1	2	Yes
15	FeLV-infection	1	1	2	Yes
16	Inflammatory disease	1	1	2	Yes
17	Neoplasia	2	2	2	Yes
18	Kidney disease	1	1	2	No
19	Diabetes mellitus	1	1	3	No

Table 5 Mean deviation of haematocrit values from the expected post-transfusion increase with SE and con-fidence intervals (CI) in patients with positive and negative cross-match (CM) results

			95% CI	
Positive CM	Mean	Std. Error	Lower	Upper
No	0.535	0.721	−0.935	2.005
Yes	−1.042	1.093	−3.271	1.187

P = 0.237

3.11 References

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4 Discussion

This dissertation reports on the examination of two pre-transfusion methods in cats. In general, transfusion medicine in veterinary practice progresses on a number of fronts, simultaneously. Such progress is influenced by the basic understanding of the biological systems involved in the variety of species encountered in veterinary medicine, but also by logistical and economic constraints. Applying novel technologies and improving their usage guidelines to expand our repertoire of tools in the clinic therefore serves both aspects of this progression. The first paper, reported here, addresses the very practical need in the clinical setting for fast, cost-effective, easy-to-use and conveniently storable blood typing tools by evaluating a novel immunochromatographic point-of-care blood typing device. The second paper examines, through serial cross-matching, an aspect of feline blood transfusion that presents a potential complication based on our understanding of the dynamics of alloantibody formation subsequent to transfusions. The current guidelines for cross-matching in feline transfusion medicine are not based on evidence as to when these antibodies are formed in cats, which may lead to exposing patients to an unnecessary risk of transfusion reactions. This paper represents a first attempt to provide an evidentiary basis for these guidelines.

4.1 Findings

4.1.1 Evaluation of a novel feline AB blood typing device

The first study prospectively evaluated an immunochromatographic cell-capture cartridge test (IC, the RapidVet-H IC Feline) that had been recently developed for the feline AB blood group system. This test is one of four commercial point-of-care devices currently available for cats in Europe and the United States (Table 3). The other three are the RapidVet-H Feline, a card-based test that uses lyophilised reagents; the Alvedia Quick Test A + B, an open cartridge test that contains an immunochromatographic test strip, for which a ‘Lab’ version exists that eliminates the cartridge but requires minimal additional laboratory equipment; and the Quickvet Feline Blood Typing cartridge for use in the company’s proprietary multipurpose analyser (Table 3). The card-based test has been evaluated in three studies, and was shown to be as reliable as manual laboratory methods in one of those studies, with the caveat that severe anaemia and the presence of FeLV might impede correct readings, (Kohn et al., 1997). Both that study and another study (Stieger et al., 2005) point to variations in the degree of agglutination on the card, the results of which are interpreted visually and may be misinterpreted when agglutination is mild. An additional study found that manual laboratory methods, as well as the now defunct gel column test both outperformed the card test (Proverbio et al., 2011). The immunochromatographic Alvedia Quick Test A + B (along with its ‘Lab’

version) was, likewise, evaluated and found to show higher performance compared with the card test (Spada et al., 2015) (see also Table 4).

In the current study, two reference methods were used to evaluate the performance of the IC test: the Pennsylvania tube agglutination method (TUBE), a laboratory method, as well as a the gel column test, representing another point-of-care method. TUBE has been cited as the 'gold standard' or 'reference' blood typing method for the feline AB blood group system (Seth et al., 2011; Stieger et al., 2005). The two reference methods showed a 100% agreement with each other while the IC test agreed with each of them at an overall rate of 96.1%, showing a 100% specificity for the A and B antigens, as well as a 95% sensitivity for the A antigen and 100% sensitivity for the B antigen. These findings are similar to those reported elsewhere for point-of-care devices for the feline AB blood group system (Table 4). Because type B cats are especially susceptible to acute haemolytic transfusion reactions due to their high titres of anti-A antibodies, it is notable that the IC test showed 100% sensitivity for the B antigen. Sample quality, an issue with other point-of-care devices (Kohn et al., 1997; Tocci & Ewing, 2009; Weingart et al., 2017), did not appear to hamper reliability of this device. Disease status may also interfere with blood typing results (Griot-Wenk & Giger, 1995; Seth et al., 2011), no evidence of which was found here.

