DETERMINATION OF FELINE AB BLOOD TYPES IN BANGKOK AND VICINITIES BY THE TUBE TEST

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การระบุหมู่เลือดชนิดเอบีของแมวในกรุงเทพมหานครและปริมณฑลด้วยการทดสอบในหลอดทดลอง

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ธีรวีร์ แสงแก้ว : การระบุหมู่เลือดชนิดเอบีของแมวในกรุงเทพมหานครและปริมณฑลด้วยการทดสอบในหลอดทดลอง. (DETERMINATION OF FELINE AB BLOOD TYPES IN BANGKOK AND VICINITIES BY THE TUBE TEST) อ.ที่ปรึกษาหลัก : ผศ. สพ.ญ. ดร.สุกัลยา ฤทธิกุลประเสริฐ

ระบบหมู่เลือดเอบีเป็นระบบเลือดที่มีความสำคัญมากที่สุดสำหรับแมว ซึ่งถูกกำหนดโดยการพบว่ามีแอนติบอดีหรือไม่ และแอนติเจนบนผิวของเม็ดเลือดแดง หมู่เลือด เอ บี และเอบี เป็นหมู่เลือดที่พบในแมวได้ทั่วโลก แมวหมู่เลือดบีทุกตัวจะมีแอนติบอดีที่ต่อต้านหมู่เลือดเออย่างรุนแรง ในขณะที่แมวหมู่เลือดเอเพียงบางตัวจะมีแอนติบอดีในระดับสูงที่จะต่อต้านหมู่เลือดบี ซึ่งเป็นสาเหตุที่ทำให้เกิดปฏิกิริยาความไม่เข้ากันของเลือด เมื่อทำการถ่ายเลือดที่ไม่ตรงหมู่เลือดให้กัน ความถี่ของหมู่เลือดในประชากรแมวจึงมีความจำเป็นในการใช้ประเมินความเสี่ยงของปฏิกิริยาความไม่เข้ากันของเลือด จุดประสงค์ของการศึกษาในครั้งนี้คือ การประเมินหาความถี่ของหมู่เลือดระบบเอบีในแมวพันธุ์พื้นเมืองและแมวพันธุ์แท้ในเขตกรุงเทพมหานครและปริมณฑล ประเทศไทย และเพื่อประเมินประสิทธิภาพของวิธีการตรวจการทดสอบการตกตะกอนในหลอดทดลอง โดยทำการเก็บเลือดจำนวน 320 ตัวอย่าง มาจากแมวมีเจ้าของซึ่งมีสุขภาพดีที่อาศัยอยู่ในเขตกรุงเทพมหานครและปริมณฑล ซึ่งถูกนำมาทำการทดสอบหมู่เลือดโดยวิธีมาตรฐานด้วยการทดสอบการตกตะกอนในหลอดทดลอง ภายหลังได้ทำการทดสอบหาความเหมาะสมของน้ำยาที่ใช้ในการตรวจ ณ ภาควิชาอายุรศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย สำหรับการทดสอบแอนติบอดีได้ทำเฉพาะในแมวที่มีผลการทดสอบการตกตะกอนในหลอดทดลองเป็นหมู่เลือดบีหรือเอบี นอกจากนี้ได้มีการทดสอบความคงตัวของน้ำยาที่ใช้ในการทดลองเช่นกัน ผลการศึกษาครั้งนี้ ประกอบด้วย แมวพันธุ์พื้นเมือง 229 ตัว และแมวพันธุ์แท้ 91 ตัว ความถี่ของหมู่เลือดเอและบีอยู่ที่ ร้อยละ 97.5 และ 2.5 ตามลำดับ ไม่พบแมวหมู่เลือดเอบี แมวพันธุ์พื้นเมืองทุกตัวเป็นหมู่เลือดเอ แมวหมู่เลือดบีพบได้ใน ร้อยละ 17.1 ของแมวพันธุ์เปอร์เซีย และร้อยละ 4 ของแมวพันธุ์สก็อตติชโฟลด์ แมวพันธุ์แท้ที่เหลือทั้งหมดเป็นหมู่เลือดเอ อย่างไรก็ดีจำนวนตัวอย่างในกลุ่มแมวพันธุ์แท้ในแต่ละสายพันธุ์ถือเป็นข้อจำกัดของการศึกษา น้ำยาที่ใช้ในการทดสอบการตกตะกอนในหลอดทดลองมีความคงตัวนานอย่างน้อย 21 วัน เมื่อเก็บรักษาไว้ที่อุณหภูมิ 4 องศาเซลเซียส การศึกษาครั้งนี้เป็นการสำรวจครั้งใหญ่เพื่อหาหมู่เลือดเอบีในแมวในเขตพื้นที่กรุงเทพมหานคร ซึ่งบ่งชี้ให้เห็นว่า หมู่เลือดเอเป็นหมู่เลือดที่พบมากที่สุดในแมวพันธุ์พื้นเมือง จึงทำให้แมวเหล่านี้มีความเสี่ยงต่ำต่อการเกิดปฏิกิริยาความไม่เข้ากันของเลือดหากมีการถ่ายเลือดไม่ตรงหมู่เลือดให้กัน และยังได้พบหมู่เลือดบีในแมวพันธุ์แท้อย่างน้อย 2 สายพันธุ์ ดังนั้นการตรวจหาชนิดของหมู่เลือดเอบีในแมวจึงมีความจำเป็นอย่างยิ่งหากจะทำการถ่ายเลือด โดยเฉพาะระหว่างกลุ่มแมวพันธุ์แท้

สาขาวิชา	อายุรศาสตร์สัตวแพทย์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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KEYWORD: AB blood group, Bangkok and vicinities, Blood typing, Cat, Tube test

Teerawee Sangkaew : DETERMINATION OF FELINE AB BLOOD TYPES IN BANGKOK AND VICINITIES BY THE TUBE TEST. Advisor: Asst. Prof. SUKULLAYA RITTHIKULPRASERT, D.V.M., M.Sc., Ph.D., D.T.B.V.M.

The most important blood group system in cats is the AB system, which is identified by the presence and absence of antibodies and the antigen on the surface of red blood cells. Blood type A, B and AB are recognized in cats worldwide. The presence of strong alloantibodies against type A in all type B cats, and those with high titer against type B in some type A cats can cause significant transfusion reactions when cats are transfused with mismatched blood. Frequencies of blood types in a cat population is essential for estimating risks of transfusion reactions. The objectives of the study were to determine the frequencies of AB blood types in purebred and non-purebred domestic cats in Bangkok and vicinities, Thailand, and to assess the performances of the standard tube agglutination test. Whole blood samples were collected from 320 clinically healthy client-owned cats that lived in Bangkok and nearby provinces. Blood typing was performed by a standard tube agglutination method following the optimization of reagents in the assay, at the Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University. The alloantibody test was performed consecutively to determine whether cats were type B or type AB. The stability of reagents used in the assay was also evaluated. Overall, 229 non-purebred (domestic shorthair) cats and 91 purebred cats were enrolled in the study. Of the cats, the frequencies of type A and type B blood were 97.5% and 2.5%, respectively. No type AB cats were identified. All domestic shorthair cats had type A blood. Type B blood was found in 17.1% of Persian and 4.0% of Scottish Fold cats. The rest of purebred cats were type A. However, the sample size of each purebred was limited in this study. The reagents in the tube test were stabilized at 4°C for at least 21 days. This is a large survey of feline AB blood types in the Bangkok area. This study suggests that type A is most common in domestic shorthair cats, so they are at low risk of developing transfusion reaction due to mismatched blood transfusion. Type B was found in at least two purebred cats. Therefore, blood typing is necessary for blood transfusion purposes, particularly among purebred cats.

Field of Study: Veterinary Medicine Student's Signature .....

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LIST OF ABBREVIATIONS

°C	Degree Celsius
μg	Microgram
μL	Microliter
CI	Confidence Interval
CMAH	Cytidine Monophospho-*N*-Acetylneuraminic acid Hydroxylase
CPDA-1	Citrate Phosphate Dextrose Adenine-1
DSH	Domestic shorthair
FeLV	Feline Leukemia Virus
FIP	Feline Infectious Peritonitis
FIV	Feline Immunodeficiency Virus
HPLC	High-Performance Thin-Layer Chromatography
IC	Immunochromatographic
kg	Kilogram
mg	Milligram
mL	Milliliter
NeuAc	*N*-acetylneuraminic acid
NeuGc	*N*-glycolylneuraminic acid
NI	Neonatal Isoerythrolysis
RBC	Red Blood Cell
PBS	Phosphate Buffered Saline
PCV	Packed Cell Volume
TLC	Thin-Layer Chromatography

CHAPTER I
INTRODUCTION

Feline AB blood group system is well recognized in veterinary medicine. There are 3 major blood types in the AB blood group system: type A, B, and AB, which are classified by the antigen on cell surface of erythrocytes (Auer and Bell, 1981). Another blood type, *Mik*, was also reported in cats (Weinstein et al., 2007). In feline medicine, anemia is one of the most common clinical illnesses that may be life-threatening in cats. Blood transfusion is therefore, a necessary procedure of treatment in critical conditions. Because cats may have naturally occurring antibodies against erythrocytes antigens that are different from their own blood types, transfusion of mismatched blood can cause incompatibility reaction. For example, transfusion of type A or AB blood to a type B cat that has high titer of anti-A antibodies will develop severe anaphylactic shock, while a type B cat with a low titer of anti-A antibodies will develop extravascular hemolysis followed by icterus (Yagi and Holowaychuk, 2016). Giger and Bucheler (1991) reported; the mean half-life of transfused type B red blood cells (RBCs) and type A RBCs to a type B cat was 34 days and 1 hours, respectively. Moreover, transfusion of blood from type B or AB cat to a type A cat can result in mild acute reaction or delayed hemolysis (Yagi and Holowaychuk, 2016). The mean half-life of transfused type A and type B RBCs to a type A cat was 32 and 2 days, respectively (Giger and Bucheler, 1991). Compatibility testing between blood of donor and recipient is therefore very important before a blood transfusion. Determination of feline blood types is also significant for cats in some pedigreed catteries or breeders which are aware of neonatal isoerythrolysis (NI) in kittens (Silvestre-Ferreira and Pastor, 2010). In cats, blood compatibility tests can be performed by blood typing, crossmatching, and antibody screening test (also known as back-typing or alloantibody test) (Yagi and Holowaychuk, 2016). Currently, blood typing of the AB blood group system is mandatory before transfusion in practice (Yagi and Holowaychuk, 2016). Nevertheless, transfusion of the same blood type in the AB blood group system does not guarantee that a transfusion reaction will not occur because of the risk for incompatible blood type of the other system. (Weinstein et al., 2007; Tasker et al., 2014; Yagi and

Holowaychuk, 2016). For this reason, crossmatching is often performed concurrently in order to confirm the blood compatibility. However, crossmatching may be limited when the cat has autoagglutination or hemolysis (Yagi and Holowaychuk, 2016). Antibody screening test can be performed using RBCs of known blood types and serum or plasma of the recipient (Milkins et al., 2013; Yagi and Holowaychuk, 2016). This method is less commonly performed. Many assays are used for blood typing, including the slide test, the tube test, the card test, the gel test, the immunochromatographic (IC) test, and the molecular technique (Seth et al., 2011; Yagi and Holowaychuk, 2016). In many previous studies, the tube test is considered to be the gold standard for feline blood typing (Auer and Bell, 1981; Griot-Wenk et al., 1993; Stieger et al., 2005).

