การตรวจวินิจฉัยแมวที่ป่วยด้วยโรคเม็ดเลือดแดงแตกจากภูมิคุ้มกันไวเกินโดยวิธี Flow cytometry

นายภากร ลิ้มเล็งเลิศ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาอายุรศาสตร์สัตวแพทย์ ภาควิชาอายุรศาสตร์
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

DETECTION OF IMMUNE-MEDIATED HEMOLYTIC ANEMIA IN CATS BY FLOW CYTOMETRY

Mr. Parkorn Limlenglert

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Medicine

Department of Veterinary Medicine
Faculty of Veterinary Science
Chulalongkorn University

Academic Year 2009

Thesis Title	DETECTION OF IMMUNE-MEDIATED HEMOLYTIC ANEMIA IN CATS BY FLOWCYTOMETRY
By	Parkorn Limlenglert
Field of Study	Veterinary Medicine
Advisor	Associate Professor Rosama Pusoonthornthum, D.V.M., Ph.D.
Co-Advisor	Associate Professor Anudep Rungsipipat, DVM., Ph.D.

Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

M. Techakamphu ..... Dean of the Faculty of Veterinary Science

(Professor Mongkol Techakamphu, Doctoratde 3e cycle)

THESIS COMMITTEE

Ratanaporn Brahmasa ..... Chairman

(Associate Professor Ratanaporn Brahmasa, D.V.M.)

Rosama Pusoonthornthum ..... Thesis Advisor

(Associate Professor Rosama Pusoonthornthum, D.V.M., Ph.D.)

..... Thesis Co-Advisor

(Associate Professor Anudep Rungsipipat, D.V.M., Ph.D.)

Achara Tawasin ..... Examiner

(Associate Professor Achara Tawasin)

Chaleow Salakij ..... External Examiner

(Associate Professor Chaleow Salakij, D.V.M., Ph.D.)

ภากร ลิ้มเส็งเลิศ : การตรวจวินิจฉัยแมวที่ป่วยด้วยโรคเม็ดเลือดแดงแตกจากภูมิคุ้มกันไวเกินโดยวิธี Flow cytometry. (DETECTION OF IMMUNE-MEDIATED HEMOLYTIC ANEMIA IN CATS BY FLOW CYTOMETRY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ร.ศ. สพ.ญ. ดร. รสมา ภู่สุนทรธรรม, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ร.ศ. น.สพ. ดร. อนุเทพ รังสีพิพัฒน์ 95 หน้า.

การศึกษาความชุกของโรคเม็ดเลือดแดงแตกจากภูมิคุ้มกันไวเกินในแมวโดยใช้ flow cytometry เป็นเครื่องมือในการตรวจหาอิมมูโนกลอบูลินจี และ อิมมูโนกลอบูลินเอ็มบนผิวเม็ดเลือดแดง เพื่อเปรียบเทียบกับ Coombs' test ในแมวปกติจำนวน 10 ตัวและแมวที่มีภาวะโลหิตจางจำนวน 19 ตัว พบแมวในกลุ่มโลหิตจางที่ให้ผลบวกต่อ Coombs' test 5 ตัว มีความชุกเท่ากับร้อยละ 26.31และการใช้ flow cytometry ให้ผลบวกต่ออิมมูโนกลอบูลินจี และ อิมมูโนกลอบูลินเอ็มในแมวทั้ง 5 ตัว นอกจากนี้ยังมีแมวอีก 7 ตัว จาก 14 ตัวที่ให้ผลลบต่อ Coombs' test แต่ให้ผลบวกจากการตรวจโดยวิธี flow cytometry ซึ่งแสดงถึงการพบอิมมูโนกลอบูลินจี และ อิมมูโนกลอบูลินเอ็ม บนผิวเม็ดเลือดแดง และน่าจะมีโรคเม็ดเลือดแดงแตกจากภูมิคุ้มกันไวเกินเกิดขึ้น พบว่าการตรวจด้วยวิธี flow cytometry มีความไวต่อโรคสูงกว่าการตรวจด้วย Coombs' test และมีค่าใช้จ่ายต่ำกว่า น่าจะมีการนำการตรวจด้วยวิธี flow cytometry นี้มาใช้ในการตรวจวินิจฉัยโรคเม็ดเลือดแดงแตกจากภาวะภูมิคุ้มกันไวเกินในแมวเพื่อทดแทนวิธีการตรวจด้วย Coombs' test ต่อไปในอนาคต

ภาควิชาอายุรศาสตร์ ลายมือชื่อนิสิต ภากร ลิ้มเส็งเลิศ
สาขาวิชาอายุรศาสตร์สัตวแพทย์ ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก
ปีการศึกษา 2552 ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม

4975568531 : MAJOR VETERINARY MEDICINE

KEYWORDS : CATS / FLOW CYTOMETRY / IMMUNE-MEDIATED HEMOLYTIC ANEMIA

PARKORN LIMLENGLERT: DETECTION OF IMMUNE-MEDIATED HEMOLYTIC ANEMIA IN CATS BY FLOW CYTOMETRY. THESIS ADVISOR: ASSOC. PROF. ROSAMA PUSOONTHORNTHUM, Ph.D., THESIS CO-ADIVSOR: ASSOC. PROF. ANUDEP RUNGSIPIPAT, Ph.D., 95 pp.

The study of the prevalence of feline immune-mediated hemolytic anemia and tested the applicability of flow cytometry for the detection of feline immunoglobulin-G (IgG) and immunoglobulin-M (IgM) on red blood cell using goat-anti-cat IgG and IgM compared with Coombs' test in ten healthy and nineteen anemic cats was performed. Of nineteen anemic cats, five cats had positive Coombs' test results for IMHA. Using flow cytometry, all five Coombs'test positive cats were significantly positive on both IgG and IgM. From seven of fourteen Coombs' test-negative cats flow cytometry showed increased IgG and IgM on the results which indicated IMHA. Thus, flow cytometric analysis is proved to be a sensitive technique which can help in detecting red blood cell-bound feline IgG and IgM. Flow cytometry provided a more sensitive and cost-effective method to quantitate erythrocyte-bound immunoglobulin than feline Coombs' test.

Department : Veterinary Medicine	Student's Signature
Field of Study : Veterinary Medicine	Advisor's Signature
Academic Year : 2009	Co-Advisor's Signature

ACKNOWLEDGEMENT

I would like to express my deep and sincere gratitude to my advisor, Associated Professor Rosama Pusoonthornthum for her kindness and logical way of thinking that have been of great value for me. Her understanding, encouraging, personal guidance and her support have provided a good basis for my thesis.

I am deeply grateful to my co-advisor, Associated Professor Anudep Rungsipipat, for his detailed and constructive comments throughout this work.

I wish to thank Associated Professor Achara Tawatsin for her guidance in statistical analysis.

I am grateful to Mr. Pitcha Pornmingmas for his aid in screening cases. During this work I have collaborated with many colleagues for whom I have great regard, and I wish to extend my warmest thanks to all those who have helped me with my work in the Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University.

I owe my loving thanks to my family. They have support me a lot during the time of my research. Without their encouragement and understanding it would have been impossible for me to finish this work. I also thanked the Graduate School, Chulalongkorn University for the research grant.

CONTENTS

LIST OF TABLES

Table page

LIST OF FIGURES

Figure page

LIST OF ABBREVIATIONS

ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
BUN	blood urea nitrogen
CBC	complete blood count
CI	confidence interval
C1	complement component 1
C3, C3b	complement component 3
DAT	direct antiglobulin test
DIC	disseminated intravascular coagulation
DSH	domestic short hair
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FC	flow cytometry
Fc	constant fragment
FeLV	feline leukemia virus
FIP	feline infectious peritonitis

FITC	fluorescein isothiocyanate
FIV	feline immunodeficiency virus
FSC	forward scatter
IgA	immunoglobulin A
IgG	imuunoglobulin G
IgM	immunoglobulin M
IMHA	immune-mediated hemolytic anemia
IMTP	immune-mediated thrombocytopenia
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MFI	mean fluorescence intensity
MM	mucous membrane
MPS	mononuclear phagocytic system
PBS	phosphate buffered saline
PRCA	pure red cell aplasia
RBC	red blood cell
SD	standard deviation
SSC	side scatter
USA	United States of America

CHAPTER 1

INTRODUCTION

Anemia is one of the most common abnormalities in dogs and cats. The primary causes of anemia are blood parasites, trauma, chronic renal failure and viral infection. However, one of the causes of anemia that often has been overlooked is immune-mediated hemolytic anemia (IMHA) (Day, 2002; Gunn-Moore *et al*., 1999; Husbands *et al*., 2002; Kohn, 2007; Mackin, 2002; Nassiri *et al*., 2005; Weiss, 2008; Zini *et al*., 2007). This abnormality has been overlooked in most small animal practice in Thailand due to the lack of appropriated diagnostic tool.

IMHA is the abnormality that red blood cells are destroyed in blood vessel (intra vascular hemolysis) and in tissue (extra vascular hemolysis) (Barker, 2000; Giger, 2001; Mackin, 2002). Chronic stimulation of red blood cell by immunoglobulin and complements cause IMHA which results in red blood cell breakdown (Day, 2002; Kohn, 2007; Mackin, 2002). This abnormality causes acute or chronic anemia and leads to multiple organ infarction and death. Besides destruction of red blood cells, immunoglobulin and complements from IMHA may cause damages in bone marrow and cause reduction in the production of red blood cell (non-regenerative anemia) (Day, 2002; Kohn, 2007; Mackin, 2002; Weiss, 2008; Zini *et al*., 2007). Early, fast and precise diagnosis of IMHA is needed in small animal medicine to lower the death of anemic cats caused by IMHA. The objective of this study is to use flow cytometry as a new possible diagnostic method for early detection of immunoglobulin on red blood cell in anemic cats with suspected IMHA.

Objectives of the study

1. To study the prevalence of immune-mediated hemolytic anemia in cats.
2. To compare the sensitivity and specificity of flow cytometry and direct antiglobulin test for feline immune-mediated hemolytic anemia.

Keywords: Immune-mediated hemolytic anemia (IMHA), flow cytometry, cats

Advantages of Study

1. Using the prevalence of immune-mediated hemolytic anemia in anemic cats at Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University and Veterinary Hospitals in Bangkok to predict cats with IMHA.
2. Be able to use flow cytometry for the diagnosis and follow up of immune-mediated hemolytic anemia in anemic cats.

CHAPTER 2

LITERATURE REVIEW

Feline anemia is one of the most frequently clinical findings in Thailand. This abnormality has been diagnosed in routine health check up by complete blood count. Anemia may also be relative or physiologic as well as pathologic. Physiologic anemia occurs when the red blood cell mass is decreased as a result of normal physiologic changes such as seen in neonates or during estrus. Relative anemia results from increased plasma volume (dilutional anemia) or sequestration of red blood cell (RBC) within the microcirculation. These are not true anemia as the functional RBC mass is not decreased. Clinical evidence of anemia is not associated with relative and physiologic anemia. (Cowgill *et al.*, 2003) It is classified into two types: Regenerative and Non-regenerative anemia.

Regenerative anemia is characterized by properly production of RBCs by the bone marrow when bloods are loss or destroyed. The excessive blood loss (hemorrhage) or RBC destruction (hemolysis) result in increased erythropoiesis with increased numbers of young red cells (reticulocytes) present in the peripheral blood. Reticulocytes appear in the blood within 2 to 4 days after blood loss or destruction with peak production occurring within 4 to 7 days. Thus erythropoiesis may appear non-regenerative early in the course of an anemia because of inadequate response time. (Cowgill *et al.*, 2003) Non-regenerative anemia is characterized by decreased or ineffective production of RBCs by the bone marrow. These may be caused by primary or, more commonly, secondary bone marrow disease.

The clinical manifestations of anemia are determined in part by their specific origin and pathogenesis. In general, clinical signs can be attributed to a reduction in oxygen-carrying capacity of the blood. Severity is determined by the rapid of onset, magnitude of decreased blood volume, and adequacy of cardiopulmonary adaptation. Some animals that have severe anemia may not show clinical signs because of chronic onset, whereas others that have anemia may be severely affected if the onset is acute.

In regenerative anemia, there are many successive treatments that can be used. While in non-regenerative anemia, multiple blood transfusion or prolong hormonal treatment have many complications and adverse effects. Advanced therapy such as bone marrow transplantation or stem cell therapies are more suitable for these patients. Non-regenerative diseases can be divided as followed (Table 1):

Table 1 Causes of non-regenerative anemia

Causes	Diseases
Hypoproliferative anemia	Renal disease, hypothyroidism, hypoadrenocorticism, panhypopituitarydism, decrease growth hormone, reduced oxygen requirements, increased oxygen release
Lack of iron	Inflammation, chronic bleeding, iron deficiency
Marrow disorders	external toxin – aplasia, myelophthistic disease, myelofibrosis, myelodysplasia, hyperestrogenism – iatrogenic, neoplasia
Infection	FeLV, FIV, FIP, Ehrlichiosis, Mycoplasmosis, immunotherapy, pure red blood cell aplasia
Ineffective erythropoiesis	*Macrocytic* : intrinsic bone marrow disease, vitamin B_{12} deficiency *Normocytic* : stromal disease (myelofibrosis), intrinsic erythroid disease *Microcytic* : nonsideroblastic – iron deficiency, sideroblastic – globin or porphyrin abnormality
Time related	Hemolysis – during the first 3-5 days at least, hemorrhage – during the first 3-5 days at least)

(Apply from: Cowgill, *et al.* (2003). "Clinical application of reticulocyte counts in dogs and cats." Vet. Clin. North Am. Small Anim. Pract. 33(6): 1223-1244).

Therefore, completed blood count, blood morphology and bone marrow evaluation are the important diagnostic tools to classify the type of anemia.

One of the most common causes of anemia in canine and feline patients is IMHA. IMHA is the state that immunoglobulin and complement attached to red blood cell surface (Barker *et al*., 1992). These two compositions induce the red blood cell to lyses by two main pathways as primary IMHA and secondary IMHA (Giger, 2005). Primary IMHA is a stage that immune system attacks the surface of normal red blood cell which found mainly in dogs more than cats (Day, 2002; Mackin, 2002; Kohn, 2007). This condition typically affects young adult and middle-aged animals. Secondary IMHA is a stage that the antigens attached to surface of red blood cell and the immune system attempt to clear these antigens from red blood cell. After the cascade, red blood cell will also be destroyed and reshaped or lyses. This is commonly found in cats. The causes of secondary IMHA in cats are feline leukemia, *Mycoplasma haemofelis*, lymphoma, neonatal isoerythrolysis, and drug induced such as sulfonamide/trimethoprim, penicillin, and vaccine (Day, 2002; Mackin, 2002; Kohn, 2007). Secondary IMHA will often response poorly to treatment or relapse unless the underlying cause is recognized and eliminated. The causes of hemolytic anemia are shown in Table 2.