Table 4 Overview of point-of-care test kits for the feline AB blood group system with list of published evaluations and performance data: agreement with other tested methods (listed in parentheses), as well as sensitivity and specificity for each of the two known RBC antigens (A and B), including AB, where reported

Name	Evaluated by (Author, Date)	Test performance						
		Agreement (%), (reference method)	Sensitivity for A (%)	Sensitivity for B (%)	Sensitivity for AB (%)	Specificity for A (%)	Specificity for B (%)	Specificity for AB (%)
Alvedia Lab Test A + B	(Spada et al.2015)	100 (back-typing)	100	100	100	100	100	100
Alvedia Quick Test A + B	(Seth et al., 2011)	94.8 (TUBE)	97.7	95.7	NR	100	97.1	NR
	(Spada et al., 2015)	100 (back-typing)	100	100	100	100	100	100
DiaMed ID-Gel Test Feline Anti A + B Typing	(Stieger et al., 2005)	100 (TUBE)	NR	NR	NR	NR	NR	NR
	(Proverbio et al., 2011)	100 (back-typing)	100	100	100	100	100	100
	(Seth et al., 2011)	99.4 (TUBE)	100	100	NR	100	99.3	NR
RapidVet-H Feline, DMS Laboratories	(Kohn et al., 1997)	96.3 (TUBE)	NR	NR	NR	NR	NR	NR
	(Stieger et al., 2005)	100 (TUBE)	NR	NR	NR	NR	NR	NR
	(Seth et al., 2011)	91.4 (TUBE)	93.2	95.7	NR	100	97.1	NR
	(Spada et al., 2015)	78.6 (back-typing)	NR	NR	NR	NR	NR	NR
	(Proverbio et al., 2011)	94.1 (back-typing)	100	100	61	100	95	100
RapidVet-H IC Feline	(Hourani et al., 2014)	96.1 (TUBE)	95	100	NR	100	100	NR
Quickvet Feline Blood Typing	(Weingart et al., 2017)	90.8 (TUBE)	100	100	NR	95	94	NR

TUBE = Pennsylvanian tube agglutination method; NR = not reported

Ease of use is an essential attribute of point-of-care laboratory devices, where the human factor, represented by the user or operator of such devices, is an important aspect of overall safety and performance. The operator needs to be able to perform a test quickly without having to consult lengthy instruction manuals and be able to have confidence in the results shown by the test. No independent studies are available at the time of writing that propose methods to evaluate the ease of use of point-of-care devices. However, a position paper is available based on the European Directive on In Vitro Diagnostic Medical Devices about safety standards for both user and patient ('Requirements for Point of Care Testing Systems,' 2016). Additionally, the user interface design community, for whom extensive observational studies of the end-user of a particular device are essential (Rubin & Chisnell, 2008), has produced text book material to help design safe point-of-care devices (Wiklund et al., 2011).

The insight gained in the present research showed that all-in-one immunochromatographic devices, such as the IC test, do achieve a high standard for ease of use, in part, because they are fast and reliable, as the data shown suggests: in most cases, results could be accurately read in less than 5 mins with minimal time needed to understand the procedure. They are also practical, since no additional equipment is required: the test kit comes in a box with all the materials needed to perform the test. One minor drawback arose from a small number of weak (in 3% of tested cartridges), as well as incomplete lines that only reached 25% of the viewing port (in 2% of tested cartridges) which may cause an operator to hesitate before determining whether a result is positive. This is an issue similar to the degree of agglutination seen on the card-based test, where 5% of tested A-samples showed only mild (1+) agglutination (Kohn et al., 1997). The IC test is also easy to store, as no refrigeration is necessary. These results make it an overall valuable device for situations where fast, reliable blood typing is needed, provided that other blood-banking and pre-transfusion safety standards are met. Such measures include, for example, reconfirming the rarer B and AB blood, if so typed by point-of-care testing, and close patient monitoring during transfusions, because no amount of pre-transfusion testing can avoid all possible scenarios in which a transfusion reaction may occur.