The distribution of frequencies of blood types in domestic shorthair (DSH) cats was previously reported worldwide, and results showed the variation of blood type frequencies among breeds of cats and different geographic regions. Blood type A is the most common type in DSH cats worldwide, ranging from 40% to 100% (Auer and Bell, 1981; Ejima et al., 1986; Giger et al., 1991a; Giger et al., 1991b; Jensen et al., 1994; Mylonakis et al., 2001; Silvestre-Ferreira et al., 2004; Arikan et al., 2006; Merbl et al., 2011; Cattin, 2016; Yagi and Holowaychuk, 2016). Cats with type B blood are less common (range, 0% to 36%), whereas type AB cats are rare (Auer and Bell, 1981; Ejima et al., 1986; Giger et al., 1991a; Giger et al., 1991b; Jensen et al., 1994; Silvestre-Ferreira et al., 2004; Malik et al., 2005; Arikan et al., 2006; Forcada et al., 2007; Merbl et al., 2011; Cattin, 2016; Yagi and Holowaychuk, 2016). In some pedigree or purebred cats, British shorthair, Rex, Turkish angora, and Turkish Van cats have higher percentage (up to 60%) of type B than that of other breeds (Giger et al., 1991a; Giger et al., 1991b; Knottenbelt et al., 1999; Arikan et al., 2003; Malik et al., 2005). In addition, several studies reported that 100% of Siamese cats had blood type A. Little is known about frequencies of blood types in purebred and non-purebred cats in Thailand. In addition, blood typing is currently performed using commercially available test kits. These test kits are expensive and may be not afforded by some owners. For this reason, the goal of this study was to perform feline blood typing using a conventional tube agglutination test, instead of commercially available test kits, in order to determine frequencies of AB blood types in domestic cats in Bangkok and vicinities, Thailand.

Objective of Study

1. To determine the frequencies of AB blood types in purebred and non-purebred domestic cats in Bangkok and vicinities, Thailand

2. To assess the performances of the standard tube agglutination test

Hypothesis

Blood types A and B are common in cats in Bangkok and vicinities while type AB is rare.

Keywords (Thai): กลุ่มหมู่เลือดเอบี กรุงเทพมหานครและปริมณฑล การตรวจหมู่เลือด แมว การทดสอบในหลอดทดลอง

Keywords (English): AB blood group, Bangkok and vicinities, Blood typing, Cat, Tube test

Advantages of Study

1. The protocol of the tube agglutination method or the tube test in this study will be used as the reference test for determination of feline AB blood types at the Chula Feline Center, the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University.

2. The tube test is valid, simple to perform by veterinarians and veterinary technicians. It is also cost effective and affordable for owners of cats.

3. Performing the tube test will reduce risks of transfusion reactions due to transfusion of incompatible blood types and will prevent feline neonatal isoerythrolysis in breeders.

4. The results of this study provide the update information regarding the frequencies of AB blood types of the cat population in Bangkok area and vicinities.

5. The list of cats with known blood types and contact information of owners were recorded. This is beneficial for finding blood donor candidates.

CHAPTER II
LITERATURE REVIEW

2.1. Feline blood types

Feline blood types were first investigated in the 1910's (Ingebrigtsen, 1912; Ottenberg and Thalhimer, 1915). Initially, they were classified by the tube test into 3 types: EF, O, and F (Holmes, 1950; Holmes, 1953). Nearly 10 years later, the feline blood group system was assigned by the antigen on the surface membrane of red blood cells (RBCs), as same as human's RBCs (Eyquem et al., 1962). The frequency of blood types showed 85% and 15% of type A and type B cats, respectively, but type AB or type O cats were not identified (Eyquem et al., 1962). Recently, a novel feline blood group system, the *Mik* group, was discovered (Weinstein et al., 2007). However, the method for detecting this blood group system is not available.

2.2. Feline antigen in the AB blood group system

The membrane of RBCs is the phospholipid bilayer that contains glycocalyx (the pericellular matrix which is a glycoprotein and glycolipid that surrounds the cell membrane). There is variation on glycocalyx among different species of mammals (Yamakawa et al., 1960; Reid and Mohandas, 2004). Cats were shown to contain more glycolipids on the erythrocyte membrane than those of other species (Yamakawa et al., 1960). There were several studies that describe feline erythrocyte antigen using numerous techniques, such as thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) (Butler et al., 1991a; Andrews et al., 1992; Griot-Wenk et al., 1993; Silvestre-Ferreira et al., 2011). The *N*-glycolylneuraminic acid-*N*-glycolylneuraminic acid-galactose-glucose-ceramide, also called $(NeuGc)_2G_{D3}$ was the major glycolipids on feline erythrocyte membranes of many cats, however, *N*-acetylneuraminic acid-*N*-acetylneuraminic acid-galactose-glucose-ceramide ($(NeuAc)_2G_{D3}$) was found in 2 Persian cats in one study (Hamanaka et al., 1979). Later, 7 gangliosides (also known as glycosphingolipid with sialic acid on the sugar chain) were discovered from the glycolipid of erythrocyte membranes in Japanese cats (Ando and Yamakawa, 1982). Eventually, the neuraminic

(or sialic) acid on gangliosides was used to define the feline AB blood group system that included type A, type B, and type AB (Andrews et al., 1992; Griot-Wenk et al., 1993; Silvestre-Ferreira et al., 2011). In type B cats, the main ganglioside is $(NeuAc)_2G_{D3}$ and the minor is *N*-acetylneuraminic acid-galactose-glucose-ceramide ($(NeuAc)GM_3$) (Silvestre-Ferreira et al., 2011). The major ganglioside of type A cats is $(NeuGc)_2G_{D3}$, however, $(NeuGc)GM_3$, $(NeuAc)_2G_{D3}$, $(NeuAc)GM_3$, NeuAc-NeuGc-G_{D3} and NeuGc-NeuAc-G_{D3} can be found in various amount (Griot-Wenk et al., 1993; Silvestre-Ferreira et al., 2011). Almost type AB cats have equal NeuAc and NeuGc (Andrews et al., 1992; Griot-Wenk et al., 1993; Silvestre-Ferreira et al., 2011).

The variation of gangliosides on the erythrocyte membrane results from the activity of enzyme (Muchmore et al., 1989; Irie et al., 1998; Bighignoli et al., 2007). The NeuAc is converted to NeuGc by catalyzation of the "cytidine monophospho-*N*-acetylneuraminic acid hydroxylase" (CMAH) enzyme (Muchmore et al., 1989). In general, NeuGc can be found in most mammals except for humans because CMAH is inactivated by the deletion of the coding region (Irie et al., 1998). The mutation of feline CMAH, including 6 single nucleotide polymorphisms (SNPs) and 18-nucleotide insertion/deletion (indel) was also found in type B cats (Bighignoli et al., 2007). SNP G139A and indel 18 were used to identify feline blood type in many laboratories, but there were discordant results between these 2 mutations in some cats. Bighignoli et al. (2007) purposed that the allele of feline AB blood type may be $A>a^{ab}>b$ and the genotype/phenotype would be AA (type A), Aa^{ab} (type A), Ab (type A), $a^{ab}b$ (type AB), $a^{ab}a^{ab}$ (type AB) and bb (type B). Later, Tasker et al. (2014) determined agreement between genotype and phenotype of feline AB blood type. The result showed 100% concordance of type A, using the SNP G139A and SNP C136T, but type AB cats also had type A genotype.

2.3. Feline naturally occurring alloantibodies in the AB blood group system

Cats have naturally occurring alloantibodies against A and B blood types which have high anti-A titers in type B and low or none anti-B titers in type A (Auer and Bell, 1981; Bucheler and Giger, 1993). In type B cats, alloantibody developed in 4-6 weeks of age and reached as adult in 12 weeks of age while type A cats developed in 12

weeks of age (Auer and Bell, 1981; Bucheler and Giger, 1993). Feline alloantibodies may be developed from the response to gastrointestinal microorganisms and food antigens as same as that was proposed for the ABO blood types in humans (Eyquem et al., 1962). This is because the neuraminic acid can be found in many organisms, but this mechanism was still unexplained (Eyquem et al., 1962; Yagi and Holowaychuk, 2016). Neonatal isoerythrolysis (NI) is a life-threatening disease that was reported from kittens due to the transfer of alloantibodies of type B queens to type A neonates via ingestion of colostrum (Eyquem et al., 1962). Because type B cats do not have NeuGc on the RBCs, anti-A antibodies that are strong IgM hemagglutinins and hemolysins will be developed when cats receive the foreign antigen of NeuGc (Yagi and Holowaychuk, 2016). There are several reports indicate that 1 in 3 of type A cats have weak IgM hemagglutinins and IgM and IgG hemolysins (Auer and Bell, 1981; Ejima et al., 1986; Bucheler and Giger, 1993). Whereas, almost 100% of type B cats have anti-A antibody (Auer and Bell, 1981). The variation of amounts of neuraminic acids on the RBCs may affect the variability of anti-B titers of type A cats (Yagi and Holowaychuk, 2016). Type AB cats do not have naturally occurring antibodies against A and B types (Yagi and Holowaychuk, 2016).

2.4. Importance of pre-transfusion compatibility testing

Blood transfusions are lifesaving procedures in veterinary medicine for treating animals with blood loss, hemolysis, and anemia. Cats may be fatal due to several hemorrhagic and hemolytic disorders, such as perioperative or postoperative bleeding, trauma, bleeding of gastrointestinal tract, abdominal neoplasia, immune mediated thrombocytopenia, coagulopathies, immune-mediated hemolytic anemia, and neonatal isoerythrolysis (Barfield and Adamantos, 2011). In addition, cats are at risk of severe anemia because of many diseases, including chronic kidney disease, heavy flea infestation, and hepatic necrosis (Barfield and Adamantos, 2011). Some infectious diseases, including feline immunodeficiency virus (FIV) infection, feline leukemia virus (FeLV) infection, and feline infectious peritonitis (FIP) can cause anemia in cats (Shelton and Linenberger, 1995; Shelton et al., 1995; Norris et al., 2005). Occurrence of blood transfusion reaction can occur when cats are transfused with incompatible blood of

their own types (Cain and Suzuki, 1985; Ejima et al., 1986; Giger and Bucheler, 1991; Casal et al., 1996; Niggemeier et al., 2000; Knottenbelt, 2002; Silvestre-Ferreira and Pastor, 2010; Yagi and Holowaychuk, 2016). Therefore, identification of feline blood type is very important. Blood compatibility testing is mandatory in feline medicine due to risk for transfusion of incompatible blood among feline recipients.