Table 2. Differential diagnostic list for hemolytic anemia

Red Blood Cells	Type of Abnormalities
Immune-mediated	***Chemical mediated***
Autoimmune hemolytic anemia	Onion, rye grass, maple
Systemic lupus erythematosus	Phenazopyridine hydrochloride
Subacute bacterial endocarditis	Chronic progressive hepatitis
Incompatible transfusions	Phenothiazines
Feline leukemia virus infection	Propylthiouracil
Neonatal isoerythrolysis (cats) Glomerulonephritis	Severe hypophosphatemia (<1.0 mg/dL)
Lymphocytic leukemia	Dirofilariasis (venal caval syndrome)
Lymphosarcoma	Topical benzocaine
Idiopathic	Vitamin K
Histopiasmosis	Propylene glycol
Reticulum cell sarcoma	Methylene blue
Ancyclostoma caninum	Castor bean Sulfonamide
	Heparin
Microangiopathic	Dipyrone Quinidine Copper
Disseminated intravascular coagulation	Zinc
Hemangiosarcoma	Trimethoprim/sulphonamide
Heartworm disease	Penicillins
Vasculitis	Cephalosporins
Hemolytic uremia syndrome	*Bee sting envenomation
Intrinsic red blood cell defects	***Red blood cell parasites***
Methemoglobin reductase deficiency	*Haemobartonella fells* (epicellular)
Phosphofructokinase deficiency	*Haemobartonella canis* (epicellular)
Idiopathic Heinz body anemia	Cytauxzoonosis (usually nonregenerative)
Pyruvate kinase deficiency	Piroplasmosis
Chondrodysplasia (in Malamutes)	*Anaplasma*
Congenital feline porphyria	*Babesia canis* (intracellular)
Spur cell anemia (liver disease)	Ehrlichia
Stomatocytosis	***Bacterial***
Elliptocytosis	Acute leptospirosis
	Endotoxemia
	Clostridium perfringens

(From: McCullough, S. (2003). "Immune-mediated hemolytic anemia: understanding the nemesis." Vet. Clin. North Am. Small Anim. Pract. 33(6): 1295-1315).

IMHA is classified as Class II hypersensitivity (Antibody-mediated cytotoxic) (Mackin, 2002; Nassiri et al., 2005). Antigen-activated immune system produces Immunoglobulin G (IgG) and Immunoglobulin M (IgM) which attach on red blood cell surface (Day, 1999).

IgG possesses two antigen-binding sites (monomeric) and usually cannot directly agglutinate red blood cells unless a large amount of antibody is present. When the red blood cell attaches to an IgG molecule, it is phagocytized by macrophages possessing multiple receptors for the constant fragment (Fc) portion of the IgG molecule. These macrophages are found primarily in the spleen; thus, active erythrophagocytosis results in splenomegaly. The more immunoglobulin production increases the more molecules are bound to the red blood cell. Erythrophagocytosis occurs within the liver, and hepatomegaly may also result (Fig.1) (McCullough, 2003).

Another key immune response in IMHA is activation of the complement system and binding of complement proteins to red blood cells. Complement activation can cause immediate intravascular lysis or enhance extravascular lysis (Day, 2002; Mackin, 2002, Kohn, 2007) and produce spherocytes by mononuclear phagocytic system. C1, a serine protease from the liver, enters the complement cascade and, through a series of enzyme reactions depicted generates a "membrane attack complex" (C8C9CSbC6C7) that attaches to the red blood cell membrane. The membrane attack complex "punches holes" in the red blood cell membrane, allowing an influx of water and electrolytes, cell swelling, and lyses (Fig. 2). The C3b-membrane complex can be removed by macrophages within the spleen (partial erythrophagocytosis) producing spherocytes. Spherocytes are more rigid and can be destroyed more quickly as they traverse through the spleen or liver. (McCullough, 2003)

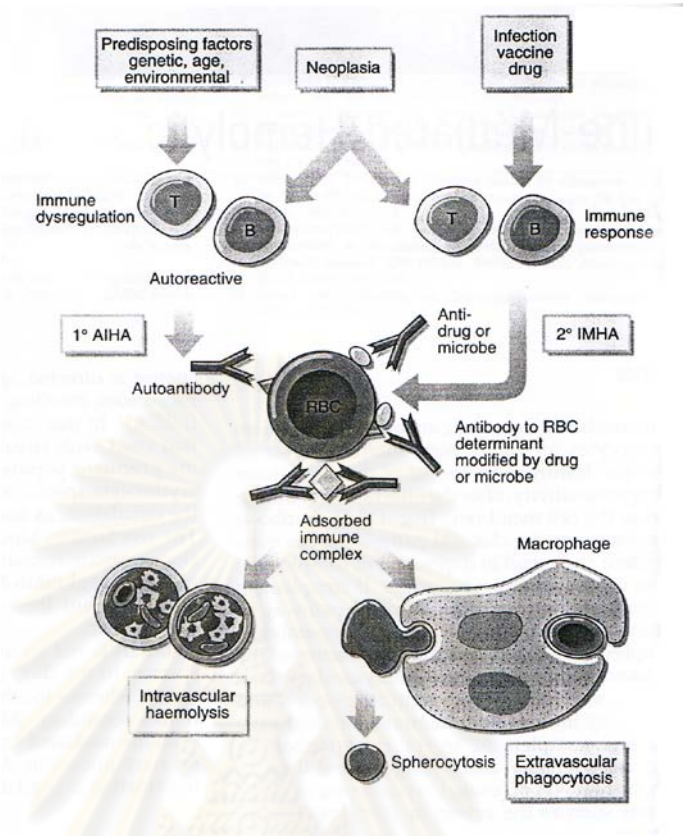
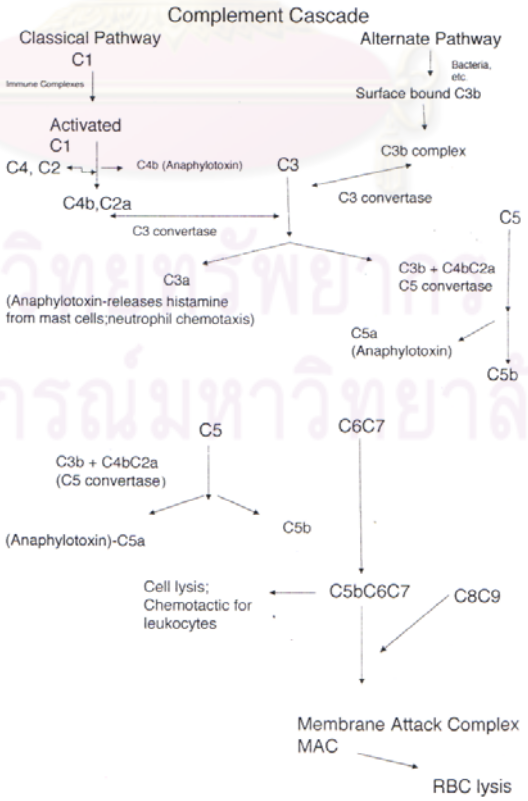


Figure 1 Primary IMHA and secondary IMHA pathway (Harvey, 2001)



IgM is a large pentameric molecule and agglutinates red blood cells. Once IgM is bound to the red blood cell, C1 can be bound, and the complement cascade is activated. IgM may also detach after complement activation and bind to other red blood cells. The red blood cell-IgM complex may bind complement and be phagocytized by macrophages (possessing the Fc receptor), most commonly in the liver, although about 20% of receptors are in the spleen (McCullough, 2003).

Antibody attachment to cell membranes triggers RBC destruction by a number of different mechanisms: With high levels of antibody attachment and, particularly, complement fixation, membranes may be damaged then extracellular water leaks into the cytoplasm, causing swelling and rupture of the RBC while it is still in the circulation, so-called intravascular hemolysis. In the absence of direct RBC lysis, antibody attachment and subsequent cell membrane damage can still lead to an accelerated rate of destruction of affected RBCs by tissue macrophages within the mononuclear phagocytic system (MPS), a process that occurs outside of the circulation which called extravascular hemolysis. MPS destruction of RBCs is mediated by Fc receptors on the macrophage surface, receptors which bind the Fc component of the antibodies attached to the RBC membranes. Since the MPS is located throughout the body, extravascular hemolysis can occur in many organs, but typically is most pronounced in the liver and the spleen. In some patients with high levels of anti-RBC antibodies, many individual antibodies can each bind to two different RBCs, a process that causes the cells to clump together (agglutinate). Patients that exhibit significant RBC agglutination at body temperature typically have an increased rate of extravascular hemolysis, since clumping of RBC slows their passage through vessels and facilitates their removal by the MPS (Mackin, 2001; McCullough, 2003).

Typically, IMHA is caused by antibodies directed against mature RBC, with the marrow response to anemia by regenerative response. (Feldman, 2001; Giger, 2001; Husbands *et al*., 2002) However, in some animals, antibodies may also be directed against marrow RBC precursors at any stage in their development. Hemolytic anemia with an inappropriately poor regenerative response will develop if antibodies are

directed against cell membrane components that are present both on mature RBC and their marrow precursors. In contrast, if antibodies are directed against membrane components that are present only on marrow precursors, and not on mature RBC, non-regenerative anemia will develop without peripheral hemolysis (Day, 2002; Kohn, 2007; Mackin, 2002; Weiss, 2008; Zini *et al*., 2007). Pure red cell aplasia (PRCA), in which all stages of marrow RBC precursor are dramatically reduced or absent, is the most extreme form of this process (Mackin, 2002; McCullough, 2003).

Categories of Immune-mediated Hemolytic Anemia (IMHA)

Typical IMHA is caused by antibodies that exert their effects at body temperature, so-called warm reactive antibodies. Some animals have anti-RBC antibodies that are only reactive at much lower temperatures. Although such cold reactive antibodies usually cause minimal harmful effects, their presence can potentially cause specific clinical syndromes, and can also lead to a false positive diagnosis of IMHA if tests such as slide agglutination are performed at cold temperatures. IMHA has been sub-divided into five main categories based on the thermal reactivity of the anti-RBC antibodies, and their major clinical effects at optimal temperature (Mackin, 2002).

1. Warm Antibody Type, Agglutination:

High levels of antibody lead to detectable autoagglutination of RBC. Agglutination is often associated with acute severe extravascular hemolysis. (Mackin, 2002)

2. Warm Antibody Type, Intravascular Hemolysis:

Intravascular hemolysis, usually associated with high levels of antibody and complement fixation, causing severe anemia with detectable hemoglobinemia and hemoglobinuria. (Mackin, 2002)

3. Warm Antibody Type, Incomplete Antibody:

Anti-RBC antibodies cause extravascular hemolysis, without detectable autoagglutination or hemoglobinemia and hemoglobinuria. Disease onset can be chronic or sub-acute, and resultant anemia varies from mild to severe. (Mackin, 2002)

4. Cold Antibody Type, Agglutination:

Anti-RBC antibodies are only reactive at cold temperatures, and agglutination does not occur at body temperature. Agglutination can occur within the vasculature of the extremities, particularly in colder weather. Obstruction of the blood supply to the peripheral vasculature due to agglutination can lead to ischemic necrosis of the ear or tail tips, the end of the nose, and the feet. (Mackin, 2002)

5. Cold Antibody Type, Non-agglutinating Hemolysis:

Antibodies are only reactive at cold temperatures, and hemolysis does not occur at body temperature. In cold weather, however, some degree of hemolysis may occur within the extremities, which manifests clinically as transient hemoglobinemia and hemoglobinuria. (Mackin, 2001)

Although the above categorisation system is derived by extrapolation from human beings, all five categories of IMHA have been reported in small animals. Agglutinating and (especially) hemolysing cold antibody types of IMHA are rare in both dogs and cats. Intravascular warm antibody type IMHA is also relatively uncommon.

Clinical signs

Signs typically associated with IMHA reflect the presence of both anemia (lethargy, weakness, pale mucous membranes, and a hemic heart murmur) and compensatory responses caused by tissue hypoxia and stimulation of the sympathetic nervous system (tachypnea, tachycardia, and bounding pulses). Some patients may also show clinical signs of an ongoing immunological or inflammatory process, such as pyrexia, anorexia and, uncommonly, lymphadenopathy. Since the Mononuclear Phagocytic System (MPS) within the spleen and liver is usually the main site of RBC destruction, organomegaly is only variably present in animals with IMHA. Patients with IMHA of acute onset tend to be very severely affected by their anemia, and are often very depressed, weak or even collapsed and death (Giger, 2001; Gunn-Moore *et al*., 1999; Kohn, 2007; Mackin, 2002; Nassiri *et al*., 2005; Weiss, 2006). Hyperbilirubinemia, bilirubinuria and tissue jaundice are often seen during acute severe episodes of IMHA

(Giger, 2001). Since intravascular hemolysis is uncommon, hemoglobinemia and hemoglobinuria are observed very infrequently. Patients with extravascular hemolysis due to sub-acute or chronic IMHA can compensate to some extent for their lack of erythrocytes, and may be remarkably bright despite the presence of severe anemia. In these patients, the liver can often cope with the extra bilirubin released by RBC breakdown, and jaundice does not occur.

Pulmonary thromboembolism is a well-recognized complication of IMHA, and is common in animals with acute severe anemia that are receiving high dose glucocorticoids. Pulmonary thromboembolism should always be suspected in those anemic animals that suddenly develop severe and persistent dyspnea, although other causes of dyspnea such as cardiogenic pulmonary edema or acute bacterial pneumonia should also be considered, especially in dogs already receiving glucocorticoid and immunosuppressive therapy. Disseminated intravascular coagulation (DIC) can also complicate severe cases of IMHA.

Diagnosis of Immune-mediated Hemolytic Anemia (IMHA)

Changes in red blood cell morphology are effective test for IMHA (Barger, 2003). Hematology in patients with IMHA typically reveals a moderate to severe anemia, which is most commonly regenerative, with anisocytosis, polychromasia, a high corrected reticulocyte count and increased numbers of nucleated RBCs. Reticulocyte counts can sometimes be inappropriately low, either because antibodies are also directed against RBC precursors, or because anemia is peracute. White cell and neutrophil counts are often moderately to markedly increased, probably in response to both non-specific marrow stimulation and the inflammatory process associated with RBC breakdown. White cell counts can be high enough to mimic myelogenous leukemia, a reaction sometimes called a 'leukemoid response'. Platelet counts are usually normal unless the animal also has immune-mediated thrombocytopenia purpura (IMTP). Hematology can often also reveal clues that suggest a specific etiological diagnosis.

1. Spherocytosis:

Spherocytes are small spherical erythrocytes that, when present in high numbers, strongly suggest a diagnosis of primary or secondary IMHA. Spherocytes are formed when macrophages remove a piece of RBC membrane without cell destruction or a significant loss of cytoplasm (Fig 3). Spherocytes can be difficult to recognize in cats, because normal feline RBCs tend to be smaller and less discoid than canine RBCs.