4.1.2 Alloimmunisation in transfused cats

The second study described in this dissertation used cross-matching in anaemic hospitalised cats as a means to find a point at which alloimmunisation might occur after an allogeneic transfusion. The hypothesis guiding this study was that cats may form alloantibodies earlier than previously assumed after a transfusion that has been deemed AB-compatible through prior blood typing. This study is the first designed to test each feline transfusion patient prior to and serially after each transfusion. Patients that had received more than one transfusion were of particular interest, as it was assumed, based on studies in human medicine (Abou-

Elella et al., 1995; Ameen et al., 2009; Tormey et al., 2008), that they are at a higher risk of developing alloantibodies. 25% of the tested cats showed a positive CM over the course of the study. The Hct rise was as expected in 51.5% of the transfusions given to the cats in the study. An association was observed between the development of a positive MCM and an inadequate rise of Hct. On average, this was 1.04 percentage points less than expected relative to those with a negative MCM, where the rise was, on average, 0.53 percentage points higher than expected. The small case number of this clinical study precluded statistical significance, but the measured association may allow clinicians to use the formula suggested here and elsewhere (Griot-Wenk & Giger, 1995; Reed et al., 2014; Weingart et al., 2004) to assess transfusion efficacy and risk of individual patients for alloimmunisation. Multiply-transfused patients and those that were transfused from more than one donor likewise had a higher occurrence (60% of cases) of a positive MCM.

The most surprising result of the second study, however, was not the detection of antibodies, as this was a well-known complication not only based on the literature but based on our experience in the clinical setting at the small animal clinic (Barfield & Adamantos, 2011; Brown 2012; Klaser et al., 2005; Tocci & Ewing, 2009; Weingart et al., 2004). It was, rather, the finding that a positive CM occurred earlier than the 4-day rule for mandatory cross-matching prior to the transfusion of a previously transfused feline patient. As noted above, no evidence base exists for this rule, therefore the guidelines for pre-transfusion testing may need to be revised in the future, in the interest of minimising risk as part of transfusion safety protocols.

4.2 Limitations

4.2.1 Evaluation of a novel feline AB blood typing device

The IC-study was designed much like other studies that evaluated point-of-care blood typing devices (Kohn et al., 1997; Proverbio et al., 2009; Proverbio et al., 2011; Seth et al., 2011; Stieger et al., 2005). However, a recent publication on quality assurance in the clinical laboratory setting recommends repeat measurements (although single measurements are considered acceptable) (Vap et al., 2012). More frequent same and next day, as well as more inter-operator repeat measurements might have improved over-all robustness of the results. The IC-study did include an inter-operator examination, whereby operator 1 was the primary investigator with in-depth knowledge of the devices and testing protocol, while operator 2 was familiar with the IC-device on a basic level and interpreted the results based solely on colour photographs. Only operator 2 results were presented in the publication because they represented a reduction in bias.

4.2.2 Alloimmunisation in transfused cats

Due to technical and logistical idiosyncrasies, the second study could not include inter-observer results. The evaluation of microscopic agglutination or lack thereof had to be performed in a time-constrained framework in a clinical setting. It was essential that microscopic evaluation was performed within a short time-window and while the incubated RBCs were put in motion (gentle tapping or rocking of the slide was required). It was only through this method that agglutination could be excluded properly. In several cases, digital photographs were taken with a special microscope connected to a computer, but they produced unsatisfactory results (Figure 1). Only filming, then, would have produced a similar scenario to that in study 1, in which a second operator could have evaluated the microscopic cross-matching results. Data collection with video or other technical advances in data processing and optical instrumentation would allow future studies to present more rigorous results in this regard.