2.5. Blood compatibility testing

Blood compatibility testing can be performed using blood typing, crossmatching, and antibody screening. Blood typing is used to determine erythrocyte antigen of donors and recipients (Holmes, 1950; Auer and Bell, 1981; Giger et al., 1989; Yagi and Holowaychuk, 2016). Due to the presence of alloantibodies against different blood types, this is the most important method that should be performed prior to blood transfusion in cats (Ejima et al., 1986; Bucheler and Giger, 1993; Knottenbelt, 2002). Crossmatching is another method that is practically used. Crossmatching is performed to evaluate blood compatibility when blood types of donors and recipients are unknown. This method consists of a test for the agglutination of donor's RBCs and recipient's serum/plasma (major crossmatching) and a test for the agglutination of donor's serum/plasma and recipient's RBCs (minor crossmatching) (Niggemeier et al., 2000). Antibody screening or alloantibody test or back-typing is the method to determine the reaction between serum/plasma of recipients and RBCs with known type of blood (Milkins et al., 2013; Yagi and Holowaychuk, 2016). In cats, alloantibody test is used to confirm the type B cats which have strong anti-A antibody. Type AB not has both anti-A and anti-B antibody. Type A generally not use because of varying degree of anti-B antibody (Auer and Bell, 1981; Ejima et al., 1986; Bucheler and Giger, 1993). Antibody screening can be used to screen *Mik* blood type in cats; if AB blood system compatibility but crossmatching incompatibility (Weinstein et al., 2007).

2.6. Determination of feline blood types

Many assays are used to determine feline blood types, including slide test, tube test, card test, gel test, immunochromatographic test, and molecular technique (or genotyping) (Seth et al., 2011; Yagi and Holowaychuk, 2016). In several studies, the tube test is considered to be the gold standard or the reference method for feline blood typing (Auer and Bell, 1981; Griot-Wenk et al., 1993; Stieger et al., 2005). Many reagents, including polyclonal antibody, monoclonal antibody, and agglutinated reagent e.g. wheat germ lectin (*Triticum vulgaris*) can be used in the slide and tube tests (Yagi and Holowaychuk, 2016). Lectins are carbohydrate-binding proteins that are found in several sources such as plants, bacteria, mold and membranes of some mammalian cells (Bird, 1989; Butler et al., 1991b). Lectins have ability to agglutinate red blood cells. Each type of lectin has blood group specific (Sharon, 1977; Bird, 1989). The reaction between lectin and carbohydrate is similar to the reaction of an antibody with an antigen (Sharon, 1977). Wheat germ lectin has ability to bind specific carbohydrates like N-acetylneuraminic acid of type B blood gangliosides, and consequently lead to strong agglutination (Nagata and Burger, 1974; Butler et al., 1991b). For this reason, wheat germ lectin can be used instead of anti-B antibody for detecting type B blood. In 1995, the card test is first recognized as a commercially available point-of-care test (Rapid Vet-H Feline, DMS Laboratories). This test is composed of lyophilized monoclonal anti-A antibody and wheat germ lectin for detecting blood type A and B, respectively (Yagi and Holowaychuk, 2016). However, misidentification of blood types can occur due to weak reaction of anti-A antibody to blood type AB, rouleaux formation, autoagglutination, and severe anemia that causes the prozone effect (Niggemeier et al., 2000; Barrs et al., 2009; Seth et al., 2011). The gel test is a gel column technique that was modified to be a commercial assay (DiaMed-Vet ID card A+B and Rapid Vet-H Gel, DMS Laboratories) using monoclonal anti-A and anti-B antibodies or monoclonal anti-A antibody and wheat germ lectin (Stieger et al., 2005; Proverbio et al., 2011; Seth et al., 2011). It has similar accuracy to the tube test (Stieger et al., 2005; Seth et al., 2011). However, it is no longer available (Yagi and Holowaychuk, 2016). The IC test is a new point-of-care test using lateral flow technique that is composed of monoclonal anti-A and anti-B antibodies (Quick Test

A+B, Alvedia and Rapid Vet H IC, DMS Laboratories). Rouleaux formation and autoagglutination did not affect the result of this test and performances were higher than those of the card test. However, weak reaction of anti-A antibody to type AB blood can occur (Knottenbelt and Mackin, 1998). False negative results of IC test maybe possible from sample of severe anemic cats. Because, degree of intensity of red color on strip depend on hemoglobin. In laboratory method, the slide and the tube tests have likely same result, but the slide test has no negative control and no concentration adjustment of RBCs. A previous study demonstrated that the slide and gel tests had 99% agreement with the tube test, while the IC and card tests had 95% and 91% agreement with the tube test, respectively (Seth et al., 2011).

2.7. Frequencies of feline blood types

There are several studies that reported frequencies of blood types in cats. Most results found that the frequency of feline blood types depends on geographical regions and breeds of cats (Silvestre-Ferreira and Pastor, 2010; Cattin, 2016; Yagi and Holowaychuk, 2016). In general, blood type A is most common in all breeds, blood type B is less common, and blood type AB is rare (Yagi and Holowaychuk, 2016). Although type B cats were usually uncommon, frequencies were reported from many countries with a wide range of distribution, including North America, South America, northern and southern England, Ireland, Italy, Greece, South Africa, Japan, Israel, Turkey, Australia, and New Zealand (Auer and Bell, 1981; Ejima et al., 1986; Giger et al., 1989; Giger et al., 1991a; Hubler et al., 1993; Jensen et al., 1994; Knottenbelt et al., 1999; Mylonakis et al., 2001; Silvestre-Ferreira et al., 2004; Malik et al., 2005; Arikan et al., 2006; Weingart et al., 2006; Forcada et al., 2007; Juvet et al., 2011; Merbl et al., 2011; Cattin, 2016; Yagi and Holowaychuk, 2016). Frequencies of blood type B varied among different countries as well as different areas of the same countries. For example, 0 – 10% of cats in most parts of North America had blood type B, while cats in the western North America had 10-20% of blood type B (Giger et al., 1991b; Yagi and Holowaychuk, 2016). Type AB of DSH was about 1% of cats (Yagi and Holowaychuk, 2016). In purebred or pedigree cats, distributions of blood types varied in individual breeds. British shorthair, Rex, Turkish angora, and Turkish Van had a high percentage of

type B (Giger et al., 1991a; Giger et al., 1991b; Knottenbelt et al., 1999; Arikan et al., 2003; Malik et al., 2005). Numerous studies reported 100% of blood type A in Siamese cats, but type B Siamese cats were also found (Yagi and Holowaychuk, 2016).

In Thailand, blood typing is limited to some veterinary hospitals where the expense can be afforded by owners. In addition, little is known about the current frequency of blood types in domestic cats in Thailand. Therefore, this study is proposed to develop a conventional tube test which is a cost-effective method for blood typing in cats. The tube test was primarily used for evaluation of the frequency of feline blood types in Bangkok and vicinities, Thailand. Hopefully, the tube test will be helpful for confirmation of blood compatibility between feline recipients and donors, decreasing the cost of blood testing, and reducing the risk for transfusion reaction.

CHAPTER III

MARERIALS AND METHODS

3.1. Conceptual framework of this study

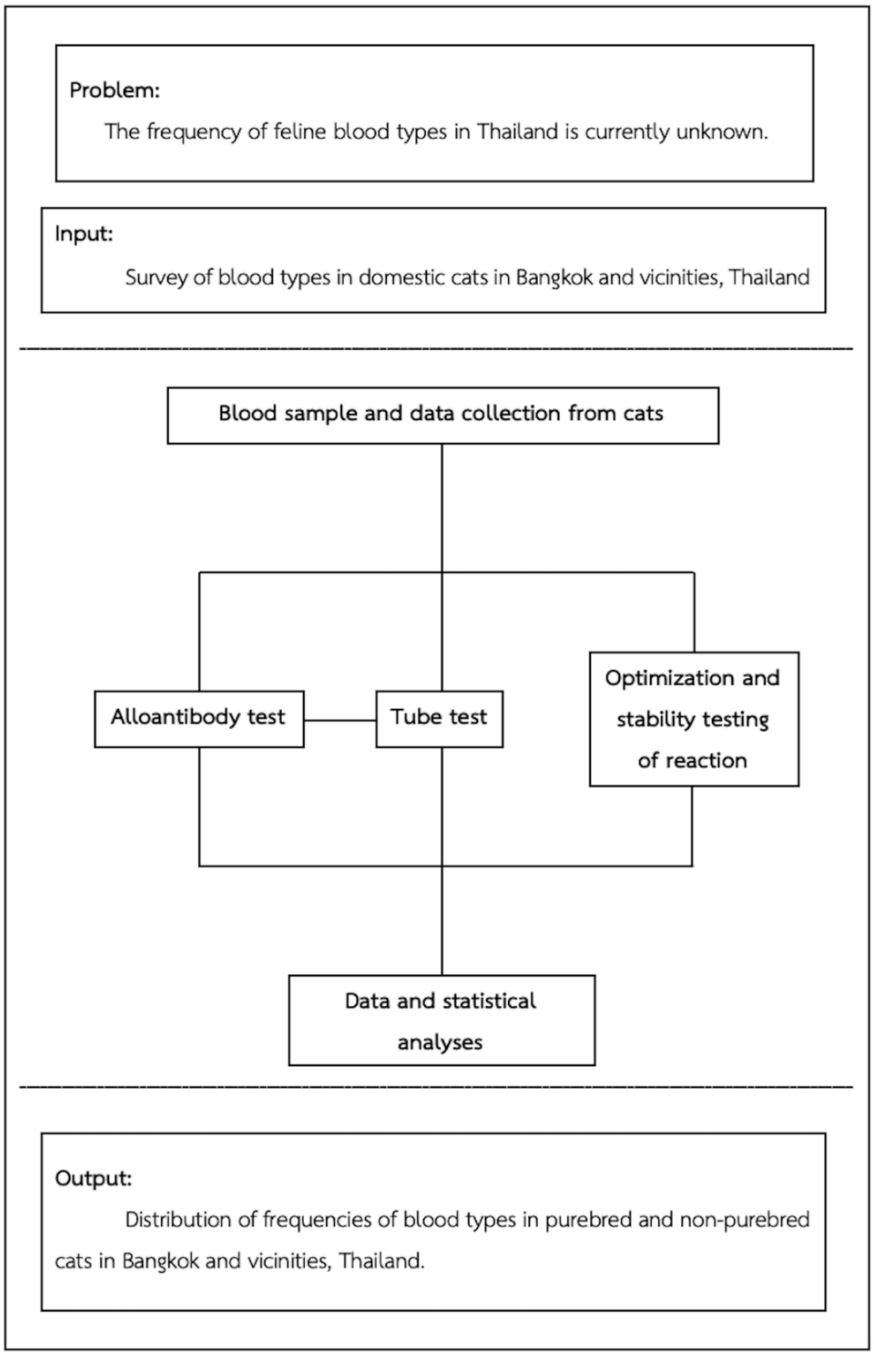

Figure 1 The conceptual framework of this study.