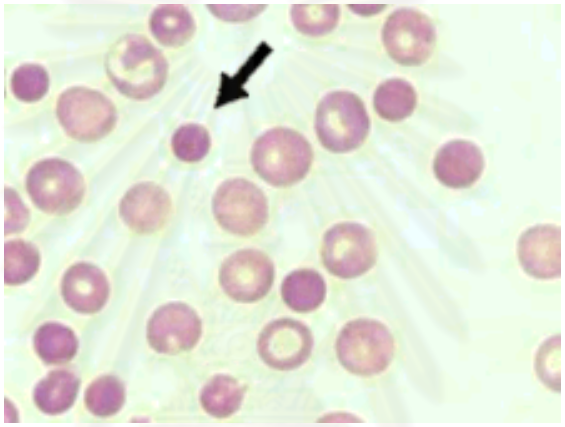


Fig. 3 Spherocyte with dense red color and spherical shape (Arrow)
Available from: http://www.marvistavet.com/html/body_imha.html

2. Agglutination:

Examination of blood smears may reveal microscopic auto-agglutination (clumping) of RBCs (Tvedten, 2004). Such agglutination can form large rafts of RBC that, when a collection tube containing anti-coagulated blood is closely inspected, are visible to the naked eye as multiple red speckles. Similar speckles can be created by rouleaux formation, a phenomenon that can occur in normal animals, especially cats. Clinicians should perform a saline dilution (one drop of RBCs to one drop of saline in dogs, one drop of RBCs to two drops of saline in cats) slide agglutination test to differentiate rouleaux from genuine auto-agglutination (Mackin, 2002). True agglutination can be seen grossly as persist despite dilution with saline, and microscopically as non-linear clumps of RBCs. A positive slide agglutination result is highly suggestive of a

diagnosis of IMHA, and also suggests that the condition is likely to be acute and severe. Negative slide agglutination does not rule out IMHA; since in fact a negative result is reported to be the most common result in small animals with IMHA because most actually have non-agglutinating antibodies. Automated hematology analyzers sometimes register a clump of agglutinated RBCs as a single cell, often of a size too large to even be recorded as a RBC at all. Resulting an artifactually high MCV or lowering of the calculated hematocrit. Since the hemoglobin within all RBCs is still measured by the analyzer, this leads to an erroneously high estimation of mean corpuscular hemoglobin concentration (MCHC). When agglutination is suspected to be the cause of a lower than expected hematocrit, packed cell volume (PCV), which is not affected by RBC clumping, should be monitored using microhematocrit tube centrifugation rather than an automated analyzer.

3. Other RBC Abnormalities:

Careful examination of RBC morphology may suggest an underlying cause of either immunological or non-immunological hemolysis. Diagnostically useful RBC abnormalities include detection of parasites such as *Mycoplasma haemofelis* (which may cause secondary IMHA), Heinz bodies (suggesting hemolysis secondary to oxidative damage) and schistocytosis (suggesting a microangiopathic hemolytic process such as DIC). Mild to moderate hyperbilirubinemia and bilirubinuria may be seen transiently in animals with acute severe anemia. Since the liver is usually able to cope with all but the transient overwhelming bilirubin loads produced by acute severe hemolysis, severe hyperbilirubinemia or persistence of jaundice for more than 3 to 5 days, even in the markedly anemic animal, usually indicates the presence of concurrent hepatic disease or biliary obstruction. Hemoglobinemia and hemoglobinuria are uncommon, transient events that indicate the presence of severe intravascular hemolysis.

Immunological Testing

Specific immunological testing can be used to support a tentative diagnosis of IMHA. The most widely used test is the direct antiglobulin test (DAT) or Coombs' test, which detects antibodies and/or complement bound to RBC membranes. A standard

DAT as provided by most laboratories typically uses a mix of antibodies directed against IgG, IgM and complement, and is performed at body temperature (Mackin, 2002). Modifications of the routine screening DAT that may increase its diagnostic value include running the test at different temperatures and titers, and using individual antibodies against IgG, IgM, IgA and complement as well as the standard polyvalent antibody/complement mix. Positive DAT results (at 4 ° Celsius) are of minimal diagnostic significance unless the patient has clinical signs consistent with cold antibody type agglutination or intravascular hemolysis. Strictly interpreted, a positive DAT supports a diagnosis of IMHA, while a negative test suggests a non-immunological cause of hemolysis. Large number of studies have shown that a DAT can often be of only mediocre diagnostic accuracy: although sensitivity and specificity undoubtedly improve with meticulous attention to test methodology (Quigley *et al*., 2001; Overmann *et al*., 2007; Wardrop, 2005), the fact remains that both false positive and false negative results do occur relatively commonly (Quigley *et al*., 2001). Veterinarians should be aware that since IMHA can occur in the presence of a negative DAT and, conversely, a positive test does not absolutely prove the presence of IMHA, sometimes a diagnosis must be made based on clinical judgment despite the presence of an apparently discrepant DAT result. However, performing a DAT is still recommended in all patients with suspected IMHA even if criteria such as spherocytosis or positive slide agglutination already strongly suggest a diagnosis, since a positive DAT will add support to the diagnosis and characterize the disease further by determining the involvement of various immunoglobulin types and complement. Various other immunological tests for detecting anti-RBC antibody have been reported, including an enzyme-linked immunosorbent assay, and a direct enzyme-linked antiglobulin test by Flow cytometry in dogs (Barta, 1981; Tarrant, 2005) but, although some of these tests may arguably be more sensitive than the DAT, they have not as yet become commonly available. Flow cytometry have more advantage than DAT due to the need less antiserum and have standardization on interpret the result (Quigley *et al*., 2001). Regardless of whether a DAT or an alternative test for ant-RBC antibody is used, clinicians should be aware that a positive result

merely records the presence of antibody, and does not determine whether IMHA is primary (AIHA) or secondary.

Identification of underlying disease

Since IMHA is often secondary, particularly in cats with an atypical signalment, confirmation of a diagnosis of IMHA is not necessarily the end of the diagnostic trail. Primary IMHA can only be diagnosed with absolute certainty once potential underlying causes have been thoroughly investigated. Standard screening tests for underlying disease which ideally should be performed in all animals with IMHA include hematology (including careful examination of a blood smear), serum biochemistry, urinalysis, thoracic and abdominal radiography and, in cats, testing for retroviruses (particularly FeLV). Serologic and/or PCR testing for RBC parasites such as *Mycoplasma haemofelis* in cats is indicated. Since arguably rickettsial diseases may also predispose to secondary IMHA, testing for *Ehrlichia* species may also be indicated in endemic areas. Further tests that might be considered in some patients, particularly in older animals in which underlying occult neoplasia (especially lymphoproliferative disease) are a real possibility, include abdominal ultrasonography, lymph node aspiration cytology, and bone marrow analysis.

Bone marrow evaluation

Bone marrow evaluation is indicated when peripheral blood abnormalities are detected. The most common indications are persistent neutropenia, unexplained thrombocytopenia, poorly regenerative anemia, or a combination. Examples of proliferative abnormalities in which bone marrow examination may be indicated include persistent thrombocytosis or leukocytosis, abnormal blood cell morphology, or the unexplained presence of immature cells in blood (e.g., nucleated erythroid cells in the absence of polychromasia or a neutrophilic left shift in the absence of inflammation).

Bone marrow is examined to stage neoplastic conditions (lymphomas and mast cell tumors); estimate the adequacy of body iron stores; evaluate lytic bone

lesions; and search for occult disease in animals with fever of unknown origin, unexplained weight loss, and unexplained malaise. Bone marrow examination can also be useful in determining the cause of a hyperproteinemia when it occurs secondarily to multiple myeloma, lymphoma, leishmaniasis, and systemic fungal diseases. It may also reveal the cause of a hypercalcemia when associated with lymphoid neoplasms, multiple myeloma, or metastatic neoplasms to bone.

Bone marrow aspirate biopsies are done more frequently than core biopsies in veterinary medicine. Aspirate biopsies are easier, faster, and less expensive to perform than are core biopsies. Bone marrow core biopsies require special needles that cut a solid core of material, which is then placed in fixative, decalcified, embedded, sectioned, stained, and examined microscopically by a pathologist. Core biopsy sections provide a more accurate way of evaluating marrow cellularity and examining for metastatic neoplasia than do aspirate smears, but cell morphology is more difficult to assess.

Bone marrow analysis (aspiration cytology and/or core biopsy histopathology) is indicated in all patients suspected to have the non-regenerative forms of IMHA (Harvey, 2001; Weiss and Aird, 2001). Pure red cell aplasia is characterized by a relative or complete lack of RBC precursors within the marrow, whereas cytological or histopathological evidence of an erythroid 'maturation arrest' (preponderance of immature precursors, with an absence of more mature RBC precursors) suggests that, rather than being directed against very early stem cells, antibodies are directed against a later stage of marrow RBC development. Marrow cytology and/or histopathology may reveal macrophages phagocytosing erythrocytes or RBC precursors. In such patients, when available, techniques such as immunofluorescent or immunoperoxidase staining of marrow samples may confirm the presence of antibodies directed against RBC precursors.

The fast and accuracy test is the important key to help patient with IMHA. Because in late stage, patient cannot cope with lack of oxygenation situation and die with severe anemia. Early treatment by using immunosuppressive drug such as

corticosteroid, cyclosporine, azathioprine can combat with IMHA and have a good result for patient.

CHAPTER 3

MATERIALS AND METHODS

Study animals: are divided into 2 groups

a) Controls consisted of ten healthy cats presented to Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University with normal CBC and blood chemistry. Cats were included with no gender and breed preference aging between 12-120 months old.

b) Anemic cases (IMHA and non-IMHA) consisted of nineteen cats with anemia presented at Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University and 3 Veterinary Hospitals in Bangkok metropolitan area with no gender and breed preference aging between 12-120 months old. Cats with chronic renal failure, blood loss anemia and/or receiving immunosuppressive treatment within one month were excluded from the study. Cats with hematocrit of lower than 27% (Couto, 2001) were divided in 2 subgroups:

   1. Regenerative anemia group: cats with absolute aggregate reticulocytes count level of 42,000 /µl and/or corrected reticulocytes count > 0.4% (Cowgill *et al*., 2003).
   2. Non regenerative anemia group: all anemic cats that had been anemic for at least 5 days with absolute aggregate reticulocytes count of less than 15,000/µl and/or corrected reticulocytes count $\leq$ 0.4%.

Samples collection

Peripheral blood was collected from cephalic or saphenous vein then placed into an EDTA tube. Cats with IMHA were treated with corticosteroid and followed-up for changes in red blood cells or complete blood count.

Diagnostic method

a) Blood sample was tested for PCV (microhematocrit test), CBC (manual count), reticulocyte count (manual count), total protein (refractometer) and blood chemistry by colorimetric method including ALT (Audit Diagnostic, Ireland), ALP (Audit Diagnostic, Ireland), BUN (Merck, Germany) and creatinine (ASRITHA, INDIA).

b) Auto-agglutination test:

   1. Macroscopic agglutination was inspected by visible of multiple red speckles on a glass slide. (Mackin, 2002)
   2. Microscopic agglutination was performed with saline dilution on a glass slide (one drop of RBCs to two drops of saline) and inspected under light microscope. (Mackin, 2002)

c) Direct agglutination test (DAT)(Coombs' test) (Quigley *et al*, 2001)

   1. 1 ml EDTA blood was centrifuged at 3000 rpm for 5 minutes for separation red blood cell from others components.
   2. Mixed 0.1 ml red blood cell with 4.9 ml phosphate buffered saline (PBS) then centrifuged at 3000 rpm for 5 minutes. Discard supernatant and repeated the procedure for 4 times.
   3. Mixed washed red blood cell 0.1 ml with PBS 4.9 ml. This would made 2% red blood cell solution.
   4. Added 0.1% bovine serum albumin for blocking antigen-antibody reaction.
   5. Filled 12 well of 96 well micro-titer plate with PBS 0.1 ml in each well and added Feline Coombs reagent (Antiserum feline IgG, IgM and C3 from VMRD Coombs' test, USA) 0.1 ml in 1st well mixed properly and transferred 0.1 ml of this solution in to next well. Repeated until well number 11 (two fold dilution). This made serial dilution from 1:1, 1:2… 1:1,024. Negative control is in well 12 (PBS only).
   6. Added 2% red blood cell solution in each well. Incubated at 37°C for 30 minutes at room temperature for 30 minutes.

7. Results interpretation:
    - Negative result contained a button of RBCs that would stream when the microtiter plate was tilted.
    - Positive result exhibited a matte formation that did not stream when tilted.
8. Results would be reported as negative or positive. Highest dilution with complete agglutination titer would be reported as part of all positive results.

d) Flow cytometry test (Quigley *et al*, 2001)

1. Diluted 2% red blood cell solution from packed RBC for Coombs' test with PBS at 1:1 ratio. This would made 1% RBC solution.
2. Prepared 1:30 dilution (PBS diluent) of fluorescein isothiocyanate (FITC)-labeled goat anti-cat IgG (heavy chain specific) (Serotec, USA), FITC-labeled goat anti-cat IgM (m chain specific) (Serotec, USA).
3. Mixed 1% red blood cell solution 50 µl with (FITC)-labeled goat anti-cat IgG (polyclonal) 50 µl and incubated at 4°C in the dark for 45 minutes.
4. Mixed 1% red blood cell solution 50 µl with (FITC)-labeled goat anti-cat IgM (polyclonal) 50 µl and incubate at 4°C in dark for 45 minutes.
5. RBCs had been washed twice and resuspended in 0.2 ml of isotonic PBS with 0.1% bovine serum albumin and 0.1 ml of 10% buffered formalin.
6. Samples were analyzed by flow cytometry (BD FACSCalibur™, USA).
7. Forward and side (orthogonal) scattering signals and fluorescein-generated fluorescence (488 nm) signals were collected in list mode. Specific gates were set to identify red cells. For each sample, the median fluorescence channel recorded for 10,000 red cells.
8. BD CellQuest Pro software was used for calculation of mean fluorescence intensity (MFI). The MFI values from 10 healthy non-anemic cats were used to establish a reference interval. A test sample considered positive if the MFI was more than 2 SD above the mean for

clinically normal cats and considered negative if the MFI fell below this value.

Data analysis

Sensitivity of the tests was defined as the number of cats with IMHA that tested positive with gold standard (DAT) and specificity was defined as the number of healthy cats that tested negative with gold standard (DAT). Positive predictive value was defined as the number of cats that tested positive that had IMHA, and negative predictive value was defined as the number of cats that tested negative that did not have IMHA.

Variables (Means of PCV, red blood cell count, corrected reticulocytes percentage, platelet count, white blood cell count, total protein, ALT, ALP, BUN, creatinine, MFI of IgG and IgM) were compared among three groups of control, non-IMHA and IMHA cats with ANOVA. Ninety-five percent confidence intervals (95% CI) are reported along with sensitivity, specificity, positive/negative predictive values, and ANOVA.

CHAPTER 4

RESULTS

Results from whole blood (EDTA) taken from 19 anemic cats (PCV 8-26 %, mean ± SD 17.00 ± 5.86 %, median 17 %, red blood cell count 1.0-6.07 X 10^6 cell/µl, mean 3.17 ± 1.60 X 10^6 cell/µl, median 3.50 X 10^6 cell/µl) referred for the diagnosis of IMHA over a time period of 11 months.

The direct flow cytometry erythrocyte immunofluorescence assay and Coombs' test were used to detect immunoglobulin bounded with red blood cells. Blood samples from 10 healthy cats were used as the controls (PCV 33-52 %, mean ± SD 41.30 ± 6.02 %, median 42 %, red blood cell count 5.50-8.66 X 10^6 cell/µl, mean ± SD 6.75 ± 0.98 X 10^6 cell/µl, median 6.655 X 10^6 cell/µl). Anemic cats consisted of 1 Siamese, 1 Persian and 16 domestic short hair, with age ranged from 12 to 120 months old. Genders of these cats were 10 male, 4 female, 4 spayed female and 1 undetermined (Fig. 4). These 19 cats suffered from viral infectious diseases and others various diseases (Table 3) (Fig. 5).