Figure 1 Microscopic photograph of an agglutination reaction without the use of a cover slip, allowing for free RBC flow in a major cross-match with a degree of agglutination of 2+

Finally, the rarity of transfusion reactions in cats (reported at 1.2–9% of the studied cases, only one of those having been fatal (Castellanos et al., 2004; Klaser et al., 2005; Weingart et al., 2004)) may be such that the routine use of both blood typing and cross-matching could be legitimately questioned, on a cost-benefit-basis, in a clinical setting. In fact, one study conducted in the UK found no evidence of non-AB incompatibilities, leading the authors to question the routine use of cross-matching (Tasker et al., 2014).

4.3 Suggestions for future research

The current issues faced in the practice of human transfusion medicine are the high cost of blood products and blood banking on the one hand (Waters 2010), and a growing population that refuses blood products for religious reasons (Schaffer 2015). In addition, certain

transfusion reactions, such as transfusion-associated circulatory overload and transfusion-related acute lung injury have not been reduced much through pre-transfusion testing and other sound blood transfusion safety protocols. Further, the improvement of oxygen delivery through transfusions has been questioned and alternatives to allogeneic BT have been examined (Pape & Habler, 2007; Prittie 2010; Waters 2010). This research has led to a rethinking regarding transfusions in human medicine, a practice called 'blood management.' One aspect of blood management is to reduce the number of transfusions given to patients, overall, as well as to individual patients; another is a general focus on expanding the use of point-of-care devices relative to laboratory methods. It is thought that point-of-care testing may avoid iatrogenic anaemia in critical care patients because these devices usually need very small amounts of blood to produce reliable results (Waters 2010). Point-of-care devices are also advocated for allowing fast decision-making in situations of acute blood loss, such as intra-operative haemorrhage and trauma related bleeding (Waters 2010).

These developments in human medicine are of great interest for further research into pre-transfusion testing in veterinary medicine. For example, ease of use is essential for speedy, reliable results. The IC test was anecdotally easy to use for laboratory personnel involved in the first study and 81.7% of tests were quantifiably read in ≤5. However, the actual speed with which it produces results was not determined, as the times measured did not take into consideration the time it takes for an average person to read through instructions, understand them, perform the test and read out results.

Further, two point-of-care cross-matching tests are currently available for companion animals: the Alvedia canine immunochromatographic cross-match test (both the bulk 'Lab' and 'Quick' versions are available) and the gel-based RapidVet-H Companion Animal Cross-match. The latter may be used for both dogs and cats and it has been recently evaluated for dogs (Guzman et al., 2016). However, the authors of that study still found that the manual laboratory method far outperformed the point-of-care kit and they did not recommend it as a sole way of detecting serological incompatibilities. Further studies are needed to evaluate this device's reliability and practicality in feline care, as well as to assess the other point-of-care kit available on the market. Additional studies are also needed to provide a more robust evidence base for the timing of pre-transfusion cross-matching in cats, as well as dogs.

The possibility that multiple transfusions may put feline patients at an increased risk for alloimmunisation, indicated in the second study reported in this dissertation by an over-representation among cases with a positive MCM, ought to be taken into consideration as part of introducing the concept of blood management in feline medicine, but also extended to veterinary medicine, in general.

Several authors recommend including both cross-matching and blood typing in pre-transfusion protocols based on unforeseen serological incompatibilities in cats that cannot be detected by blood typing, alone (Davidow 2013; Giger 2009; Weinstein et al., 2007). However, in some settings, following these recommendations is not logistically possible, whereas in others, the additional cost may be prohibitive. If reliable point-of-care cross-matching devices were to be developed and become readily available for use in feline patients, they could supplant blood typing in pre-transfusion testing in certain settings, for example, in emergency situations, thus improving safety without greatly increasing costs.