3.2. Animals

This study was approved by the Chulalongkorn University Animal Care and Use Committee (protocol number 1831083). The sample size calculation was demonstrated in the section of statistical analysis. Client-owned cats that visited the Small Animal Teaching Hospital of Faculty of Veterinary Science, Chulalongkorn University and some participated private clinics from January to June 2019, were included after consents signed by owners. The inclusion criteria of the cats were clinically healthy cats that were older 3 months old and had body weight more than 1.0 kg. Cats were excluded if they had history of transfusion with blood products or they were clinically ill. Medical records, including signalment, address, reasons for visiting, were recorded from all cats.

3.3. Sample collection

Whole blood 1.5 mL was collected from a peripheral vein of each cat and collected in an EDTA-containing blood tube. The packed cell volume (PCV) was measured by the centrifugation method which blood was filled in a capillary tube and placed in a hematocrit centrifuge. The blood sample was centrifuged for 5 minutes at 15,300 x *g* at room temperature (20-25°C). The value of PCV was expressed as percentage (%) of the red blood cells in the blood for preparing of 2-5% RBC suspension. The remaining volume (1 mL) of blood samples were stored at 4°C until used within 24 hours.

3.4. Blood typing by the tube test

3.4.1. Preparation of lectin (*Triticum vulgaris*) solution (Anti-B reagent)

This procedure was performed following the procedure in previous studies (Griot-Wenk et al., 1993; Stieger et al., 2005; Seth et al., 2011; Yagi and Holowaychuk, 2016). Ten mg of lyophilized powder of *Triticum vulgaris* (Sigma-Aldrich, St. Louis, Missouri, USA) were dissolved in phosphate buffered saline (PBS) to prepare the stock solution with concentration of 0.1 mg/mL, then aliquoted to 1 mL and frozen at -20°C until used. For preparation of the working solution, 1 mL of stock solution was diluted

with 11.5 mL of PBS (1:11.5) to achieve the concentration of 8 μg/mL. The working solution of lectin was refrigerated and used within 1 week.

3.4.2. Preparation of anti-A reagent from a cat with blood type B

Whole blood (10 mL) was withdrawn from the jugular vein of a cat with known blood type B (assessed by lectin solution agglutination and back typing test). Blood was collected in a plain tube without an anticoagulant, left at the room temperature for 30 minutes for blood clotting. Then, blood was centrifuged for 10 minutes at 1,000 x *g* at room temperature. Serum was separated and diluted at 1:8 with 7 mL of PBS. Diluted serum was aliquoted to 0.5 mL and store at -20°C for being used in the tube test (Stieger et al., 2005; Yagi and Holowaychuk, 2016).

3.4.3. Preparation of 2-5% RBC suspension of blood sample

For each blood sample (from the section 3.3), 1 mL of blood was transferred to a new 10-mL tube and centrifuged for 2 minutes at 1,000 x *g* at room temperature. Plasma was separated and stored at -20°C for being tested in the back-typing test, as described below. The RBC pellet was washed by adding 5 mL of PBS in the tube which was vortexed and centrifuged for 2 minutes at 1,000 x *g* at room temperature. The supernatant was discarded. The washing step was repeated twice. After the second washing step, the RBC pellet was diluted to 2-5% suspension by addition of appropriate volume of PBS solution in the tube, which was vortexed gently (Stieger et al., 2005; Seth et al., 2011).

3.4.4. Evaluation of feline blood types by the tube test

The following protocol was performed according to previous reports (Stieger et al., 2005; Seth et al., 2011; Yagi and Holowaychuk, 2016). At the beginning, 3 test tubes (Hycon® Polystyrene tube 12x75 mm, BIOMED CO., LTD, Bangkok, Thailand) were prepared that included the control tube, used for detection of autoagglutination; the test tube 1, used for testing of type B blood; and the test tube 2, used for testing of type A blood. The control tube was prepared by addition of 50 μL of PBS solution

and 25 μL of 2-5% RBC suspension of blood sample. The test tube 1 was prepared by addition of 50 μL of (8 μg/mL) lectin solutions and 25 μL of 2-5% RBC suspension of blood sample. The test tube 2 was prepared by addition of 50 μL of anti-A reagent and 25 μL of 2-5% RBC suspension of blood sample. Then, all tubes were gently agitated and incubated for 15 minutes at room temperature. After incubation, the tubes were centrifuged for 15 seconds at 1,000 x *g* at room temperature. The tubes were gently agitated again, followed by an observation for agglutination over white background.

Test results were read by appearance of the agglutination reaction macroscopically that include "Negative", tiny or none aggregates of RBCs on turbid red background; "Agglutination 1+", small aggregates of RBCs on turbid red background; "Agglutination 2+", medium aggregates of RBCs on clear background; and "Agglutination 3+", many large aggregates of RBCs on clear background; "Agglutination 4+", one solid aggregates on clear background (Yagi and Holowaychuk, 2016). Type A blood was strong agglutinated (3+ or 4+) with anti-A reagent or serum of type B cat but was non-agglutinated with negative control solution (PBS solution) and anti-B reagent (Lectin solution). Type B blood was strong agglutinated (3+ or 4+) with anti-B reagent or Lectin solution but was non-agglutinated with negative control solution (PBS solution) and anti-A reagent (serum of type B cat). However, type AB blood was moderate agglutinated (2+ or 3+) with anti-A reagent or serum of type B cat and anti-B reagent or Lectin solution but was non-agglutinated with negative control solution (PBS solution).

3.5. Test for feline alloantibody (back typing test)

The back-typing test was performed on all blood samples that were identified as type B and type AB form the tube test for confirmation of B or AB blood type. Two tubes were prepared by adding 50 μL of plasma sample (from the section 3.4.3 of each cat) in both tubes, followed by adding 25 μL of 2-5% RBC suspension with known types A and B in tubes 1 and 2, respectively. The tubes were agitated gently and incubated for 15 minutes at room temperature. After incubation, the tubes were

centrifuged for 15 seconds at 1,000 x *g*, at room temperature, and agitated gently again. The agglutination reaction was observed over the white background. The results were read and interpreted following the criteria of the tube test (Section 3.4.4). Plasma of type B blood had strong aggregation (+3 or +4) with type A RBC suspension, while plasma of type AB blood had no agglutination both type A and B RBC suspension.

3.6. Optimization and stability testing of reaction

3.6.1. The stability of blood typing results after testing

After the tube test was performed, a set of 10 tubes were stored at 4°C throughout the experiment. The tubes were rechecked or re-evaluated for the agglutination reaction every day for a total of 7 days.

3.6.2. The stability of lectin solution (Anti-B reagent)

Whole blood (14 mL) was collected from a peripheral or jugular vein of 2 individual cats, which had blood type A and blood type B. Blood of each cat was collected in a citrate phosphate dextrose adenine (CPDA-1)-containing blood tube (1 mL CPDA-1: 7 mL whole blood) and stored at 4°C throughout the experiment. The stock solution of lectin was diluted to working solution and aliquoted to 1 mL and stored at 4°C for 21 days. The blood type A and type B were withdrawn (1 mL each) from the CPDA-1 bags and used in the tube test following the protocol in the section 3.4.4. The tube test was performed on day 0, 1, 2, 3, 4, 5, 6, 7, 14, and 21. The agglutination reaction was read and recorded.

3.6.3. Optimization of lectin concentration (Anti-B reagent)

The stock solution of lectin (1 mL) was diluted. Finally, there were 3 different concentration of lectin solution (8, 4 and 2 μg/mL). Each concentration of lectin solution was used in test with 2-5% RBC from blood stored in CPDA-1 (in the section 3.6.2).

3.6.4. The stability of anti-A reagent

The anti-A reagent collected from the section 3.4.2 was divided to 10 tubes (0.1 mL/tube) and stored at 4°C. The tube tests were repeated at day 0, 1, 2, 3, 4, 5, 6, 7, 14 and 21, using a mixture of 50 μL of anti-A reagent and 25 μL of 2-5% RBC from blood stored in CPDA-1 (in the section 3.6.2).

3.6.5. Optimization of anti-A reagent concentration

The serum (0.5 mL) was collected from type B cat. Then, the serum was diluted by 2-fold dilution with PBS solution. Thus, there were three concentration of anti-A reagent (1:8, 1:16 and 1:32). Each concentration of anti-A reagent was used in test with 2-5% RBC from blood stored in CPDA-1 (in the section 3.6.2).

3.7. Statistical analysis

For sample size calculation, frequencies of blood types in the AB blood group system were previously reported from many countries (Yagi and Holowaychuk, 2016). Most studies found that 99% of DSH cats were estimated to have blood type A, 1% of DHS cats had blood type B, and less than 1% of DSH cats had blood type AB. However, limited data were reported from Thailand. For this reason, a statistical program (EpiTools epidemiological calculators, Ausvet 2019) was used to calculate the sample size with these following parameters: Estimated true proportion, 0.01 (1% of B+AB); Desired precision, 0.05; Confidence level, 0.95; Population size, 50,000. The calculated output showed that the maximum number of 385 cats was appropriate for this study.

Descriptive analyses were used for data relating to ages, breeds, and sexes of cats, and frequency of all blood types. Age of cats was expressed as median and range. Breeds, sexes, and frequency of blood types was presented as percentage and 95% confidence interval (95% CI) of cats from the total number of the cats, as shown in the tables. Distribution of blood types according to residential areas of cats were presented as percentage, as shown in the map chart. Statistical analyses were performed using a statistical software (IBM SPSS Statistics 22, IBM Corporation).

CHAPTER IV
RESULTS

4.1. Demographic distribution

The population of study included 320 cats that were 229 DSH cats (71.6%) and 91 purebred cats (28.4%). The proportion between DSH and purebreds in Bangkok was approximately 3 : 1 (201 DSH cats: 67 purebred cats), and in vicinities was approximately 1.2 : 1 (28 DSH cats: 24 purebred cats). Purebred cats were Persian (n=41), Scottish Fold (n=25), American shorthair (n=5), British shorthair (n=4), Exotic shorthair (n=4), Maine Coon (n=4), Siamese (n=2), Sphynx (n=2), American Curl (n=1), Munchkin (n=1), Norwegian Forest Cat (n=1) and Ragdoll (n=1). The median age was 3 years old (range, 3 months to 25 years). There were 151 female (47.2%) and 169 male (52.8%) cats. Mean of PCV was 38.9% $\pm$ 6.9 (range 20% to 55%). Cats were brought to the hospitals due to health checkup (77.5%) and presence of illnesses (22.5%) which were not associated with hematologic or emergency diseases. The illnesses that presented were dermatological (n=14), gastrointestinal (n=11), respiratory tract (n=18) and urinary tract diseases (n=29).