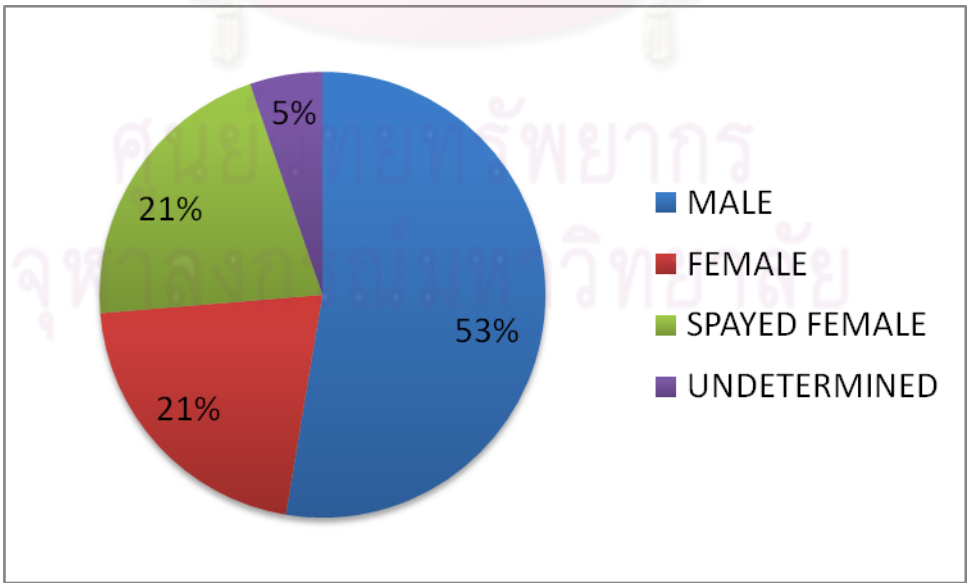


Fig. 4 Percentage of anemic cats according to gender (n=19).

Table 3 Diseases status of anemic cats (n=19)

	Coombs' test	
	Positive	Negative
FeLV	2	6
FIV	2	0
FIP + FeLV	1	0
Calici virus	0	1
Unknown	0	7
Total Number of Cats	5	14

Note: FeLV and FIV were diagnosed with ELISA test kit (IDEXX, USA). FIP and calici virus were suspected by clinical signs and fluid analysis. No definitive diagnostic test was done.

FeLV = Feline Leukemia Virus FIV = Feline Immunodeficiency Virus FIP = Feline Infectious Peritonitis

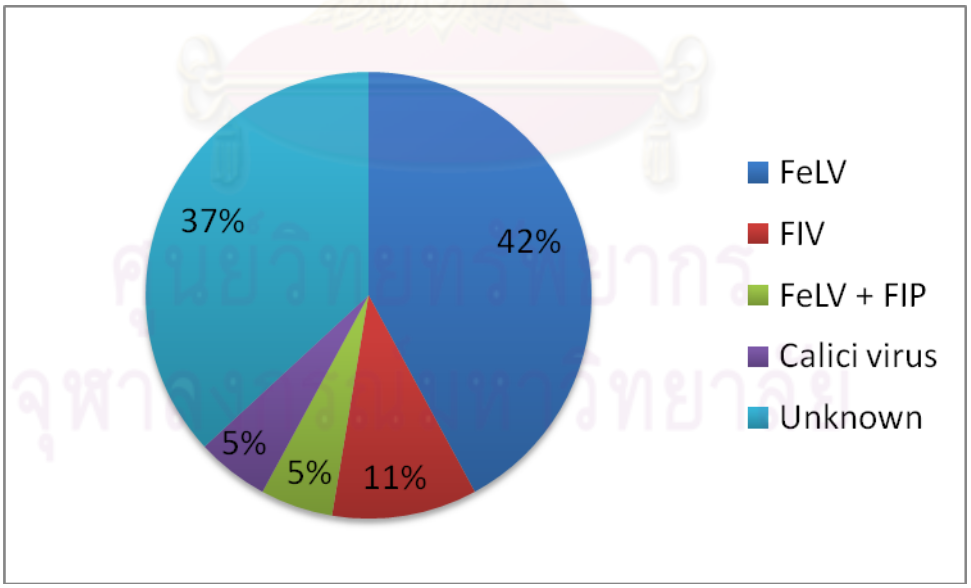


Fig. 5 Diseases status of all anemic cats (n=19).

Note:

FeLV = Feline Leukemia Virus FIV = Feline Immunodeficiency Virus FIP = Feline Infectious Peritonitis

The results of this study showed that the prevalence of feline IMHA presents in cats with PCV less than 27% at Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University and veterinary hospitals in Bangkok area between October 2008 and August 2009 was 26.31% (5/19) by using Coombs' test (Fig. 6).

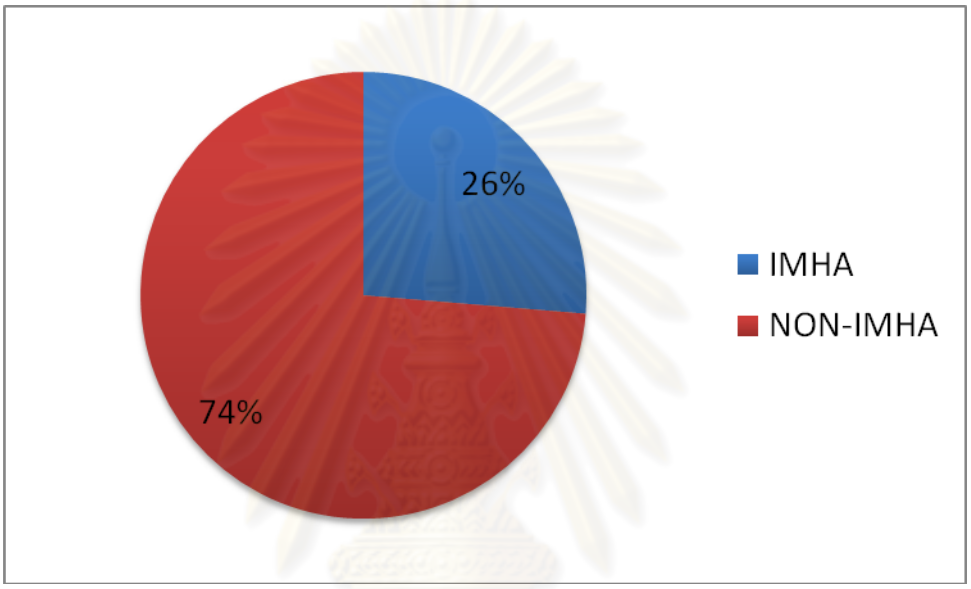


Fig. 6 Percentage of anemic cats with IMHA found in Small Animal Hospital and Veterinary Hospitals in Bangkok metropolitans area.

The IMHA cats were 2 FeLV, 2 FIV, 1 FeLV and FIP combination (Fig. 7). Mean age of cats with IMHA was 4.4 years old. Five IMHA cats consisted of 1 Siamese, 1 Persian and 3 domestic short hair (Fig. 8) with no gender predisposition (Fig. 9).

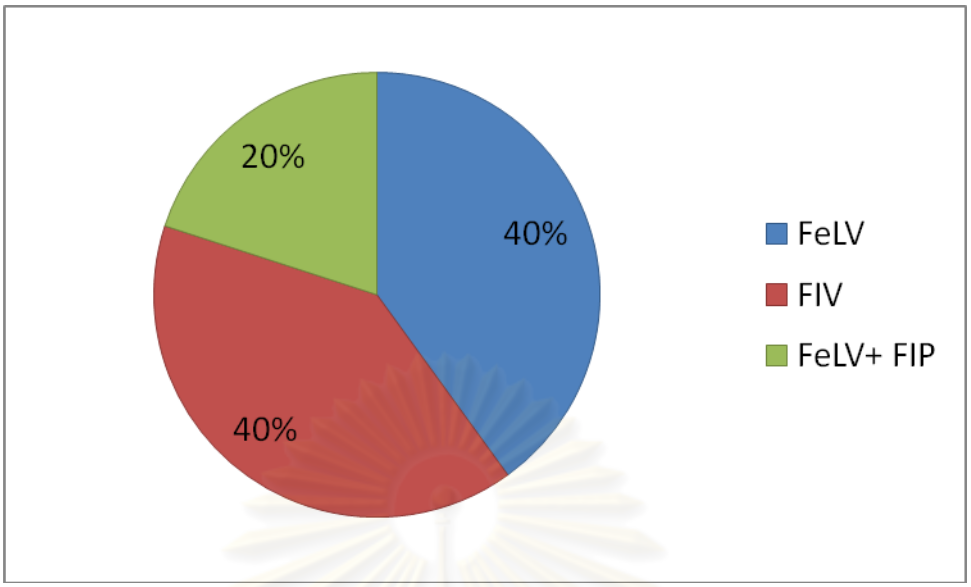


Fig. 7 Causes of IMHA in five positive IMHA cats.

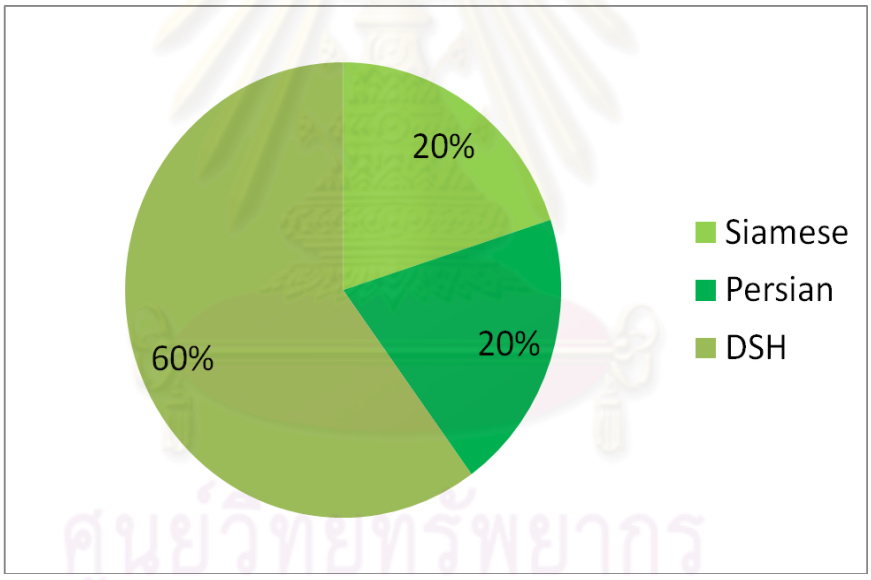


Fig. 8 Percentage of cats with IMHA according to breed.

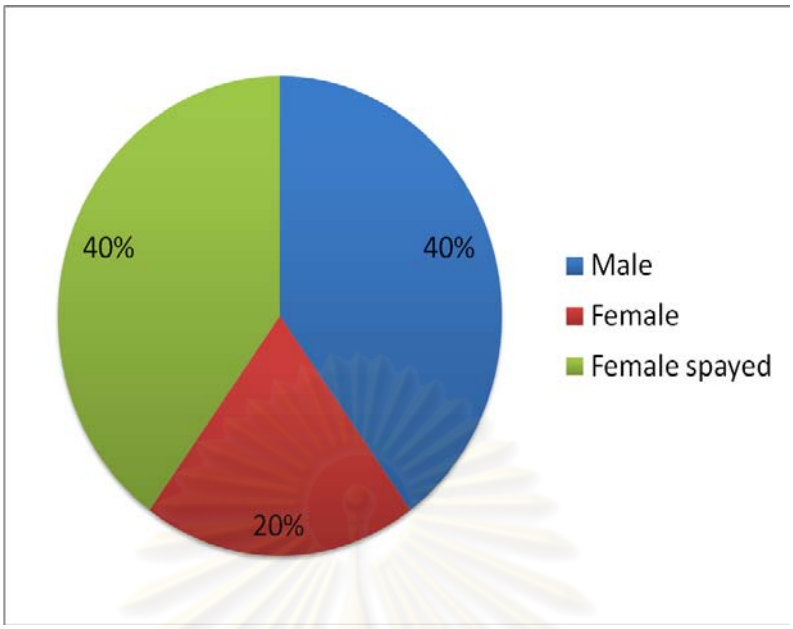


Fig. 9 Percentage of IMHA cats according to gender.

IMHA positive cats had RBC range 1.48-4 X 10^6 cell/µl, mean ± SD 2.84 ± 1.25 X 10^6 cell/µl, median 3.55 X 10^6 cell/µl and PCV range between 12% - 26%, mean ± SD 17.20 ± 5.80%, median 17% with evidence of regenerative anemia in only one cat. Thrombocytopenia (mean platelet value ± SD was 0.84 ± 0.46 X 10^5 cell/µl) was found in all of cats with IMHA ranged from 49,000 to 148,000 cell/µl (median, 53,000 cell/µl) (Table 5).

White blood cell counts varied depend on each cat and its stage of the disease. Mean white blood count and blood chemistry results of all cats were within normal range. Total protein, alkaline phosphatase, alanine transaminase, creatinine and blood urea nitrogen were normal (Table 6). Among the IMHA positive cats, three cats were seropositive for FeLV (60%) and two cats for FIV (40%).

Sensitivity and specificity of flow cytometry (FC) method were 100% and 50% compared with DAT method. Positive predictive value is 41.66% and negative predictive value is 100%. These values from FC were compared with DAT. PCV from control, IMHA and anemic group ± SD were 41.30 ± 6.02%, 17.20 ± 5.80% and 16.93 ± 6.10% (Table 5). PCV and red blood cell count were statistically significant difference between

controls and 19 anemic cats ($P<0.01$). Regenerative anemia was found only in 20% of IMHA (corrected reticulocytes count 0-0.8%, mean ± SD 0.24 ± 0.32%, median 0.13 %). This may be due to the time of detection that passed the regenerative phase (4-7 days) for a period of time instead of these cats had bone marrow abnormality. This study showed the lack of aggregate reticulocytes but many of punctuate reticulocytes found in reticulocytes count which confirmed that the regenerative phase of IMHA had been passed for 14-21 days. All IMHA cats had thrombocytopenia (platelet count 0.49-1.48 X 10^5 cell/µl, mean ± SD 0.84 ± 0.46 X 10^5 cell/µl, median 0.53 X 10^5 cell/µl). Platelet was statistically significant different between control and anemic cats ($P<0.05$). The white blood cell counts (total white blood cell count 6,000-24,500 cell/µl, mean ± SD 15,160 ± 8,371 cell/µl, median 18,700 cell/µl) were unremarkable different. FeLV and/or FIV were diagnosed in all IMHA cats (FeLV 60%, FIV 40%) (Table 5).

Blood chemistry values were also unremarkable (ALT 25-98 U/L, mean ± SD 45.34 ± 30.11 U/L, median 33 U/L, ALP 23.7-66 U/L, mean ± SD 44.85 ± 29.91, median 44.85, BUN 14.2 -31.4 mg/dl, mean ± SD 22.8 ± 12.16mg/dl, median 22.8 mg/dl, creatinine 0.7-1.74 mg/dl, mean ± SD 1.22 ± 0.39 mg/dl, median 1.30 mg/dl) (Table 6).

Abnormal blood morphology were also observed such as stomatocytes, schistocytes, poikilocytosis, anisocytosis, ploychromasia, hypochromasia and rouleaux formation that were found in many anemic cats in this study due to various causes of anemia. Autoagglutination was negative in all 5 IMHA cats and positive in 1 of 14 non-IMHA anemic cats (7.14%) (Table 7). DAT was positive in 5 IMHA cats and had titer from 1:1 to 1:128 in IMHA group (table 4). All of non-IMHA cats were negative for DAT. Due to low sensitivity; negative results were not expected to be true negative.