4.4 Conclusions

Fast, low-cost, easy-to-use, reliable point-of-care pre-transfusion testing devices will be needed in veterinary transfusion medicine in general and feline practice, in particular. One of these devices has been presented in this dissertation and it was concluded that it can be recommended because it delivers on the expectations of everyday veterinary practice. Alloimmunisation is a complication in transfusion medicine and one way to detect it is through cross-matching. The second study presented here recognises that this complication should be expected earlier than previously thought when transfusing feline patients, and may be addressed by including cross-matching in routine pre-transfusion testing. Both studies contribute to an increased awareness of ways to improve patient safety for cats receiving allogeneic blood and blood products.

5 Summary

Transfusion medicine has greatly improved our ability to treat anaemia in human and veterinary medicine. The safety and efficacy of transfusion medicine has advanced as both our understanding of the relevant biological systems has improved and as our tools and procedures for carrying out transfusions have become more sophisticated. This dissertation reports on two prospective clinical studies examining specific aspects of feline pre-transfusion testing methods with the potential to improve the safety and efficacy of the subsequent transfusions.

In the first study, the novel immunochromatographic point-of-care blood typing device (RapidVet-H IC Feline, DMS Laboratories) for the feline AB blood group system was assessed for reliability by comparing it to two other tests as reference methods, the Pennsylvania tube agglutination and a gel column test. Blood samples from 105 sick and healthy cats were included. The samples were between 0 and 10 days old (median 3 days) and were tested for haemolysis and agglutination; the haematocrit ranged from 0.07 to 0.57 l/l (median 40 l/l). The reference methods showed a 100% agreement with each other. 85 samples were determined blood type A by the two reference methods, 80 of which were correctly identified by the immunochromatographic test. Four were misidentified as AB and one was rated inconclusive. All B samples were correctly typed. Two of the three AB samples were correctly identified by the immunochromatographic test and one was rated inconclusive. The sample quality had no influence on test performance. Of 30 repeats, 28 were readable and showed agreement in 27 cases. The agreement of the immunochromatographic test with the control methods was 96.1% for the 103 conclusive tests, and it showed high sensitivity and specificity for A and B antigen detection, of note being the 100% sensitivity for B antigen detection. Based on the data, it is still suggested that AB results be reconfirmed with a laboratory method and that a 'back-typing' be performed with plasma from B-typed samples, ensuring that cats with blood type B not be given A-type blood. The novel immunochromatographic test device showed very good performance and ease of use, as well as convenient storage requirements.

The second study was designed to detect a positive cross-match in cats after receiving an AB-compatible blood transfusion, potentially indicating that alloimmunisation had taken place in those patients. Twenty-one hospitalised anaemic recipients (blood type A: n = 20; blood type B: n = 1) received 1–4 (median 2) whole-blood transfusions over 1–6 days (median 2 days) in 33 transfusion instances. The manual laboratory tube cross-matching method, including major, minor and recipient control, was used to serially test transfusion patients for macroscopic and microscopic agglutination reactions. No clinical signs of an acute transfusion reaction were observed. A total of 63 cross-matches were performed. The minor cross-match was negative

in all cases. A positive major cross-match was observed in five cases after 2–10 days (median 5 days) after an initial blood transfusion. These five cats had received a total of 1–4 (median 2) blood transfusions. In 51.5% (17/33) of transfusion instances, the cat's haematocrit increased as expected, with cats with a positive major cross-match at 40% (4/10) vs 56.5% (13/23) if the major crossmatch was negative. The five positive major cross-match results likely represent feline recipients which developed alloantibodies as early as two days after the initial blood transfusion. Cross-matching is currently only recommended as part of pre-transfusion testing for repeat transfusions in cats four days after having received an initial transfusion. The second study adds data to an evidentiary basis for recommendations to include cross-matching in routine pre-transfusion screening tests.

Both studies contribute to an increased awareness of ways to improve patient safety for cats receiving allogeneic blood and blood products.