Residential areas of cats in this study were displayed in Figure 2. Cats resided in 39 of 50 districts of Bangkok and 12 districts in 4 nearly provinces. In Bangkok, cats were from Bang Khen (n=40), Khlong Sam Wa (n=40), Prawet (n=27), Ratchathewi (n=14), Sathon (n=12), Huai Khwang (n=11), Khlong Toei (n=11), Suan Luang (n=10), Yan Nawa (n=10), Bang Rak (n=9), Bang Na (n=7), Phra Khanong (n=7), Watthana (n=6), Dusit (n=5), Phasi Charoen (n=5), Chatuchak (n=4), Chom Thong (n=4), Khan Na Yao (n=4), Pathum Wan (n=4), Pom Prap Sattru Phai (n=4), Thon Buri (n=4), Thung Khru (n=4), Bang Bon (n=3), Bang Kho Laem (n=3), Min Buri (n=3), Rat Burana (n=2), Sai Mai (n=2), Taling Chan (n=2), Bang Khae (n=1), Bang Khun Thian (n=1), Bang Phlat (n=1), Bang Sue (n=1), Bangkok Yai (n=1), Bueng Kum (n=1), Don Mueang (n=1), Lat Phrao (n=1), Nong Khaem (n=1), Phaya Thai (n=1) and Samphanthawong (n=1). There were 4 provinces of sampling area in vicinities. First, Pathum Thani included Lam Luk Ka (n=19) and Mueang Pathum Thani (n=5). Second, Samut Prakan included Bang Phli (n=9), Mueang Samut

Prakan (n=3), Bang Bo (n=2), Phra Samut Chedi (n=2) and Phra Pradaeng (n=1). Third, Nonthaburi included Mueang Nonthaburi (n=5), Pak Kret (n=3), Bang Bua Thong (n=1) and Bang Kruai (n=1). Lastly, Samut Sakhon included Mueang Samut Sakhon (n=1).

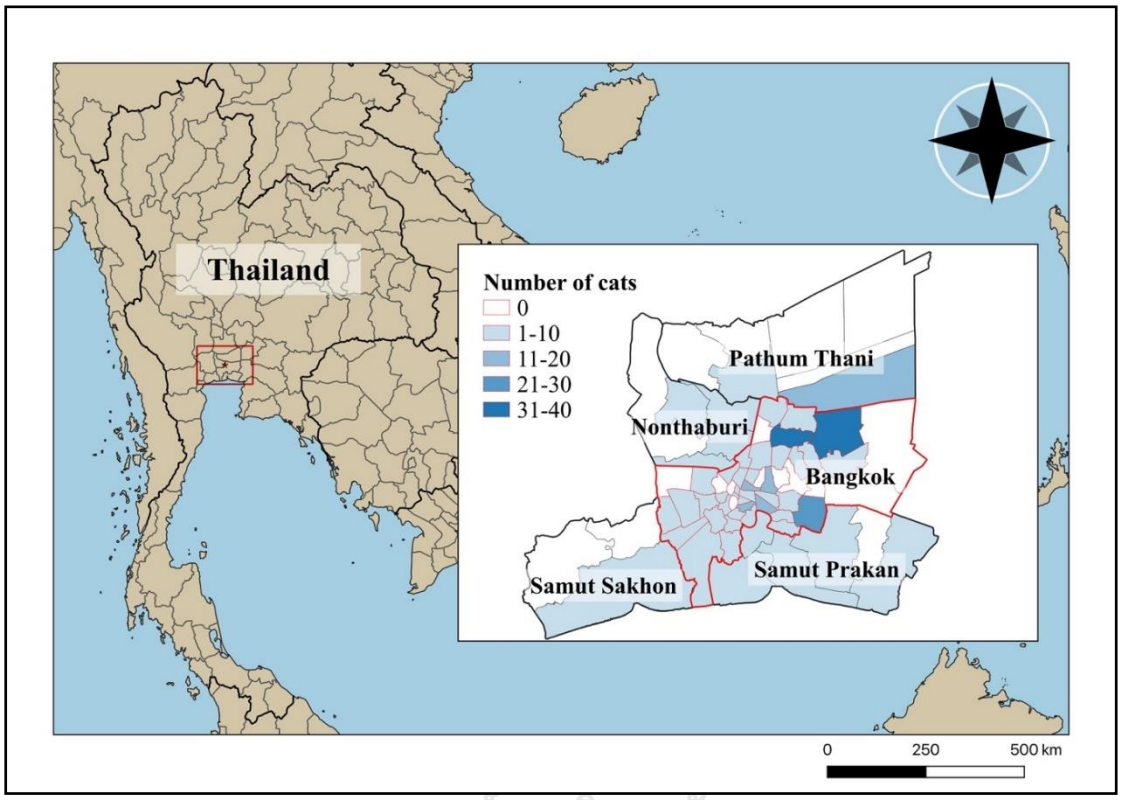

Figure 2 Distribution of locations of cats. The large map represented the map of Thailand. The red star indicated Bangkok. The red rectangle located the Bangkok and vicinity area. The small map displayed the distribution of residential areas of 320 cats. The red line indicated the boundary of the Bangkok area.

4.2. Frequencies of feline blood types

The results of the tube test showed strong agglutination reaction (3+ to 4+) for type A and type B cats. Type A blood was agglutinated with anti-A reagent or serum of type B cat (Figure 3) but was non-agglutinated with negative control solution (PBS solution) and anti-B reagent (Lectin solution). However, type B blood was agglutinated

with anti-B reagent or lectin solution (Figure 4) but was non-agglutinated with negative control solution (PBS solution) and anti-A reagent (serum of type B cat). Type AB not found in this study. Alloantibody tests had strong agglutination reaction (3+ to 4+) in all type B cats.

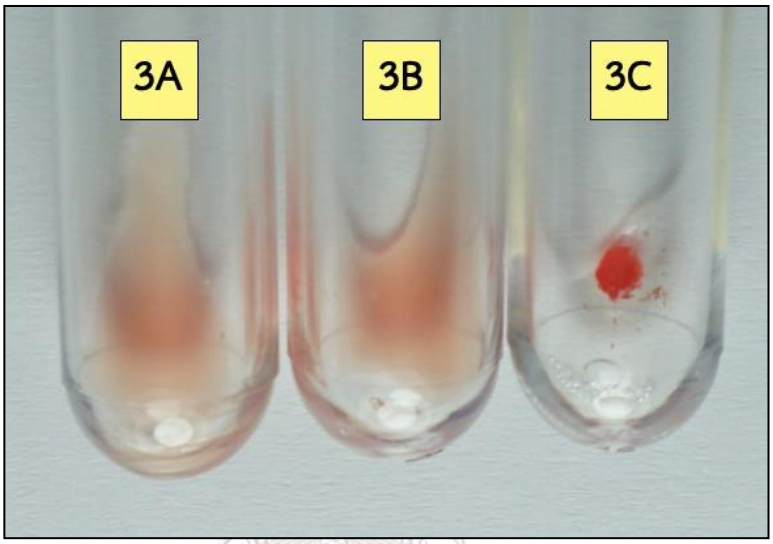

Figure 3 Macroscopic agglutination reaction of the tube test of a cat with blood type A. 3A, negative control (PBS solution); 3B, negative result (lectin solution); 3C, agglutination 4+ (anti-A reagent).

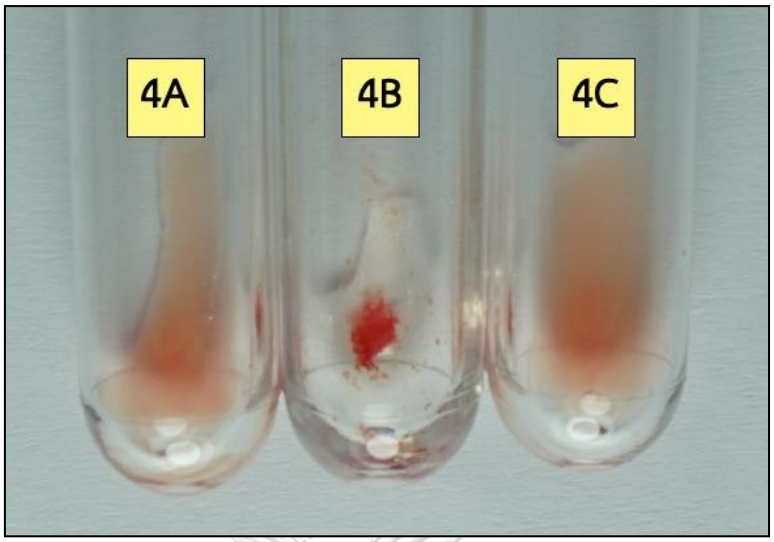

Figure 4 Macroscopic agglutination reaction of the tube test of a cat with blood type B. 4A, negative control (PBS solution); 4B, agglutination 4+ (lectin solution); 4C, negative result (anti-A reagent).

The overall blood types were displayed in Table 1. There were 97.5% of type A cats, 2.5% of type B cats, and 0% of type AB cats. Type A blood was found in DSH (100%), Persian (82.9%), Scottish Fold (96.0%), American shorthair (100%), British shorthair (100%), Exotic shorthair (100%), Maine Coon (100%), Siamese (100%), Sphynx (100%), American Curl (100%), Munchkin (100%), Norwegian Forest (100%) and Ragdoll cats (100%). However, type B blood was found in Persian (17.1%) and Scottish Fold cats (4.0%). Type A cats were distributed in 50 districts of Bangkok and vicinities as shown in Figure 5. Type B cats were found in 7 districts of Bangkok and vicinities, including Khlong Sam Wa (n=2), Chom Thong (n=1), Sathon (n=1), Taling Chan (n=1), Thung Khru (n=1), Yan Nawa (n=1) and Bang Kruai (n=1) (Figure 5).

Table 1 The number, percentage, and 95% confidence interval of each feline blood types and breeds from Bangkok and vicinities.

Area		Bangkok			Vicinities			Overall		
Blood types		A	B	AB	A	B	AB	A	B	AB
Breed	Number									
Total	320	261	7	0	51	1	0	312 (97.5%: 95.8-99.2)*	8 (2.5%: 0.8-4.2)	0
Domestic shorthair	229	201	0	0	28	0	0	229 (100%)	0	0
Persian	41	27	6	0	7	1	0	34 (82.9%: 70.9-94.9)	7 (17.1%: 5.0-29.0)	0
Scottish Fold	25	14	1	0	10	0	0	24 (96.0%: 87.7-104.3)	1 (4.0%: 0-12.3)	0
American shorthair	5	5	0	0	0	0	0	5 (100%)	0	0
British shorthair	4	4	0	0	0	0	0	4 (100%)	0	0
Exotic shorthair	4	0	0	0	4	0	0	4 (100%)	0	0
Maine Coon	4	4	0	0	0	0	0	4 (100%)	0	0
Siamese	2	1	0	0	1	0	0	2 (100%)	0	0
Sphynx	2	2	0	0	0	0	0	2 (100%)	0	0
American Curl	1	1	0	0	0	0	0	1 (100%)	0	0
Munchkin	1	1	0	0	0	0	0	1 (100%)	0	0
Norwegian Forest	1	0	0	0	1	0	0	1 (100%)	0	0
Ragdoll	1	1	0	0	0	0	0	1 (100%)	0	0

* Number (percentage: 95% confidence interval)