Red blood cells were detected by flow cytometry (Fig. 10, 11, 12). Total positive result was 12 from 19 anemic cats. All IMHA cats were positive for both IgG and IgM detected on red blood cell surface (MFI of IgG 43.46-79.53, mean ± SD 61.01 ± 13.98, median 57.25, MFI of IgM 37.92-54.88, mean ± SD 47.90 ± 6.69, median 48.71). Six of non-IMHA cats which were positive from flow cytometry test had insignificant higher level of MFI for IgG or IgM than control cats. After staining with (FITC)-labeled goat anti-cat IgG and IgM, MFI in IMHA cats were more than mean + 2SD of control group (cut

point). Cut point was 27.20 for IgG and 25.51 for IgM (Table 7). Difference between staining intensities of IMHA group and control group were statistically significant ($P<0.01$) and also different between IMHA group and non IMHA ($P<0.01$).

Three of IMHA cats survive. One of IMHA cat which positive for FeLV (cat number 5) was treated with prednisolone and recover from IMHA. MFI of IgG and IgM reduced from 54.99 and 85.09 on the first day of diagnose of IMHA to 17.72 and 18.42 after treatment (Fig. 13).

Table 4 Serum titer of five IMHA cats diagnosed with Coombs' test

Cat's Name	Serum Titer
NO.1 (TUA-TUNG)	1: 128
NO. 2 (TUA-LEK)	1:1
NO.3 (TA-TOE)	1:4
NO. 4 (SI-RI)	1:4
NO. 5 (MOKKY)	1:2

Note: Serum titer, feline IgG, IgM and C3 were used in serial dilution agglutination assays. Coombs' test was positive in 5 of 19 patients.

Table 5 Comparison of packed red blood cell volume, corrected reticulocytes percentage, red blood cell, white blood cell and platelet in control, IMHA and non-IMHA cats

PARAMETER	CONTROL GROUP (n=10) (mean ± SD)	ANEMIC GROUP	
		IMHA (n=5) (mean ± SD)	NON-IMHA (n=14) (mean ± SD)
PCV (%)	41.30 ± 6.02	17.20 ± 5.80[aa]	16.93 ± 6.10 [bb]
CORRECTED RETICULOCYTE (%)	0.08 ± 0.11	0.24 ± 0.32	0.34 ± 0.50
RED BLOODCELL COUNT (cell/µl)	6.75 ± 0.98 X 10^6	2.84 ± 1.25 X 10^{6aa}	3.29 ± 1.73 X $10^{6\,bb}$
PLATELET COUNT (cell/µl)	2.59 ± 1.38 X 10^5	0.84 ± 0.46 X $10^{5\,a}$	1.12 ± 1.10 X $10^{5\,b}$
WHITE BLOOD CELL COUNT (cell/µl)	10,723 ± 3,402	15,160 ± 8,371	13,817 ± 12,004

Note [a] $P < 0.05$ when compared between controls and IMHA cats [b] $P < 0.05$ when compared between non IMHA and control cats

[aa] $P < 0.01$ when compared between controls and IMHA cats [bb] $P < 0.01$ when compared between non IMHA and control cats

IMHA = Immune-mediated hemolytic anemia Non-IMHA = Non-immune mediated hemolytic anemia

Table 6 Comparison of total protein, alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine in control, IMHA and non IMHA cats

PARAMETER	CONTROL GROUP (n=10) (mean ± SD)	ANEMIC GROUP	
		IMHA (n=5) (mean ± SD)	NON-IMHA (n=14) (mean ± SD)
TOTAL PROTEIN (g/dl)	7.41 ± 0.73	7.74 ± 0.50	7.54 ± 1.40
ALT (U/L)	35.72 ± 14.31	45.34 ± 30.11	126.21 ± 232.65
ALP (U/L)	89.46 ± 56.52	44.85 ± 29.91	39.85 ± 53.79
BUN (mg/dl)	31.17 ± 8.13	22.80 ± 12.16	20.91 ± 26.58
CREATININIE (mg/dl)	1.86 ± 0.34	1.23 ± 0.4	0.98 ± 0.49

Note: ALT = Alanine aminotransferase
ALP = Alkaline phosphatase
BUN =Blood urea nitrogen
IMHA = Immune-mediated hemolytic anemia
Non-IMHA = Non-immune mediated hemolytic anemia

Table 7 Comparison of the results auto-agglutination test, Coombs' test and mean fluorescence intensity (MFI) from flow cytometry in controls, IMHA and non-IMHA cats

PARAMETER		CONTROL GROUP (n=10)	ANEMIC GROUP	
			IMHA (n=5)	NON-IMHA (n=14)
AUTO AGGLUTINATION		0% (0/10)	0% (0/5)	7.14% (1/14)
COOMBS' TEST		0% (0/10)	100% (5/5)	0% (0/14)
FLOW CYTOMETRY (MFI)	IgG (mean ± SD)	19.20 ± 4.00	61.01± 13.98[aa]	25.84 ± 7.22[a]
	CUT POINT	27.20		
	IgM (mean ± SD)	17.87 ± 3.82	47.90 ± 6.69[aa]	22.72 ± 6.51[a]
	CUT POINT	25.51		

Note [a] $P < 0.05$ when compared between controls and non-IMHA cats

[aa] $P < 0.01$ when compared between IMHA and control cats

IMHA = Immune-mediated hemolytic anemia

Non-IMHA = Non-immune mediated hemolytic anemia

MFI = Mean fluorescence intensity

IgG = Immunoglobulin-G

IgM = Immunoglobulin-M

CUT POINT = Mean + 2SD of controls

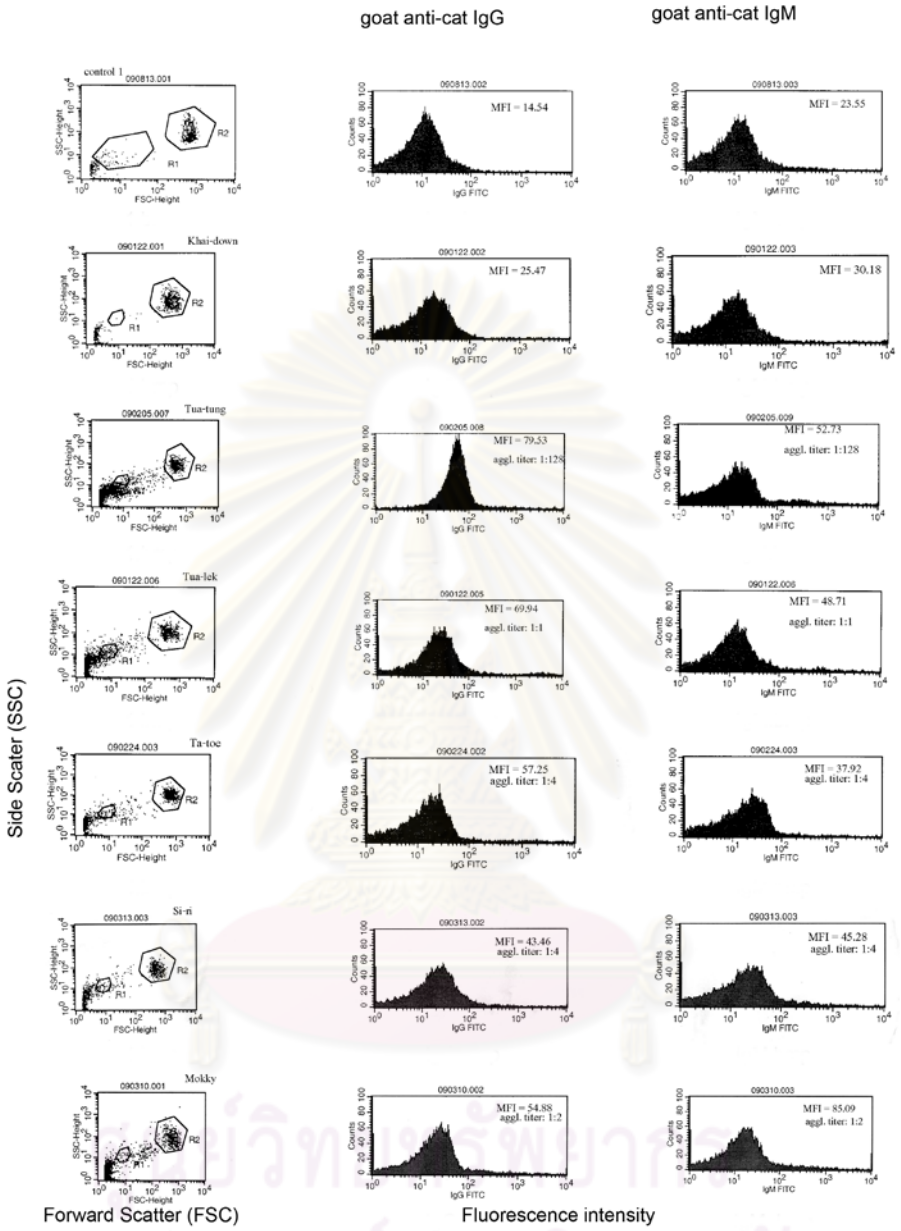


Fig. 10 Flow cytometry evaluation of RBCs from healthy, non IMHA and IMHA cats after staining with IgG and IgM-specific secondary reagent. FSC/SSC dot-plots and fluorescence histogram are shown for one healthy control cat, one non IMHA anemic cat and five IMHA anemic cats after incubation with goat anti-cat IgG (1:30) and goat anti-cat IgM (1:30). Histogram of fluorescence profiles were generated after gating on RBC in FSC/SSC dot-plots (region R2). Mean fluorescence intensities (MFI) are noted within each histogram; in anemic cats agglutination titer (AT) is also shown.

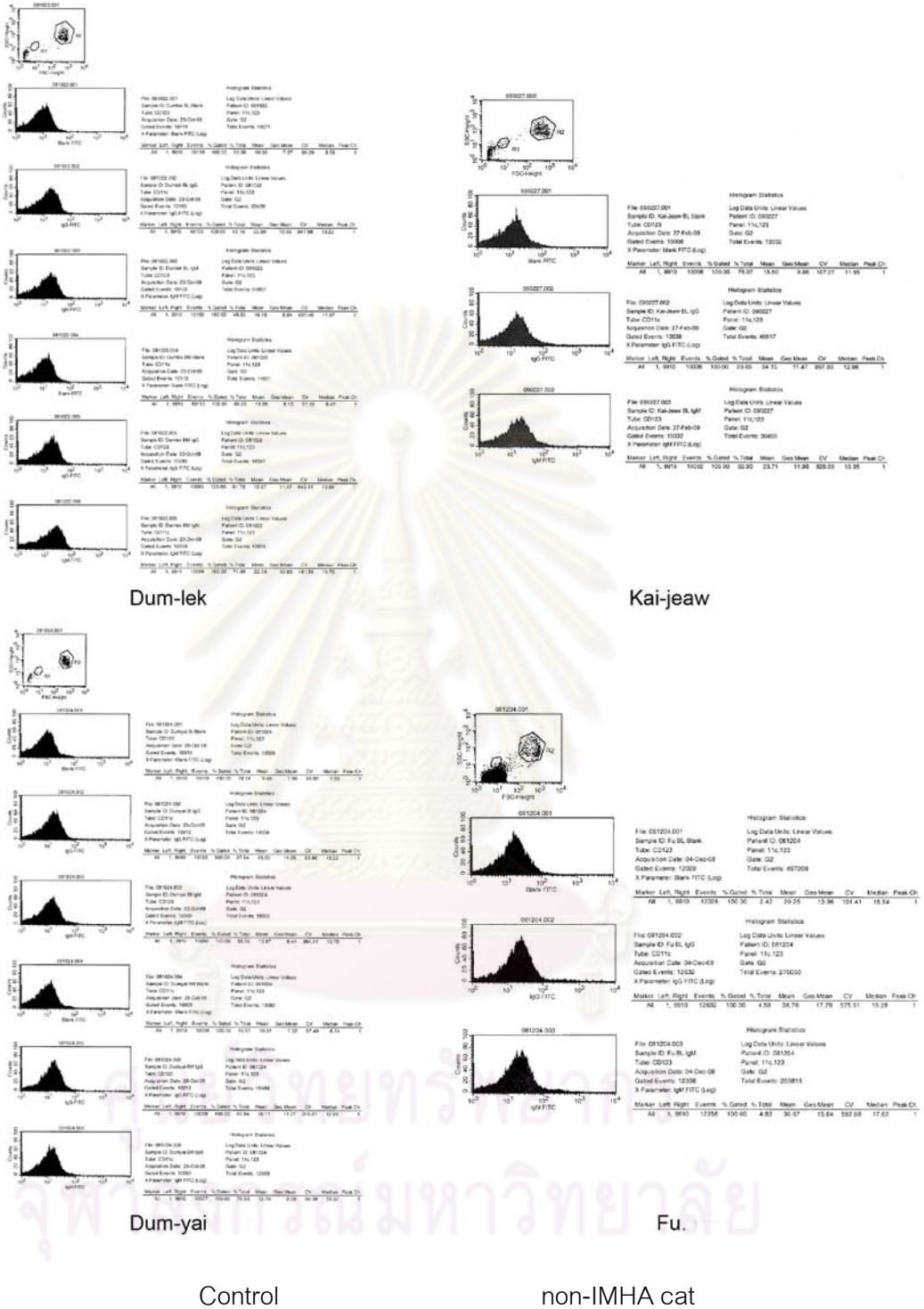


Fig. 11 Flow cytometry compared between controls and non-IMHA cats shows gating of red blood cells and fluorescence related to red blood cells.