6 Zusammenfassung

Studien zur Prätransfusionsdiagnostik bei Katzen: Evaluierung eines neuen Schnelltests zur Blutgruppenbestimmung und Kreuzproben bei Transfusionspatienten

Die Transfusionsmedizin hat sowohl in der Human- als auch in der Veterinärmedizin zu einer Weiterentwicklung der Therapie von Anämien geführt. Ein besseres Verständnis der involvierten biologischen Systeme und der in der Transfusionsmedizin angewandten Techniken und Vorgehensweisen führte zu Fortschritten in der Transfusionssicherheit. Diese Dissertation berichtet über zwei prospektive klinische Studien, in denen Testmethoden der Prätransfusionsdiagnostik bei Katzen überprüft wurden, welche die Möglichkeit bieten, die Sicherheit von Transfusionen und deren Erfolg zu erhöhen.

In der ersten Studie wurde ein neuer patientennaher immunchromatographischer Schnelltest (RapidVet-H IC Feline, DMS Laboratories) für das AB Blutgruppensystem bei Katzen auf Verlässlichkeit der Testergebnisse evaluiert, indem dieser mit zwei anderen Tests als Referenzmethoden verglichen wurde: der Röhrchen-Agglutinations-Methode und einer Säulen-Agglutinations-Methode. Blutproben von 105 gesunden und kranken Katzen wurden verwendet. Die Proben waren zwischen 0 und 10 Tage alt (Median 3 Tage) und wurden auf Hämolyse und Agglutination überprüft. Der Hämatokrit reichte von 0,07 l/l bis 0,57 l/l (Median 0,40 l/l). Die Referenzmethoden zeigten untereinander eine Übereinstimmung von 100%. 85 Proben wurden von beiden Referenzmethoden als Blutgruppe A typisiert. 80 dieser 85 Proben wurden vom immunchromatographischen Test korrekt bestimmt. Vier Proben wurden falsch als AB typisiert. Eine Probe war nicht auswertbar. Alle B-Proben wurden korrekt typisiert. Zwei der drei AB-Proben wurden vom immunchromatographischen Test korrekt bestimmt und eine Probe war nicht auswertbar. Die Probenqualität hatte keinen Einfluss auf die Testergebnisse. Von 30 Testwiederholungen waren 28 auswertbar, wovon 27 mit dem Erstergebnis übereinstimmten. Für die 103 auswertbaren Tests lag die Übereinstimmung des immunchromatographischen Tests mit den Kontrollmethoden bei 96,1%. Die Ergebnisse zeigten eine hohe Sensitivität und Spezifität für die Erkennung der A und B-Antigene. Wichtig dabei war vor allem die 100%ige Sensitivität für das B-Antigen. Auf der Grundlage der Daten wird weiterhin empfohlen, dass AB-Ergebnisse durch ein Referenzlabor bestätigt werden und ein sogenanntes Backtyping mit Plasma von B-Proben durchgeführt wird, um so zu verhindern, dass Katzen mit Blutgruppe B Blut der Blutgruppe A bekommen. Der neue immunchromatographische Test zeigte sehr gute Testeigenschaften, war einfach durchzuführen und ließ sich unkompliziert lagern.

Die zweite Studie war so angelegt, dass eine positive Kreuzprobe bei Katzen festgestellt werden konnte nachdem diese eine AB-kompatible Bluttransfusion erhalten hatten, was auf