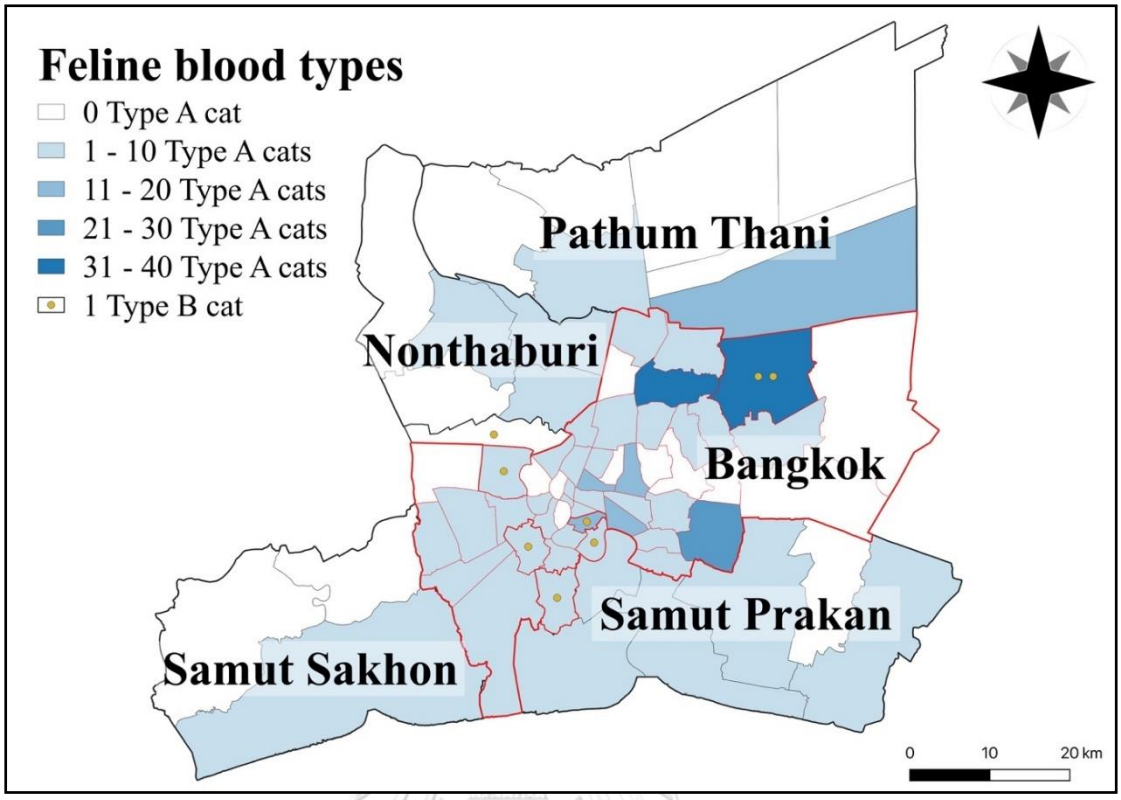

Figure 5 Distribution of feline blood type A and B in Bangkok and vicinities of Thailand. The blue areas were the districts where type A cats lived. The different shades of blue color indicated different number of type A cats. Yellow dots located the place of type B cat. Red lines were the Bangkok area.

4.3. Optimization and stability testing of reaction

4.3.1. The stability of blood typing results after testing

After the tube test was performed, a set of 10 tubes of samples were re-evaluated for the agglutination reaction. In this experiment, the results of all tubes were the same being stored at 4°C for a total of 7 days.

4.3.2. The stability of lectin solution (Anti-B reagent)

The agglutination reaction throughout the experiment showed that there were no differences in results that were performed consecutively until day 21 (Figure 6 and 7). The lectin solution was stabilized for 21 days.

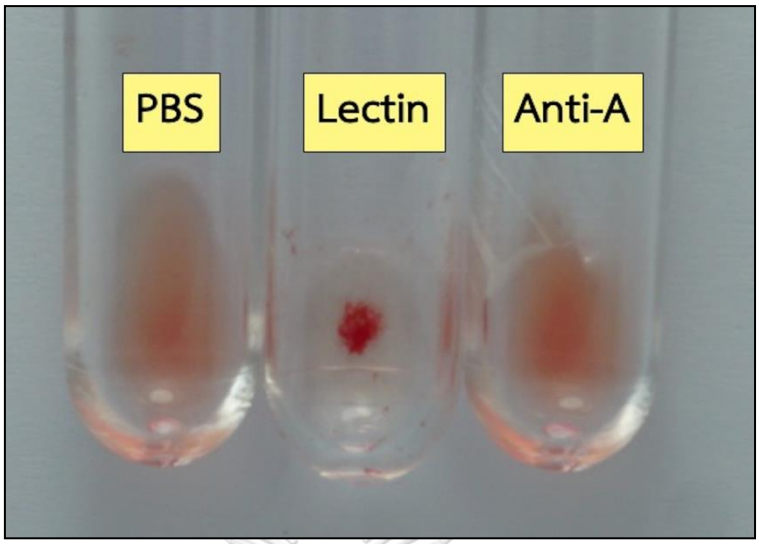

Figure 6 The agglutination reaction result of stability of lectin solution on Day 0.

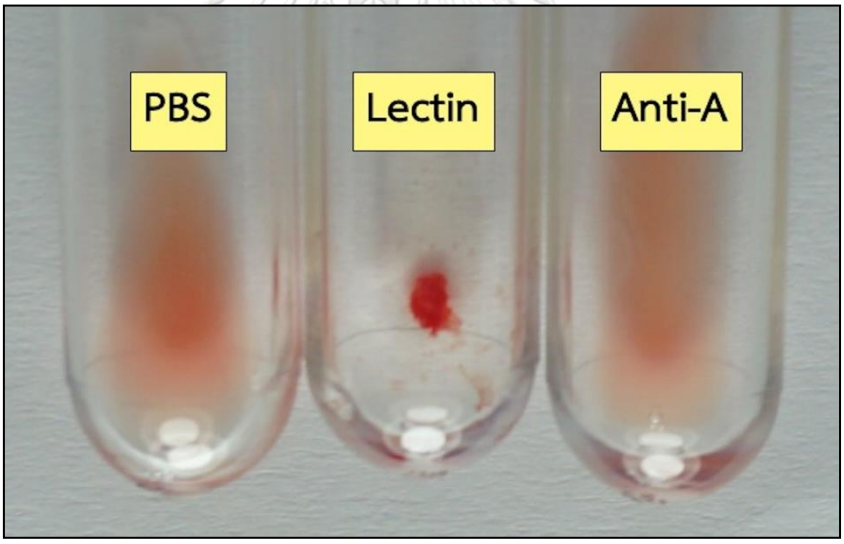

Figure 7 The agglutination reaction result of stability of lectin solution on Day 21.

4.3.3. Optimization of lectin concentration (Anti-B reagent)

The 3 different concentrations of lectin solution (8, 4 and 2 μg/mL) was used in test. First, the test result of the concentration of 8 μg/mL showed grade 4+ agglutination reaction. Second, the tube of 4 μg/mL was grade 2+ (medium aggregates, clear background). Finally, the test result was negative in the tube of 2 μg/mL of concentration. The results were shown in Figure 8. This experiment showed that the concentration of lectin solution at 8 μg/mL was the most optimized concentration in the tube test.

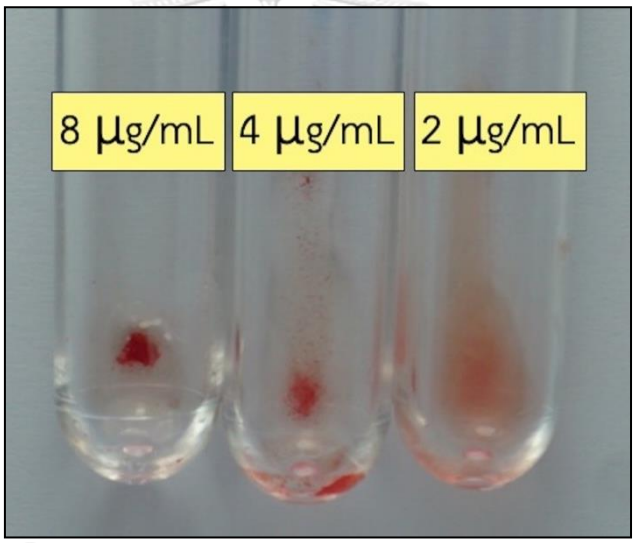

Figure 8 The result of agglutination reaction in each concentration of lectin solution.

4.3.4. The stability of anti-A reagent

The results from Day 0 to Day 21 were the same (Figure 9 and 10). The anti-A reagent was stabilized for 21 days.

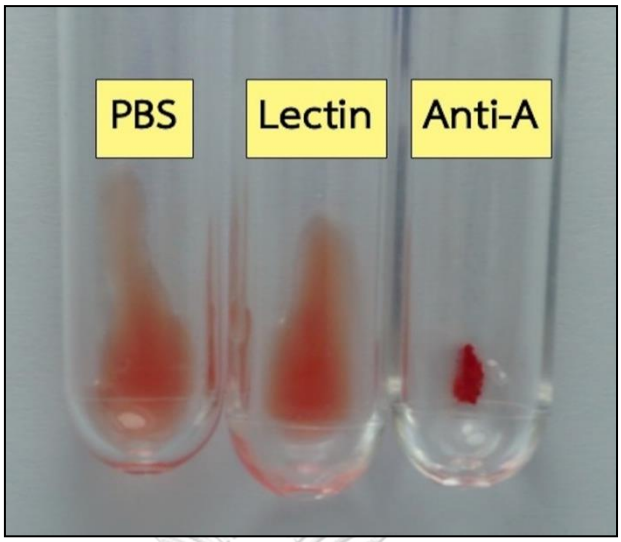

Figure 9 The agglutination reaction result of stability of anti-A reagent on Day 0.

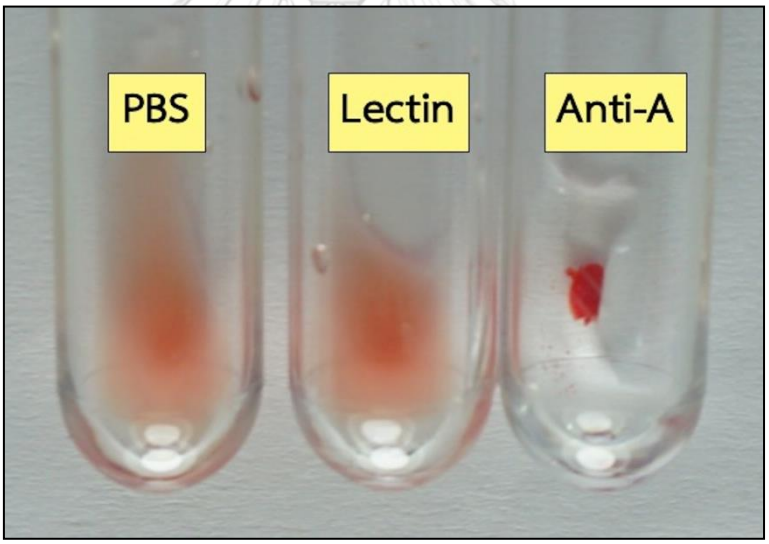

Figure 10 The agglutination reaction result of stability of anti-A reagent on Day 21.

4.3.5. Optimization of anti-A reagent concentration

The 3 different concentrations of anti-A reagent (1:32, 1:16 and 1:8) were used in the tube test. First, the concentration of 1:8 was grade 4+ of agglutination reaction. Second, the tube of 1:16 was grade 2+. Finally, there was negative result in 1:32 of concentration. The result was shown in Figure 11. This experiment showed that the concentration of anti-A reagent at 1 : 8 was the most optimized concentration in the tube test.