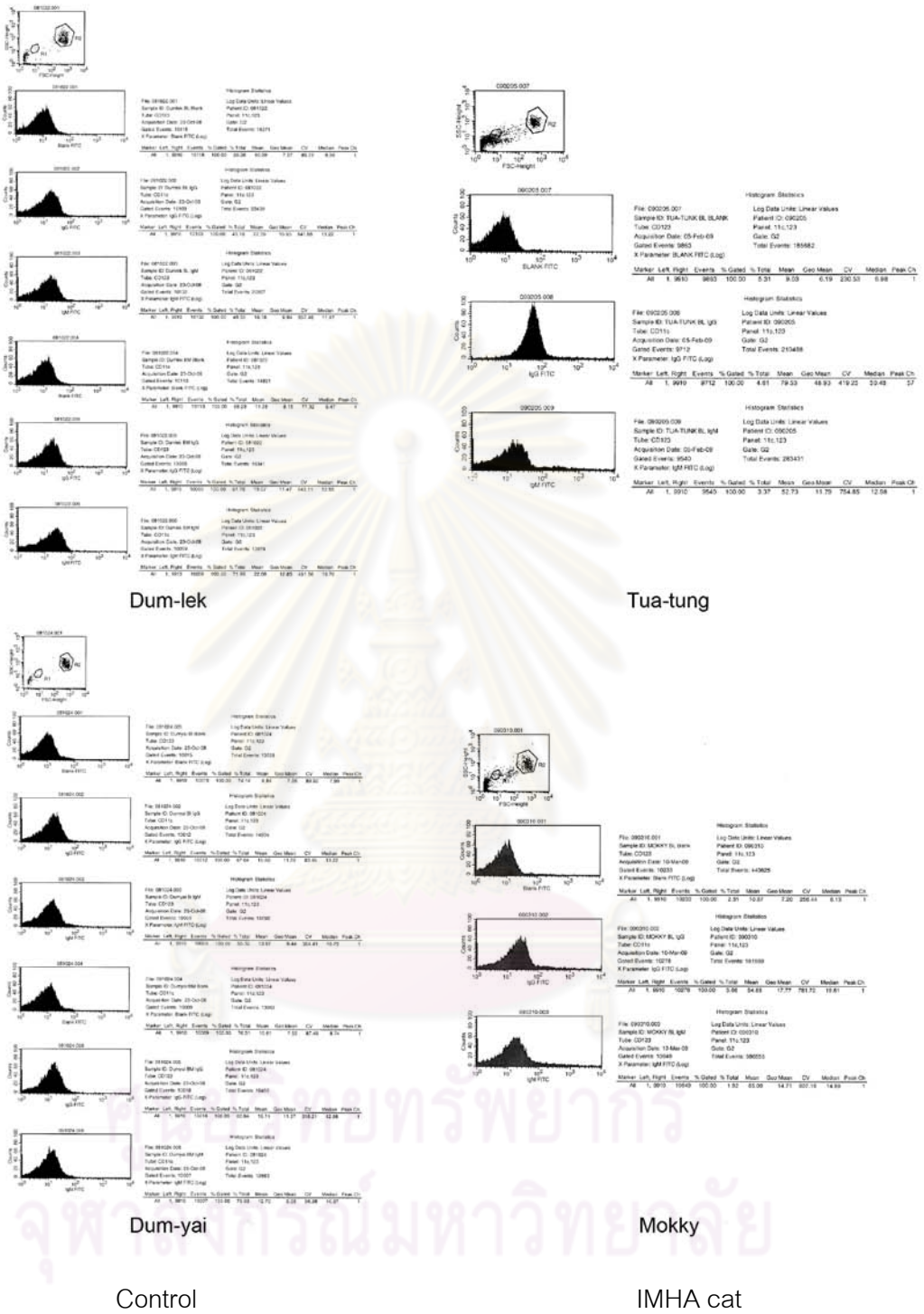


Fig. 12 Flow cytometry compared between controls and IMHA cats shows difference of fluorescence dissemination in red blood cell group.

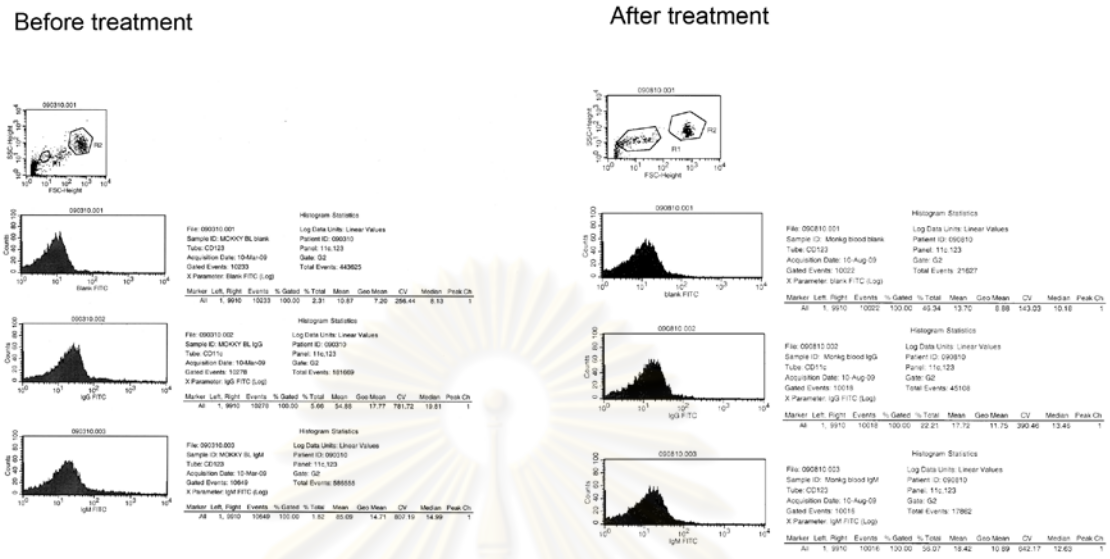


Fig. 13 Comparison of flow cytometry results before and after treatment of IMHA cat. MFI of IgG and IgM reduced from 54.99, 85.09 to 17.72 and 18.42 in phase of 5 months.

CHAPTER 5

DISCUSSION AND CONCLUSION

The prevalence of IMHA in cats of PCV less than 27% from Small Animal Hospital, Chulalongkorn University and 3 veterinary hospitals in Bangkok is 26.31% by Coombs' test. Our results are consistent with previous reports that the incidence of feline IMHA ranged between 13% in 2006 (Kohn *et al*., 2006) and 24% in 2007 from cat with anemia at Small Animal Hospital Free University of Berlin Germany (Kohn 2007).

This study also had shown that cats with IMHA are sero-positive for FeLV or FIV. Our findings showed that 3 out of 5 cats (60%) were FeLV positive and 2 (40%) were FIV positive by ELISA test kit (IDEXX, USA). As in others studies, most IMHA cases are secondary IMHA in cat (Day 1996; Gunn-Moore *et al*, 1999). It may be from the chronicity of FeLV infection that increases the amount of immunoglobulin which induced formation of IMHA. The result is immunoglobulins that bind to affected red blood cell surface resulting in IMHA. Non self antigen from FeLV and FIV infection may coated red blood cell surface and caused immune system to attack the red blood cell.

Feline IMHA has been reported in young male cats (McCullough, 2003). However, in this study there are no differences between age and gender in five positive cats. In canine, there were breeds reported with higher incidence such as Doberman Pinschers, Cocker Spaniels, Miniature Poodles, Irish Setters, Collies, English Springer Spaniels, Old English Sheepdogs and American Cocker Spaniels (McCullough, 2003).

As feline IMHA is mostly secondary, ruling out others causes are necessary to conclude that primary IMHA is found. In one retrospective study, Kohn, *et al*. (2006) found 19 cats with primary IMHA. This is contrast to canine patient that primary IMHA is more commonly found than secondary IMHA. The estimation of primary canine IMHA is 60-75% (Piek *et al*., 2008).

Not only RBC, but platelets are frequently decreased in IMHA cats. This may be due to immune system that is not only attack red blood cell but also damaged platelets cells (immune mediated thrombocytopenia purpura, IMTP) as well. Evan's syndrome has been called for patient with both IMHA and IMTP.

Three out of five IMHA cats survived in this study. The mortality rate is 40% (2/5). The survival rate of IMHA is 30-70% (McCullough, 2003). This outcome indicates the importance of IMHA diagnosis in cats with anemia. An earlier diagnosis can give a better prognosis for IMHA cats.

Our results also found that autoagglutination is negative in all IMHA cats and positive in one non-IMHA cat. This may suggested that autoagglutination is not a good screening test for feline IMHA due to the very low sensitivity (0%). It is contrast to canine IMHA which most of severe IMHA dogs may have auto-agglutination.

Using Coombs' test (DAT method), we could detect 5 IMHA cats from 19 anemic cats. Aggultination titer from DAT test ranged from 1:1 to 1:128 in the five positive cats. Cat No.1 had a highest titer due from the result of number of antigen (IgG,IgM and C3) on red blood cells that much more than others 4 positive cats. Results from other studies showed that DAT had low sensitivity due to loss of antigen on red blood cell surface during washing red blood cells procedure (high false negative) but high specificity (true negative) (Kucinskiene *et al*., 2005; Quiqley *et al*., 2001). Even though, our IMHA negative cats may be negative by DAT, others diagnostic method or serial testing may be needed to confirm these results. Other various factors may affect the result of DAT test. False positive rarely found but hemolytic sample is the main pathological abnormality that leading to false positive DAT results.

The FC method in this study had higher detection rate than Coombs' test when the cut point was set at mean + 2SD of the mean value in the control group. All IMHA cats had MFI more than 40 for IgG and 37 for IgM while none of the non-IMHA cats had this high value. In cats' number 9, 12, 13, 14, 15, 16 and 18 of non-IMHA group, there were negative results with Coombs' test but positive by using FC method and the level of MFI were not exceeded minimal level of IMHA group. These cats may be in the early stage of IMHA and a serial testing is recommended for follow up before final diagnosis. The results in this study are similar to those previously reported in dogs and humans, in which FC has been found to be more sensitive than Coombs' test (DAT). This may be from incomplete of antibody detected by FC, since antibody for complement is not

included in this study. Complement is one of three parts in pathophysiology in IMHA and should be included in diagnostic material.

Immunoglobulin M and G are both detected by FC method. There are consistent in each other positive IMHA cat in this study with high mean fluorescence intensity value. But in negative IMHA group, there were some cats that have slightly high IgG. These cats are suspicious of having subclinical IMHA and should required further investigation.

IMHA cats in this study were treated with prednisolone dose 2 mg/kg twice a day and taper dose to minimum for reducing side effect. Cat number 1 presented with pale mucous membrane and diarrhea. He died a week later after the diagnosis. This may be from high Coombs' test titer (1:128) which means that enormous inflammation occurs in his body and may lead to serious overwhelming process and at last systemic inflammatory response syndrome and multiple organ dysfunction syndrome. However, the correlation of titer and severity still unclear (Day, 2000). Cat number 3 died because of failure of drug deliver (owner could not give medication). Cat number 2 and 5 response to prednisolone but recurrence occurs after tapering prednisolone. This may be from too quick to reduce dosage or the underlying causes were not treated. Only cat number 4 response well and does not relapse since the lymphoma which may be the cause of IMHA was treated (Table 22, 23, 24).

One of the main advantages of FC is that it requires much less sample quantity than DAT method. It is more cost effectiveness than DAT method due to amount of anti-antigen used in each test. Our results show that flow cytometry is a good diagnostic tool for feline IMHA and can be used for follow up as a prognostic indicator for cats with IMHA. But DAT can tell only positive or negative IMHA result. The only disadvantage of FC method is the availability of Flow cytometry in only some research facilities due to the high cost of equipment.

At the present, there is little information of the application of direct flow cytometry method to detect red blood cell bound immunoglobuluin in cats. Our study was the first

report in cats which has shown that this technique has a promising result and should be developed as a tool for detect IMHA in cat in the future.

REFERENCES

Barger, A.M. 2003. The complete blood cell count: a powerful diagnostic tool. Vet. Clin. North Am. Small Anim. Pract. 33(6): 1207-1222.

Barker, R.N. 2000. Anemia associated with immune responses. In: Schalm's Veterinary Hematology. Feldman, B.F., Zinkl, J.G., and Jain, N.C. (5th ed.).Baltimore: Lippincott Williams & Wilkins. 169-177.

Barker, R.N., Gruffydd-Jones, T.J., Stokes, C.R., and Elson, C.J. 1992. Autoimmune haemolysis in the dog: relationship between anaemia and the levels of red blood cell bound immunoglobulins and complement measured by an enzyme-linked antiglobulin test. Vet. Immunol. Immunopathol. 34(1-2): 1-20.

Barta, O. 1981. Laboratory techniques of veterinary clinical immunology: A review. Comp. Immunol. Microbiol. Infect. Dis. 4(2): 131-160.

Couto, C.G. 2001. Update on feline haematology. J. Feline Med. Surg. 3(4): 177-179.

Cowgill, E.S., Neel, J. A., and Grindem, C.B. 2003. Clinical application of reticulocyte counts in dogs and cats. Vet. Clin. North Am. Small Anim. Pract. 33(6): 1223-1244.

Day, M.J. 1996. Diagnostic assessment of the feline immune system, part2. Feline Pract. 24: 14-25.

Day, M.J. 1999. Antigen specificity in canine autoimmune haemolytic anaemia. Vet. Immunol. Immunopathol. 69(2): 215-224.

Day, M.J. 2000. Immune-mediated hemolytic anemia. In: Schalm's Veterinary Hematology. Feldman, B.F., Zinkl, J.G., and Jain, N.C. (5th ed.).Baltimore: Lippincott Williams & Wilkins. 799-806.

Day, M.J. 2002. Immune mediated hemolytic anemia and thrombocytopenia. Proceeding of the 74th Western Veterinary Conference 2002, Nevada, USA, February 10-14 [Online]. Available:http://www.vin.com/Members/Proceedings/Proceedings.plx?CID=wvc2002&PID=pr00677&O=VIN

Feldman, B.F. 2001. Feline anemia: practical investigation and management. Proceeding of the 26th World Small Animal Veterinary Association World Congress 2001, Vancouver, Canada, August 8-11 [Online].

Available:http://www.vin.com/Members/Proceedings/Proceedings.plx?CID=wsava2001&PID=pr00043&O=VIN

Giger, U. 2001. Diagnosis of hemolytic anemias. Proceeding of the 26th World Small Animal Veterinary Association World Congress 2001, Vancouver, Canada, August 8-11 [Online]. Available:http://www.vin.com/Members/Proceedings/Proceedings.plx?CID=wsava2001&PID=pr00152&O=VIN

Giger, U. 2005. Regenerative anemias caused by blood loss or hemolysis. In: Textbook of Veterinary Internal Medicine. Ettinger, S.J., and Feldman, E.C. (6th ed.) St. Louis: Elsvier Saunders. 1886-1907.

Gunn-Moore, D.A., Day, M.J., Graham, M.E.A., Cue, S.M., and Harbour, D.A. 1999. Immune-mediated haemolytic anaemia in two sibling cats associated with multicentric lymphoblastic infiltration. J. Feline Med. Surg. 1(4): 209-214.

Harvey, J.W. 2001. Atlas of Veterinary Hematology. Philadephia: W.B. Saunders. 3-17, 93-106.

Husbands , B.D., Smith, S.A., and Weiss, D.J. 2002. Idiopathic immune-mediated hemolytic anemia (IMHA) in 25 Cats. Proceeding of the 20th American College of Veterinary Internal Medicine 2002, Texas, USA, May 29 – June 1 [Online]. Available:http://www.vin.com/Members/Proceedings/Proceedings.plx?CID=acvm2002&PID=pr01745&O=VIN

Kohn, B. 2007. Immune-mediated hemolytic anemia in cats. Proceeding of the 17th The European College of Veterinary Internal Medicine - Companion Animals Congress, Budapest, Hungary, September 13-15 [Online]. Available:http://www.vin.com/Members/Proceedings/Proceedings.plx?CID=ecvim2007&PID=pr18520&O=VIN

Kohn, B., Linden, T., and Leibold, W. 2006. Platelet-bound antibodies detected by a flow cytometric assay in cats with thrombocytopenia. J. Feline Med. Surg. 8(4): 254-260.

Kucinskiene G., Schuberth H.J., Leibold W., and Pieskus J. 2005. Flow cytometric evaluation of bound IgG on erythrocytes of anemic dogs. Vet. J.169: 303-307.

Mackin, A. 2002. Immune-mediated hemolytic anemia: Pathophysiology and

diagnosis. Proceeding of the 20th American College of Veterinary Internal Medicine 2002, Texas, USA, May 29 – June 1 [Online]. Available:http://www.vin.com/Members/Proceedings/Proceedings.plx?CID=acvim2002&PID=pr01915&O=VIN

McCullough, S. 2003. Immune-mediated hemolytic anemia: understanding the nemesis. Vet. Clin. North Am. Small Anim. Pract. 33(6): 1295-1315.

Nassiri, S. M., Shirani, D., Khazrainia, P., Hajmohammadali, A., Sharifi, H. 2005. The investigation of the prevalence of immune-mediated hemolytic anemia (IMHA) in anemic dogs referred to the Veterinary Teaching Hospital of the University of Tehran. Comp. Clin. Pathol. 14(3): 121-124.