eine potentielle Alloimmunisation bei den betroffenen Patienten deuten kann. Einundzwanzig anämische stationäre Patienten (Blutgruppe A: n = 20; Blutgruppe B: n = 1) erhielten 1–4 (Median 2) Vollbluttransfusionen über 1–6 Tage (Median 2 Tage) in 33 Transfusionsfällen. Das Blut der Transfusionspatienten wurde wiederholt mittels manueller Röhrchen-Kreuzprobe mit Major- und Minor-Kreuzproben und einer Empfängerkontrolle auf makroskopische und mikroskopische Agglutination überprüft. Es wurden keine Symptome akuter Transfusionsreaktionen beobachtet. Insgesamt wurden 63 Kreuzproben durchgeführt. Die Minorprobe war in allen Fällen negativ. Eine positive Majorprobe wurde in fünf Fällen innerhalb von 2–10 Tagen (Median 5 Tage) nach der Ersttransfusion beobachtet. Diese fünf Katzen hatten 1–4 (Median 2) Vollbluttransfusionen erhalten. Bei 51,5% (17/33) der durchgeführten Transfusionen erhöhte sich der Hämatokrit erwartungsgemäß. Bei Katzen mit einer positiven Majorprobe waren dies 40% (4/10) im Vergleich zu 56,5% (13/23) bei Katzen mit negativer Majorprobe. Die fünf positiven Majorproben-Ergebnisse deuten darauf hin, dass die Empfänger bereits zwei Tage nach der Ersttransfusion Alloantikörper entwickeln können. Derzeit wird eine Kreuzprobe vor einer geplanten weiteren Transfusion bei Katzen nur dann durchgeführt, wenn eine initiale Transfusion vier Tage zurückliegt. Aufgrund der Ergebnisse der zweiten Studie können Empfehlungen für Kreuzproben als Screeningtests vor geplanten Transfusionen ausgesprochen werden.

Beide Studien tragen dazu bei, die Patientensicherheit in der Transfusionsmedizin bei Katzen zu verbessern.

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8 List of Publications

The results of this dissertation have been peer reviewed and published as follows:

- **Alloimmunisation in transfused patients: serial cross-matching in a population of hospitalised cats**. Hourani L, Weingart C, Kohn B. J Feline Med Surg 2017, Dec;19(12):1231-1237
- **Evaluation of a novel feline AB blood typing device**. Hourani L, Weingart C, Kohn B. J Feline Med Surg 2014, Oct;16(10):826-31.

The following parts of this dissertation were presented at conferences (poster presentations):

- **Serial crossmatching in feline transfusion patients**
  Hourani L, Weingart C, Kohn B. 25th annual congress of the European College of Veterinary Medicine – Companion Animals, Lisbon, Portugal, September 10th – 12th 2015

- **Kreuzproben bei felinen Transfusionspatienten**
  Hourani L, Weingart C, Kohn B. 23rd annual symposium of the German Veterinary Association's expert committee on internal medicine and laboratory diagnostics (*23. Jahrestagung der FG „Innere Medizin und klinische* Labordiagnostik *der DVG“* (*InnLab*)), Leipzig, Germany, January 23rd – 24th 2015 (1st prize)

- **Blutgruppenbestimmung bei der Katze: Evaluierung eines Schnelltests (RapidVet-H IC feline**) **zur Bestimmung der Blutgruppen A, B und AB**
  Hourani L, Weingart C, Kohn B. 59th annual congress of the German Veterinary Association (*59. Jahreskongress der Deutschen Veterinärmedizinischen Gesellschaft*), Berlin, Germany, November 7th – 10th 2013 (2nd prize)

- **Evaluation of a Novel Feline AB Blood-Typing Device**
  Hourani L, Weingart C, Kohn B. 2013 American College of Veterinary Internal Medicine Forum, Seattle, Washington, USA, June 12th – 15th 2013

- **Evaluation of a Novel Feline AB Blood-Typing Device**
  Hourani L, Weingart C, Kohn B. 21st annual symposium of the German Veterinary Association's expert committee on internal medicine and laboratory diagnostics (*21. Jahrestagung der FG „Innere Medizin und klinische Labordiagnostik der DVG“* (*InnLab*)), Munich, Germany, February 1st – 2nd 2013

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10 Declaration of independent scholarship

I hereby declare that this doctoral thesis is an original report of my research and has been written by me. Due references have been provided on all supporting literatures and resources.

Layla Hourani
Berlin, the 19th of July 2017