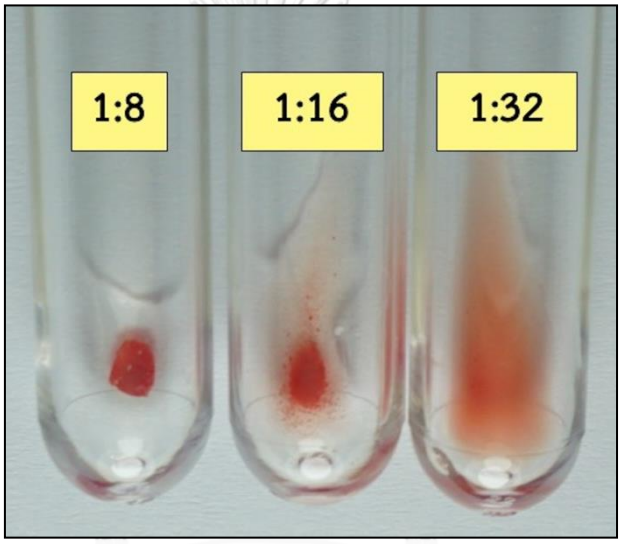

Figure 11 The result of agglutination reaction in each concentration of anti-A reagent.

CHAPTER V
DISCUSSION

In this study, there were 201 DSH cats from 32 districts of Bangkok and 28 DSH cats from 4 districts of nearby provinces. Additionally, there were 67 purebred cats in 23 districts of Bangkok and 24 purebred cats in 10 districts of nearby provinces. To the best of our knowledge, this study is the largest survey for frequencies of blood types in healthy cats in Thailand. In Bangkok, the proportion of DSH cats were 3 times higher than the proportion of purebred cats, while the proportion was similar between DSH and purebred cats (1.2 : 1) in nearby provinces. This might suggest the preference or the trend of having DSH and purebred cats of people in various locations. In cats, the opened system blood collection is a routine method for blood transfusion. Blood which is collected by the opened system should immediately be transferred to a recipient because of contaminated risk (Yagi and Holowaychuk, 2016). Due to a lack of feline blood bank in clinical settings, stored blood or processed blood products, such as packed red cells, fresh frozen plasma, etc. are usually unavailable for being used in an emergency condition. Thus, it is suggested that making lists of donor cats with known blood types by veterinary hospitals will be helpful to find a donor cat that is urgently needed.

The tube test is the gold standard to detect feline blood types (Auer and Bell, 1981; Griot-Wenk et al., 1993; Stieger et al., 2005). The advantages of the tube test that are superior to other methods such as slide test, card test and IC test, are many reasons. First, the blood samples will be washed with PBS or normal saline before using to decrease false positive from rouleaux formation. Second, the concentration of RBCs is adjusted to decrease the false negative results from polycythemic or anemic blood. Third, the tube agglutination test includes negative control which can identify autoagglutination. Finally, the result of tube test or the agglutination reaction can be interpreted by naked eyes or macroscopic method without the requirement of an equipment such as light microscope. The limitations of the tube test are time-consuming and requirement of many equipment and experienced persons (Yagi and Holowaychuk, 2016). Additionally, the volume of samples in the tube test is needed

more than other methods. Thus, the tube test is not always needed in all situations, particularly in emergency condition. It is suitable to used to determine blood type for health checkup or mating cats to prevent NI or mismatch blood transfusions.

In this study, the results in the tube test showed strong agglutination reaction (3+ to 4+) in all positive tubes similar to previous experiments (Stieger et al., 2005; Zheng et al., 2011). Lectin has strong reaction with antigen of type B and has no reaction with antigen of type A (Nagata and Burger, 1974; Butler et al., 1991b). On the other hand, anti-A antibodies of type B cat had strong reaction with antigen of type A and was not reactive to the antigen of type B (Yagi and Holowaychuk, 2016). It suggests that the reagents in this study can detect the antigen on the surface of RBCs which was similar to standard tube agglutination test in other studies. However, this study did not find a cat with AB blood type which has weak agglutination reaction than type A and B.

Back typing test or alloantibody test is commonly used to confirm type B cats from the result of antigen test, because type B cats have strong anti-A antibody and type AB cats do not have antibodies (Auer and Bell, 1981; Ejima et al., 1986; Bucheler and Giger, 1993). In this study, the results of back typing test were consistent to the tube test and revealed a strong agglutination reaction (3+ to 4+) with type-A RBCs similar to previous studies, because type B cats have strong anti-A antibody in serum or plasma (Stieger et al., 2005; Zheng et al., 2011). Due to back typing test can differentiate type B from AB. Thus, it should be performed test to confirm when type B and AB are found in the tube test.

In this study, the frequency of blood type in all DSH cats in Table 2 was 100% type A. This result was the same as reports from some countries such as Hungary and Philadelphia of USA (Bagdi et al, 2001; Giger et al., 1989). However, most of other countries such as Australia, China, Greece, Ireland, Israel, Italy, New Zealand, Portugal, UK and another part of USA had varied proportion in the AB blood types (Cattin, 2016; Forcada et al., 2007; Giger et al., 1991b; Juvet et al., 2011; Knottenbelt et al., 1999; Malik et al., 2005; Merbl et al., 2011; Mylonakis et al., 2001; Proverbio et al., 2011; Silvestre-Ferreira et al., 2004; Zheng et al., 2011). It indicates that DSH cats have a variation of the AB blood type depending on geographical locations. The extremely

high type A DSH cats in Thailand is not clearly expained in this study. The supposed origin of Thai DSH cats is Siamese cats that have 100% blood type A (Giger et al., 1991a; Malik et al., 2005; Silvestre-Ferreira et al., 2004; Yagi and Holowaychuk, 2016). From the result of this study, DSH cats in this area should have low risks of mismatched transfusion reactions and NI.

However, Suparp et al. (2017) found that frequencies of blood types in DSH cats in Bangkok, Thailand were 98.4% of type A, 0.5 % of type B and 1.0% of type AB. The difference between this previous study and the present study was the method for detection of blood type and cat population. The previous study (Suparp et al., 2017) performed blood testing using a commercial immunochromatographic test (Quick Test A+B, Alvedia) and included cats with healthy and ill conditions. Some diseases such as FeLV was reported to be a cause of erroneous blood type resulting from anemia or autoagglutination (Seth et al., 2011). Furthermore, the formation of antigenic mimicry B antigen on the surface of type A blood or the reduction of Cytidine monophospho-*N*-acetylneuraminic acid hydroxylase (CMAH) activity may be the cause of erroneous blood type (Seth et al., 2011). From a previous study, type A blood has both NeuGc and NeuAc on the erythrocytic membrane (Silvestre-Ferreira et al., 2011). For this reason, the methods that use monoclonal antibodies for detection of feline AB blood type may be erroneous. In human medicine, some diseases like colorectal malignancy can change surface antigen on RBCs and cause misidentifying ABO blood type from type-A to type AB (Garratty et al., 1996). Mistyping in this report could cause fatal hemolytic transfusion reaction in transfusion patients, because of false detection of a monoclonal anti-B on acquired B antigen (Garratty et al., 1996). Due to the possible error from the commercially available test kit, the use of monoclonal antibodies may occur. All cases that blood typing results are B or AB from the test kits, should be re-evaluated by tube agglutination test or back typing test.

The most blood type of purebred cats in Bangkok and vicinities, Thailand was type A. There was a higher frequency of blood type B in purebred cats in this study, compared to that of other countries such as Ireland, Israel and Portugal, but a lower frequency of type B in purebred cats than the present study was found in other countries such as Australia, Turkey, UK and USA (Table 2). In this study, type B cats

were found in 17.1% (41 cats) of Persian and 4.0% (25 cats) of Scottish Fold cats. The proportion of type B in Persian cats was higher than the survey of other countries. For instance, the percentage of type B in Persian cats was 3.6% (56 cats) in Denmark (Jensen et al., 1994), 9.1% (11 cats) in Japan (Ejima et al., 1986) and 9.6% (230 cats) in USA (Giger et al., 1991b), but the result was lower than in USA (24.1%, 170 cats) (Giger et al., 1991a). In Scottish Fold cats, this study was lower than in USA (14.8%, 27 cats) (Giger et al., 1991a). It indicates that the proportion of feline AB blood types has geographic variations even within the same breeds. All of Siamese cats were type A that were similar to the other reports (Bagdi et al., 2001; Forcada et al., 2007; Knottenbelt et al., 1999; Malik et al., 2005; Silvestre-Ferreira et al., 2004). It may be indicated that Siamese cats are 100% blood type A. All of American shorthair and Exotic shorthair cats were type A as same as previous studies (Giger et al., 1991a; Jensen et al., 1994; Juvet et al., 2011). British shorthair, Maine Coon, Norwegian Forest, Ragdoll and Sphynx cats in this study were type A while previous studies had variation of type B frequencies (Giger et al., 1991a; Giger et al., 1991b; Jensen et al., 1994; Juvet et al., 2011; Knottenbelt et al., 1999; Weingart et al., 2006). All of American Curl and Munchkin cats were type A. These breeds (American Curl and Munchkin) are the first report about blood typing. However, there was a small number of samples that is too difficult to summarize the frequencies in these purebred cats (American Curl, American shorthair, British shorthair, Exotic shorthair, Maine Coon, Munchkin, Norwegian Forest Cat, Ragdoll and Sphynx). Due to the frequencies of type B in purebred cats were relatively high, the risks of mismatched transfusions or NI in this area might be high. Thus, all purebred cats in this area must be tested for blood type before blood transfusion or mating.

Table 2 Frequency of feline AB blood type in each country.