Overmann, J.A., Sharkey, L.C., Weiss, D.J., and Borjesson, D.L. 2007. Performance of 2 microtiter canine coombs' tests. Vet. Clin. Pathol. 36(2): 179-183.

Piek C.J., Junius G., Dekker A., Schrauwen E., Slappendel R.J., and Teske E. 2008. Idiopathic immune-mediated hemolytic anemia: treatment outcome and prognostic factors in 149 dogs. J. Vet. Intern. Med. 22(2): 366-373.

Quigley, K.A., Chelack, B.J., Haines, D.M., and Jackson, M.L. 2001. Application of a direct flow cytometric erythrocyte immunofluorescence assay in dogs with immune-mediated hemolytic anemia and comparison to the direct antiglobulin test. Vet. Diag. Invest.13(4): 297-300.

Tarrant, J.M. 2005. The role of flow cytometry in companion animal diagnostic medicine. The Vet. J. 170(3): 278-288.

Tvedten, H. 2004. Diagnosis of hemolytic anemia. Proceeding of the 76th Western Veterinary Conference 2004, Nevada, USA, February 16-19 [Online]. Available: http://www.vin.com/Members/Proceedings/Proceedings.plx?CID=wvc2004&PID=pr05751&O=VIN

Wardrop, K.J. 2005. The Coombs' test in veterinary medicine: past, present, future. Vet. Clin. Pathol. 34(4): 325-334.

Weiss, D.J. 2006. Aplastic anemia in cats - clinicopathological features and associated disease conditions 1996-2004. J. Feline Med. Surg. 8(3): 203-206.

Weiss, D.J. 2008. Bone marrow pathology in dogs and cats with non-regenerative

immune-mediated haemolytic anaemia and pure red cell aplasia. J. Comp. Pathol. 138(1): 46-53.

Wiess, D.J., and Aird, B. 2001. Cytologic Evaluation of primary and secondary myelodysplastic syndromes in the dog. Vet. Clin. Pathol. 30(2): 67-75.

Zini, E., Hauser, B., Meli, M.L., and Glaus, T.M. 2007. Immune-mediated erythroid and megakaryocytic aplasia in a cat. J. Am. Vet. Med. Assoc. 230(7): 1024-1027.

APPENDIX

Appendix 1

Table 8 Data of breed, gender, age versus FeLV and FIV status in IMHA cats

NUMBER	BREED	GENDER	AGE (months)	FeLV, FIV STATUS
1	Siamese (Vicheanmas)	Male	12	FeLV + FIP
2	Persian	Male	84	FIV
3	DSH	Female	35	FeLV
4	DSH	Female Spayed	unknown	FeLV
5	DSH	Female Spayed	84	FIV

Note: DSH = Domestic short hair
FeLV = Feline Leukemia Virus
FIV = Feline Immunodeficiency Virus

Appendix 2

Table 9 Breed, gender, age versus clinical signs in IMHA cats

NUMBER	BREED	GENDER	AGE (months)	CLINICAL SIGNS
1	Siamese (Vicheanmas)	Male	12	Diarrhea, anemia
2	Persian	Male	84	anemia
3	DSH	Female	35	anemia
4	DSH	Female Spayed	unknown	Anorexia, pale pink mucous membrane, 10% dehydrate, fever, nasal discharge, lymphadenopathy, alopecia, lymphoma
5	DSH	Female Spayed	84	Pale mucous membrane, oral ulcer, mucous nasal discharge, flea infestation

Note: DSH = Domestic short hair
FeLV = Feline Leukemia Virus
FIV = Feline Immunodeficiency Virus

Appendix 3

Table 10 PCV, corrected reticulocytes percentage, red blood cell count and platelet count in control group

PATIENT NAME	PACKED CELL VOLUME (PCV) (%)	CORRECTED RETICULOCYTE (%)	RED BLOOD CELL COUNT (cell/μl)	PLATELET COUNT (cell/μl)
DUM-LEK	36	0	7.01×10^6	1.68×10^5
DUM-YAI	45	0	7.53×10^6	2.35×10^5
JEFFREY	44	0	6.15×10^6	1.98×10^5
OVALTINE	42	0	5.68×10^6	1.88×10^5
OYUA	42	0	6.64×10^6	5.86×10^5
SONG-SEE	33	0.18	6.02×10^6	3.48×10^5
DUM	46	0	7.67×10^6	3.14×10^5
DHANG	40	0.21	6.67×10^6	1.52×10^5
POP	33	0.18	5.50×10^6	2.98×10^5
BOON-ROD	52	0.28	8.66×10^6	1.05×10^5
MEAN ± SD	41.3 ± 6.02	0.08 ± 0.11	$6.75 \pm 0.98 \times 10^6$	$2.59 \pm 1.38 \times 10^5$

Appendix 4

Table 11 Total white cell count, neutrophil, band neutrophil, eosinophil, lymphocyte and monocyte in control group

PATIENT NAME	WHITE BLOOD CELL COUNT (cell/µl)	NEUTROPHIL (cell/µl)	BAND (cell/µl)	EOSINOPHIL (cell/µl)	LYMPHOCYTE (cell/µl)	MONOCYTE (cell/µl)
DUM-LEK	9,430	5,796	0	1,278	2,356	0
DUM-YAI	9,050	5,520	0	1,448	2,082	0
JEFFREY	10,000	6,100	0	1,100	2,800	0
OVALTINE	6,400	3,456	0	1,216	1,664	0
OYUA	9,950	5,174	0	1,094	3,682	0
SONG-SEE	15,050	8,579	150	1,204	3,762	1,355
DUM	6,300	3,213	0	441	2,520	126
DHANG	11,500	6,555	0	575	3,795	575
POP	16,800	9,912	0	2,856	3,528	504
BOON-ROD	12,750	7,140	0	1,785	3,697	128
MEAN ± SD	10,723 ± 3,402	6,144 ± 2,070	15 ± 47	1,300 ± 670	2,988 ± 800	268 ± 438

Appendix 5

Table 12 Serum color, total protein, alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine and FeLV/FIV status in control group

PATIENT NAME	SERUM COLOR	TOTAL PROTEIN (g/dl)	ALT (U/L)	ALP (U/L)	CREATININE (mg/dl)	BUN (mg/dl)	SEROLOGY
DUM-LEK	CLEAR	6.2	21.9	179	1.29	26.5	ND
DUM-YAI	CLEAR	7.2	57.8	83.2	2.18	32.9	ND
JEFFREY	CLEAR	8.6	28.2	121	1.80	26.5	ND
OVALTINE	CLEAR	7.6	46.1	63.5	2.21	25.6	ND
OYUA	CLEAR	7.0	20.7	10.3	1.54	29.6	ND
SONG-SEE	CLEAR	6.8	49.6	114	1.97	32.3	ND
DUM	CLEAR	8.0	22.9	31.5	1.69	26	ND
DHANG	CLEAR	8.3	38.6	83.2	2.18	50	ND
POP	CLEAR	7.4	32	97.3	1.2	18	ND
BOON-ROD	CLEAR	7.0	21.5	80.6	1.2	27	ND
MEAN ± SD		7.41 ± 0.73	33.93 ± 13.40	89.36 ± 50.00	1.72 ± 0.40	29.44 ± 8.32	

Note: ND = Not determine

Appendix 6

Table 13 Auto-aggulutination test, Coombs' test and mean fluorescence intensity (MFI) form flow cytometry in control group

PATIENT NAME	AUTO AGGLUTINATION TEST		COOMBS' TEST		FLOW CYTOMETRY (Mean Fluorescence Intensity)	
	GROSS	MICROSCOPIC	GROSS	MICROSCOPIC	IgG	IgM
DUM-LEK	-	-	-	-	22.59	16.18
DUM-YAI	-	-	-	-	15.50	13.87
JEFFREY	-	-	-	-	22.21	19.86
OVALTINE	-	-	-	-	19.88	15.14
OYUA	-	-	-	-	24.23	23.19
SONG-SEE	-	-	-	-	24.69	21.66
DUM	-	-	-	-	14.76	13.68
DHANG	-	-	-	-	14.54	23.55
POP	-	-	-	-	17.58	16.11
BOON-ROD	-	-	-	-	16.04	15.41
MEAN ± SD					19.20 ± 4.00	17.87 ± 3.82

Note: IgG = Immunoglobulin G
IgM = Immunoglobulin M

Appendix 7

Table 14 PCV, corrected reticulocytes precentage, red blood cell count and platelet count in IMHA group

PATIENT NAME	PACKED CELL VOLUME (PCV) (%)	CORRECTED RETICULOCYTE %	RED BLOOD CELL COUNT (cell/µl)	PLATELET COUNT (cell/µl)
TUA-TUNG	26	0	4.00×10^6	1.48×10^5
TUA-LEK	19	0.10	3.70×10^6	0.51×10^5
TA-TOE	12	0.13	1.48×10^6	0.53×10^5
SI-RI	17	0.18	3.55×10^6	0.49×10^5
MOKKY	12	0.8	1.49×10^6	1.19×10^5
MEAN ± SD	17.20 ± 5.80	0.24 ± 0.32	$2.84 \pm 1.25 \times 10^6$	$0.84 \pm 0.46 \times 10^5$

Appendix 8

Table 15 Total white cell count, neutrophil, band neutrophil, eosinophil, lymphocyte and monocyte in IMHA group

PATIENT NAME	WHITE BLOOD CELL COUNT (cell/µl)	NEUTROPHIL (cell/µl)	BAND (cell/µl)	EOSINOPHIL (cell/µl)	LYMPHOCYTE (cell/µl)	MONOCYTE (cell/µl)
TUA-TUNG	18,700	17,204	0	0	1,122	374
TUA-LEK	6,000	2,340	0	180	3,000	480
TA-TOE	19,999	13,799	800	400	4,799	200
SI-RI	6,603	2,707	0	0	3,896	0
MOKKY	24,500	12,985	0	2,695	6,615	2,205
MEAN ± SD	15,160± 8,371	9,807 ± 6,835	160 ± 357	655 ± 1,152	3,886 ± 2,043	651 ± 887

Appendix 9

Table 16 Serum color, total protein, alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine and FeLV/FIV status in IMHA group

PATIENT NAME	SERUM COLOR	TOTAL PROTEIN (g/dl)	ALT (U/L)	ALP (U/L)	CREATININE (mg/dl)	BUN (mg/dl)	SEROLOGY
TUA-TUNG	RED	7.2	25	ND	1.4	ND	FeLV
TUA-LEK	CLEAR	8.4	28.7	23.7	1.00	14.2	FIV
TA-TOE	RED	8.1	98	ND	1.3	ND	FeLV
SI-RI	CLEAR	7.4	42	ND	0.7	ND	FeLV
MOKKY	CLEAR	7.6	33	66	1.74	31.4	FIV + FIP
MEAN ± SD		7.74 ± 0.5	45.34 ± 30.11	44.85 ± 29.91	1.23 ± 0.4	22.80 ± 12.16	

Note: ND = Not determined
FeLV = Feline Leukemia Virus
FIV = Feline Immunodeficiency Virus

Appendix 10

Table 17 Auto-aggulutination test, Coombs' test and mean fluorescence intensity (MFI) form flow cytometry in IMHA group

PATIENT NAME	AUTO AGGLUTINATION TEST		COOMBS' TEST		FLOW CYTOMETRY (Mean Fluorescence Intensity)	
	GROSS	MICROSCOPIC	GROSS	MICROSCOPIC	IgG	IgM
TUA-TUNG	-	-	1:128	+	79.53	52.73
TUA-LEK	-	-	1:1	+	69.94	48.71
TA-TOE	-	-	1:4	+	57.25	37.92
SI-RI	-	-	1:4	+	43.46	45.28
MOKKY	-	-	1:2	+	54.88	85.09
MEAN ± SD					61.01± 13.98	47.90 ± 6.69

Note: IgG = Immunoglobulin G
IgM = Immunoglobulin M

Appendix 11

Table 18 PCV, corrected reticulocytes percentage, red blood cell count and platelet count in non-IMHA group

PATIENT NAME	PACKED CELL VOLUME (PCV) (%)	CORRECTED RETICULOCYTE %	RED BLOOD CELL COUNT (cell/µl)	PLATELET COUNT (cell/µl)
HUA-TOE	23	0	5.36×10^6	0.56×10^5
KAI-JEAW	20	0.22	5.28×10^6	0.1×10^5
MOMO	9	0	1.24×10^6	0.22×10^5
TONG-TAE	14	0.3	2.80×10^6	1.18×10^5
MAEW	8	0	1.00×10^6	0.28×10^5
DANG	26	0.7	5.00×10^6	4×10^5
MIMI	24	0.26	4.71×10^6	2.75×10^5
KHAI-DOWN	18	0.004	3.60×10^6	0.20×10^5
PE-TAI-NOI	12	0.13	2.13×10^6	0.43×10^5
FU	10	0.21	1.6×10^6	0.92×10^5
NONG-PHUN	14	0.38	1.59×10^6	0.76×10^5
KHAW	19	1.95	3.50×10^6	1.78×10^5
SLIP	25	0.54	6.07×10^6	1.14×10^5
DUM	15	0.16	2.20×10^6	1.41×10^5
MEAN ± SD	16.92 ± 6.10	0.34 ± 0.50	$3.29 \pm 1.73 \times 10^6$	$1.12 \pm 1.10 \times 10^5$

Appendix 12

Table 19 Total white cell count, neutrophil, band neutrophil, eosinophil, lymphocyte and monocyte in non-IMHA group

PATIENT NAME	WHITE BLOOD CELL COUNT (cell/µl)	NEUTROPHIL (cell/µl)	BAND (cell/µl)	EOSINOPHIL (cell/µl)	LYMPHOCYTE (cell/µl)	MONOCYTE (cell/µl)
HUA-TOE	7,900	7,584	0	0	316	0
KAI-JEAW	300	-	-	-	-	-
MOMO	3,900	1,950	156	39	1,716	39
TONG-TAE	20,450	14,110	1,227	0	2,659	3,067
MAEW	2,700	135	0	0	2,430	135
DANG	21,400	18,404	0	642	2,354	0
MIMI	32,300	29,393	0	646	1,938	646
KHAI-DOWN	600	-	-	-	-	-
PE-TAI-NOI	40,300	33,046	806	0	2,261	3,224
FU	4,600	2,990	46	0	1,196	460
NONG-PHUN	17,800	13,706	0	178	3,560	356
KHAW	11,500	10,580	0	0	460	690
SLIP	13,100	11,528	524	262	786	0
DUM	16,600	11,122	0	332	4,980	166
MEAN ± SD	13,817 ± 12,004	12,879 ± 10,135	230 ± 407	174 ± 248	2,054 ± 1,331	702 ± 1,169

Appendix 13

Table 20 Serum color, total protein, alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine and FeLV/FIV status in non-IMHA group