Country	Breeds	Number of cats	Blood types			Reference
			A	B	AB	
Thailand	Non-pedigree*	229	100%	0%	0%	This study
	Pedigree**	91	91.2%	8.8%	0%	
Australia	Non-pedigree	186	62.4%	36.0%	1.6%	Malik et al., 2005
	Pedigree	166	65.7%	32.5%	1.8%	
Canada	Non-pedigree	400	96%	4%	0%	Fergal et al., 2020
China	Non-pedigree	262	88.2%	11.4%	0.4%	Zheng et al., 2011
France	Non-pedigree	320	83.8%	14.4%	1.9%	Alexandra et al., 2019
	Pedigree	37	89.2%	10.8%	0%	
Greece	Non-pedigree	207	78.3%	20.3%	1.4%	Mylonakis et al., 2001
Ireland	Non-pedigree	137	84.7%	14.6%	0.7%	Juvet et al., 2011
	Pedigree	39	97.4%	2.6%	0%	
Israel	Non-pedigree	213	69.5%	16%	14.5%	Merbl et al., 2011
	Pedigree	29	96.6%	3.4%	0%	
Italy	Non-pedigree	140	90.7%	7.1%	2.1%	Proverbio et al., 2011
New Zealand	Non-pedigree	245	85.3%	13.9%	0.8%	Cattin, 2016

Portugal	Non-pedigree	159	89.3%	4.4%	6.3%	Silvestre-Ferreira et al., 2004
	Pedigree	26	90.3%	3.8%	5.9%	
Portugal	Non-pedigree	771	97.3%	2.7%	0%	Sandrina et al., 2017
	Pedigree	155	96.8%	3.2%	0%	
Spain	Non-pedigree	56	91.1%	8.9%	0%	Sandrina et al., 2017
	Pedigree	88	93.2%	6.8%	0%	
Turkey	Pedigree	113	43.4%	56.6%	0%	Arikan et al., 2003
UK	Non-pedigree	139	87.1%	7.9%	5.0%	Knottenbelt et al., 1999
	Pedigree	207	54.6%	40.1%	5.3%	
UK	Non-pedigree	105	67.6%	30.5%	1.9%	Forcada et al., 2007
	Pedigree	51	82.4%	13.7%	3.9%	
USA	Non-pedigree	3785	98.1%	1.7%	0.1%	Giger et al., 1991b
	Pedigree	748	73.8%	26.2%	0%	

* Non-pedigree = DSH, DLH or/and Mixed breeds

** Pedigree = Purebreds

From the results of optimization and stability testing, the blood typing results could be stored at 4°C and be interpreted within 7 days. The prolonged stability of the reactions provides some benefits in practice. For example, the tested tube can be stored and be evaluated in the next few days. Additionally, when a transfusion reaction occurs, the blood typing result can be rechecked if false interpretation is due to human error. The results of stability test of lectin solution (Anti-B reagent) and anti-A reagent

showed no differences in consecutive testing over 21 days. Thus, both reagents can be stored at 4°C for at least 21 days. From the optimization test, the appropriate concentrations of lectin solution (Anti-B reagent) and anti-A reagent were 8 μg/mL and 1:8, respectively that were consistent to the previous studies (Griot-Wenk et al., 1993; Stieger et al., 2005; Seth et al., 2011; Yagi and Holowaychuk, 2016).

There were some limitations in this study. First, there were the small number of the purebred cats sampled. Therefore, it was difficult to summarize the frequencies in purebred cats. In the further study, more purebred cats should be recruited for determining the true frequency of AB blood type. Second, this study investigated only in Bangkok and vicinities. It may be not representative data of all regions in Thailand. Another area of Thailand should be more investigated. From the results, 100% of type A in DSH cats does not mean that another area in Thailand will have as same as result of this study. The further investigation is needed.

In conclusions, overall frequencies of feline blood types of DSH and purebreds in Bangkok and vicinities, Thailand were 97.5% of type A cats, 2.5% of type B cats and 0% of type AB cats. Type A was the most common blood type, B was rare, and AB was none. The frequency of blood type in DSH cats indicates that they are at very low risk of AB blood type incompatibility because all DSH cats is type A. However, at least 2 purebred cats may develop severe adverse reactions if given incompatible blood because there are type A and type B cats in these groups of cats. Therefore, blood typing is considered a necessary procedure before blood transfusion in cats, particularly among purebred cats in this region. The tube agglutination test is recommended for AB blood typing in a non-emergent situation.

APPENDIX I

Table 3 Distribution of feline AB blood types in Bangkok.

Bangkok																
Area	Blood Type	American Curl	American shorthair	British shorthair	Domestic shorthair	Exotic shorthair	Maine Coon	Munchkin	Norwegian Forest	Persian	Ragdoll	Scottish Fold	Siamese	Sphynx	Total
Bang Bon	A	-	-	-	3	-	-	-	-	-	-	-	-	-	3
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Kapi	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Khae	A	-	-	1	-	-	-	-	-	-	-	-	-	-	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Bang Khen	A	-	-	-	31	-	2	-	-	6	-	-	1	-	40
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Kho Laem	A	-	-	-	3	-	-	-	-	-	-	-	-	-	3
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Khun Thian	A	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Na	A	1	-	-	2	-	-	-	-	2	-	2	-	-	7
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Phlat	A	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Rak	A	-	-	-	9	-	-	-	-	-	-	-	-	-	9
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Sue	A	-	-	-	1	-	-	-	-	-	-	-	-	-	1

	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bangkok Noi	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bangkok Yai	A	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bueng Kum	A	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chatuchak	A	-	-	-	1	-	-	-	-	1	-	2	-	-	4
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chom Thong	A	-	-	-	2	-	-	-	-	-	-	1	-	-	3
	B	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Din Daeng	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Don Mueang	A	-	-	-	-	-	-	-	-	-	-	-	-	1	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dusit	A	-	-	-	5	-	-	-	-	-	-	-	-	-	5
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Huai Khwang	A	-	-	-	11	-	-	-	-	-	-	-	-	-	11
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Khan Na Yao	A	-	-	-	4	-	-	-	-	-	-	-	-	-	4
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Khlong Sam Wa	A	-	2	-	28	-	-	-	-	8	-	-	-	-	38
	B	-	-	-	-	-	-	-	-	1	-	1	-	-	2
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Khlong San	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Khlong Toei	A	-	-	-	8	-	-	-	-	2	-	-	-	1	11
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lak Si	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lat Krabang	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lat Phrao	A	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Min Buri	A	-	-	3	-	-	-	-	-	-	-	-	-	-	3
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nong Chok	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nong Khaem	A	-	-	-	1	-	-	-	-	-	-	-	-	-	1

	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Pathum Wan	A	-	-	-	3	-	-	-	-	1	-	-	-	-	4	
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Phasi Charoen	A	-	1	-	4	-	-	-	-	-	-	-	-	-	5	
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Phaya Thai	A	-	-	-	1	-	-	-	-	-	-	-	-	-	1	
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Phra Khanong	A	-	-	-	5	-	-	-	-	1	1	-	-	-	7	
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Phra Nakhon	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Pom Prap Sattru Phai	A	-	-	-	4	-	-	-	-	-	-	-	-	-	4	
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prawet	A	-	-	-	23	-	1	-	-	3	-	-	-	-	27
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rat Burana	A	-	-	-	1	-	-	-	-	1	-	-	-	-	2
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ratchathewi	A	-	-	-	12	-	1	-	-	-	-	1	-	-	14
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sai Mai	A	-	-	-	-	-	-	1	-	-	-	1	-	-	2
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Samphan thawong	A	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saphan Sung	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Sathon	A	-	1	-	7	-	-	-	-	-	-	3	-	-	11
	B	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Suan Luang	A	-	-	-	9	-	-	-	-	1	-	-	-	-	10
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Taling Chan	A	-	1	-	-	-	-	-	-	-	-	-	-	-	1
	B	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thawi Watthana	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thon Buri	A	-	-	-	3	-	-	-	-	-	-	1	-	-	4
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thung Khru	A	-	-	-	2	-	-	-	-	-	-	1	-	-	3
	B	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Wang Thonglang	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Watthana	A	-	-	-	6	-	-	-	-	-	-	-	-	-	6
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yan Nawa	A	-	-	-	8	-	-	-	-	-	-	1	-	-	9
	B	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Overall	**A**	**1**	**5**	**4**	**201**	**-**	**4**	**1**	**-**	**27**	**1**	**14**	**1**	**2**	**261**
	B	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**6**	**-**	**1**	**-**	**-**	**7**
	AB	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

Table 4 Distribution of feline AB blood types in Samut Prakan province.

Samut Prakan																
Area	**Blood Type**	**American Curl**	**American shorthair**	**British shorthair**	**Domestic shorthair**	**Exotic shorthair**	**Maine Coon**	**Munchkin**	**Norwegian Forest**	**Persian**	**Ragdoll**	**Scottish Fold**	**Siamese**	**Sphynx**	**Total**
Bang Bo	A	-	-	-	-	-	-	-	-	-	-	2	-	-	2
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Phli	A	-	-	-	4	4	-	-	-	-	-	1	-	-	9
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Sao Thong	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mueang Samut Prakan	A	-	-	-	2	-	-	-	1	-	-	-	-	-	3
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phra Pradaeng	A	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phra Samut Chedi	A	-	-	-	1	-	-	-	-	1	-	-	-	-	2
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Overall	**A**	**-**	**-**	**-**	**8**	**4**	**-**	**-**	**1**	**1**	**-**	**3**	**-**	**-**	**17**
	B	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
	AB	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

Table 5 Distribution of feline AB blood types in Samut Sakhon province.

Samut Sakhon																
Area	**Blood Type**	**American Curl**	**American shorthair**	**British shorthair**	**Domestic shorthair**	**Exotic shorthair**	**Maine Coon**	**Munchkin**	**Norwegian Forest**	**Persian**	**Ragdoll**	**Scottish Fold**	**Siamese**	**Sphynx**	**Total**
Ban Phaeo	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Krathum Baen	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mueang Samut Sakhon	A	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Overall	**A**	**-**	-	-	1	-	-	-	-	-	-	-	-	-	1
	B	**-**	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	**-**	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6 Distribution of feline AB blood types in Nonthaburi province.

Nonthaburi																
Area	**Blood Type**	**American Curl**	**American shorthair**	**British shorthair**	**Domestic shorthair**	**Exotic shorthair**	**Maine Coon**	**Munchkin**	**Norwegian Forest**	**Persian**	**Ragdoll**	**Scottish Fold**	**Siamese**	**Sphynx**	**Total**
Bang Bua Thong	A	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Kruai	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bang Yai	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mueang Nonthaburi	A	-	-	-	-	-	-	-	-	3	-	2	-	-	5
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pak Kret	A	-	-	-	1	-	-	-	-	2	-	-	-	-	3
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sai Noi	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Overall	**A**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**6**	**-**	**2**	**-**	**-**	**9**
	B	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**1**
	AB	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

Table 7 Distribution of feline AB blood types in Pathum Thani province.

Pathum Thani																
Area	**Blood Type**	**American Curl**	**American shorthair**	**British shorthair**	**Domestic shorthair**	**Exotic shorthair**	**Maine Coon**	**Munchkin**	**Norwegian Forest**	**Persian**	**Ragdoll**	**Scottish Fold**	**Siamese**	**Sphynx**	**Total**
Khlong Luang	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lam Luk Ka	A	-	-	-	17	-	-	-	-	-	-	1	1	-	19
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lat Lum Kaeo	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A	-	-	-	1	-	-	-	-	-	-	4	-	-	5
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Mueang Pathum Thani	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nong Suea	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sam Khok	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thanyaburi	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Overall	**A**	**-**	**-**	**-**	**18**	**-**	**-**	**-**	**-**	**-**	**-**	**5**	**1**	**-**	**24**
	B	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
	AB	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

APPENDIX II

Preparation of Phosphate buffer saline (PBS)

PBS will be prepared following the formula in a previous study (Dulbecco and Vogt, 1954).

One liter of 10X PBS consists of:

- NaCl 80 g
- KCl 2 g
- $Na_2HPO_4 \bullet 2H_2O$ 14.4 g
- KH_2PO_4 2.4 g

Then, dissolved with distilled water until 1 liter.

To prepare 1 L of 1X PBS

- Diluting 100 mL of 10X PBS in 800 mL of distilled water
- Adjusting the pH to 7.4 with HCl
- Adding distilled water to 1 L.

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