PATIENT NAME	SERUM COLOR	TOTAL PROTEIN (g/dl)	ALT (U/L)	ALP (U/L)	CREATININE (mg/dl)	BUN (mg/dl)	SEROLOGY
HUA-TOE	ICTERIC +1	6.2	200	ND	1.2	ND	NEGATIVE
KAI-JEAW	ICTERIC +4	7.6	45	87	0.82	12.2	ND
MOMO	ICTERIC +2	7.1	107	ND	0.7	101.5	FeLV
TONG-TAE	ICTERIC +1	11.0	53.3	83.3	1.14	20.7	ND
MAEW	CLEAR	8.0	7	20	0.8	20	ND
DANG	ICTERIC +1	8.2	8	30	1.6	28	ND
MIMI	CLEAR	8.7	28.7	178	1.1	18.4	NEGATIVE
KHAI-DOWN	CLEAR	7.8	12	30	1.8	21	FeLV
PE-TAI-NOI	ICTERIC +3	5.7	46	ND	0.5	19.1	FeLV
FU	ICTERIC +2	6.6	89	ND	ND	ND	ND
NONG-PHUN	RED	7.3	55	ND	0.6	ND	ND
KHAW	ICTERIC +1	5.9	911	50	1.6	31	ND
SLIP	ICTERIC +2	8.4	51	ND	1.2	ND	FeLV
DUM	CLEAR	7.4	154	ND	0.7	ND	FeLV
MEAN ± SD		7.54 ± 1.40	126.21 ± 232.65	39.86 ± 53.79	0.98 ± 0.49	20.92 ± 26.58	

Note: ND = Not determined FeLV = Feline Leukemia Virus FIV = Feline Immunodeficiency Virus

Appendix 14

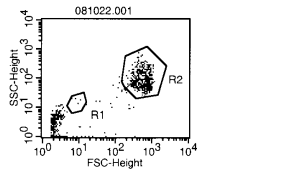
Table 21 Auto-aggulutination test, Coombs' test and mean fluorescence intensity (MFI) form flow cytometry in non-IMHA group

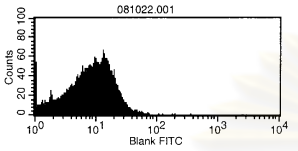
PATIENT NAME	AUTO AGGLUTINATION TEST		COOMBS' TEST		FLOW CYTOMETRY (Mean Fluorescence Intensity)	
	GROSS	MICROSCOPIC	GROSS	MICROSCOPIC	IgG	IgM
HUA-TOE	-	-	-	-	21.34	22.21
KAI-JEAW	-	-	TRACE	-	24.15	23.71
MOMO	-	-	-	-	21.84	20.80
TONG-TAE	-	-	-	-	25.37	29.34
MAEW	+	+	-	-	20.65	17.70
DANG	+	-	-	-	20.74	16.85
MIMI	-	-	TRACE	-	38.63	24.33
KHAI-DOWN	-	-	-	-	25.47	30.18
PE-TAI-NOI	-	-	-	-	32.02	21.88
FU	-	-	-	-	38.76	30.67
NONG-PHUN	-	-	-	-	29.42	11.70
KHAW	-	-	-	-	15.71	19.74
SLIP	-	-	-	-	30.60	34.11
DUM	-	-	-	-	16.99	14.94
MEAN ± SD					25.84± 7.22	23.72 ± 6.51

Note: IgG = Immunoglobulin G IgM = Immunoglobulin M

Appendix 15

Flow cytometry results of control group

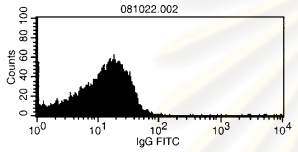




Histogram Statistics

File: 081022.001 | Log Data Units: Linear Values
Sample ID: Dumlek BL Blank | Patient ID: 081022
Tube: CD123 | Panel: 11c,123
Acquisition Date: 23-Oct-08 | Gate: G2
Gated Events: 10118 | Total Events: 18271
X Parameter: Blank FITC (Log)

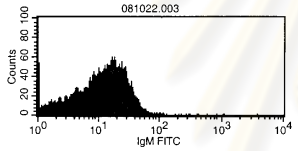
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10118	100.00	55.38	10.39	7.57	86.29	8.58	1



Histogram Statistics

File: 081022.002 | Log Data Units: Linear Values
Sample ID: Dumlek BL IgG | Patient ID: 081022
Tube: CD11c | Panel: 11c,123
Acquisition Date: 23-Oct-08 | Gate: G2
Gated Events: 10103 | Total Events: 23439
X Parameter: IgG FITC (Log)

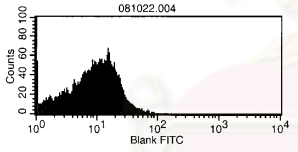
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10103	100.00	43.10	22.59	10.95	941.88	13.22	1



Histogram Statistics

File: 081022.003 | Log Data Units: Linear Values
Sample ID: Dumlek BL IgM | Patient ID: 081022
Tube: CD123 | Panel: 11c,123
Acquisition Date: 23-Oct-08 | Gate: G2
Gated Events: 10132 | Total Events: 20957
X Parameter: IgM FITC (Log)

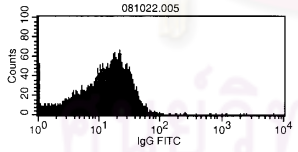
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10132	100.00	48.35	16.18	9.94	657.48	11.97	1



Histogram Statistics

File: 081022.004 | Log Data Units: Linear Values
Sample ID: Dumlek BM Blank | Patient ID: 081022
Tube: CD11c | Panel: 11c,123
Acquisition Date: 23-Oct-08 | Gate: G2
Gated Events: 10113 | Total Events: 14821
X Parameter: Blank FITC (Log)

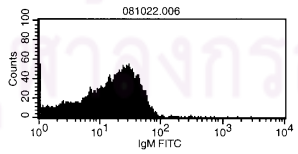
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10113	100.00	68.23	11.26	8.15	77.32	9.47	1



Histogram Statistics

File: 081022.005 | Log Data Units: Linear Values
Sample ID: Dumlek BM IgG | Patient ID: 081022
Tube: CD123 | Panel: 11c,123
Acquisition Date: 23-Oct-08 | Gate: G2
Gated Events: 10095 | Total Events: 16341
X Parameter: IgG FITC (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10095	100.00	61.78	19.07	11.47	643.11	13.95	1



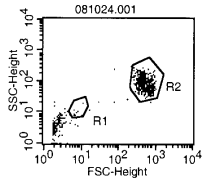
Histogram Statistics

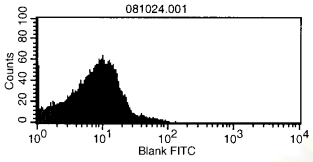
File: 081022.006 | Log Data Units: Linear Values
Sample ID: Dumlek BM IgM | Patient ID: 081022
Tube: CD11c | Panel: 11c,123
Acquisition Date: 23-Oct-08 | Gate: G2
Gated Events: 10059 | Total Events: 13974
X Parameter: IgM FITC (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10059	100.00	71.98	22.08	12.83	481.56	16.70	1

Fig. 14 control cat number 1

Appendix 16



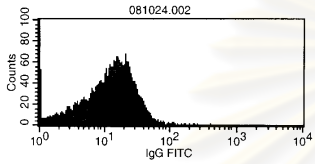


Histogram Statistics

File: 081024.001
Sample ID: Dumyai Bl Blank
Tube: CD123
Acquisition Date: 25-Oct-08
Gated Events: 10015
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 081024
Panel: 11c,123
Gate: G2
Total Events: 13508

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10015	100.00	74.14	9.84	7.06	89.92	7.99	1

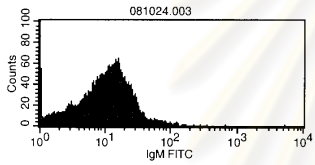


Histogram Statistics

File: 081024.002
Sample ID: Dumyai Bl IgG
Tube: CD11c
Acquisition Date: 25-Oct-08
Gated Events: 10012
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 081024
Panel: 11c,123
Gate: G2
Total Events: 14934

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10012	100.00	67.04	15.50	11.29	83.96	13.22	1

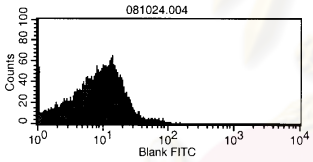


Histogram Statistics

File: 081024.003
Sample ID: Dumyai Bl IgM
Tube: CD123
Acquisition Date: 25-Oct-08
Gated Events: 10009
X Parameter: IgM FITC (Log)

Log Data Units: Linear Values
Patient ID: 081024
Panel: 11c,123
Gate: G2
Total Events: 18092

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10009	100.00	55.32	13.87	9.44	364.41	10.75	1

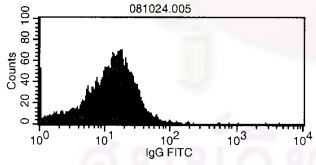


Histogram Statistics

File: 081024.004
Sample ID: Dumyai BM Blank
Tube: CD11c
Acquisition Date: 25-Oct-08
Gated Events: 10009
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 081024
Panel: 11c,123
Gate: G2
Total Events: 13082

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10009	100.00	76.51	10.61	7.52	87.49	8.74	1

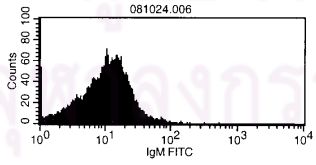


Histogram Statistics

File: 081024.005
Sample ID: Dumyai BM IgG
Tube: CD123
Acquisition Date: 25-Oct-08
Gated Events: 10018
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 081024
Panel: 11c,123
Gate: G2
Total Events: 16466

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10018	100.00	60.84	16.11	11.37	318.21	12.98	1



Histogram Statistics

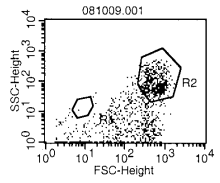
File: 081024.006
Sample ID: Dumyai BM IgM
Tube: CD11c
Acquisition Date: 25-Oct-08
Gated Events: 10007
X Parameter: IgM FITC (Log)

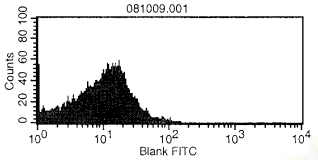
Log Data Units: Linear Values
Patient ID: 081024
Panel: 11c,123
Gate: G2
Total Events: 12663

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10007	100.00	79.03	12.70	9.28	96.38	10.37	1

Fig. 15 control cat number 2

Appendix 17

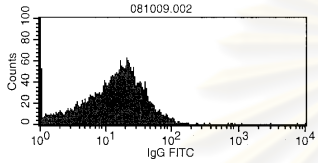




Histogram Statistics

File: 081009.001 | Log Data Units: Linear Values
Sample ID: BL Blank Jeffrey | Patient ID: 081009
Tube: CD123 | Panel: 11c,123
Acquisition Date: 10-Oct-08 | Gate: G2
Gated Events: 10005 | Total Events: 24387
X Parameter: Blank FITC (Log)

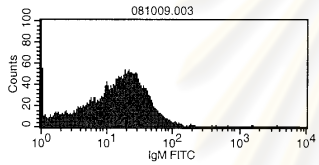
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10005	100.00	41.03	13.25	8.84	95.03	10.37	1



Histogram Statistics

File: 081009.002 | Log Data Units: Linear Values
Sample ID: BL IgG Jeffrey | Patient ID: 081009
Tube: CD11c | Panel: 11c,123
Acquisition Date: 10-Oct-08 | Gate: G2
Gated Events: 9761 | Total Events: 17760
X Parameter: IgG FITC (Log)

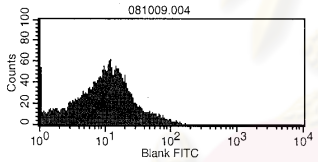
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9761	100.00	54.96	22.21	12.62	730.03	14.86	1



Histogram Statistics

File: 081009.003 | Log Data Units: Linear Values
Sample ID: BL IgM Jeffrey | Patient ID: 081009
Tube: CD123 | Panel: 11c,123
Acquisition Date: 10-Oct-08 | Gate: G2
Gated Events: 9727 | Total Events: 17015
X Parameter: IgM FITC (Log)

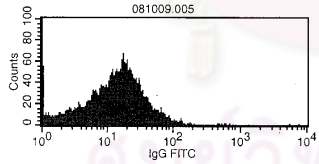
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9727	100.00	57.17	19.86	12.47	224.66	15.12	1



Histogram Statistics

File: 081009.004 | Log Data Units: Linear Values
Sample ID: BM Blank Jeffrey | Patient ID: 081009
Tube: CD11c | Panel: 11c,123
Acquisition Date: 10-Oct-08 | Gate: G2
Gated Events: 9811 | Total Events: 16042
X Parameter: Blank FITC (Log)

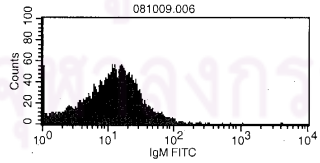
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9811	100.00	61.16	13.61	8.90	103.86	10.00	1



Histogram Statistics

File: 081009.005 | Log Data Units: Linear Values
Sample ID: BM IgG Jeffrey | Patient ID: 081009
Tube: CD123 | Panel: 11c,123
Acquisition Date: 10-Oct-08 | Gate: G2
Gated Events: 9744 | Total Events: 18566
X Parameter: IgG FITC (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9744	100.00	52.48	20.16	11.93	671.36	13.82	1



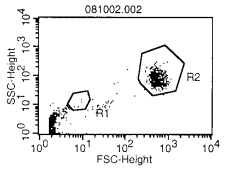
Histogram Statistics

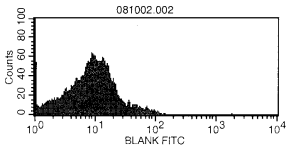
File: 081009.006 | Log Data Units: Linear Values
Sample ID: BM IgM Jeffrey | Patient ID: 081009
Tube: CD11c | Panel: 11c,123
Acquisition Date: 10-Oct-08 | Gate: G2
Gated Events: 9716 | Total Events: 18168
X Parameter: IgM FITC (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9716	100.00	53.48	17.89	10.32	647.07	11.76	1

Fig. 16 control cat number 3

Appendix 18



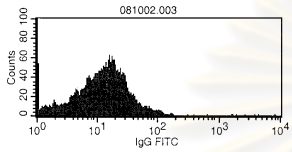


Histogram Statistics

File: 081002.002
Sample ID: Ovaltine Blood Blank
Tube: CD11c
Acquisition Date: 03-Oct-08
Gated Events: 9978
X Parameter: BLANK FITC (Log)

Log Data Units: Linear Values
Patient ID: 081002
Panel: 11c,123
Gate: G2
Total Events: 49379

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9978	100.00	20.21	12.08	8.15	174.97	8.82	1

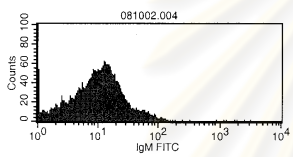


Histogram Statistics

File: 081002.003
Sample ID: Ovaltine Blood Blank
Tube: CD123
Acquisition Date: 03-Oct-08
Gated Events: 9972
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 081002
Panel: 11c,123
Gate: G2
Total Events: 58243

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9972	100.00	17.12	19.88	11.28	640.36	12.63	1

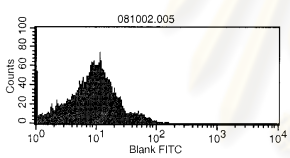


Histogram Statistics

File: 081002.004
Sample ID: Ovaltine Blood Blank
Tube: CD123
Acquisition Date: 03-Oct-08
Gated Events: 9981
X Parameter: IgM FITC (Log)

Log Data Units: Linear Values
Patient ID: 081002
Panel: 11c,123
Gate: G2
Total Events: 40026

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9981	100.00	24.94	15.14	9.44	332.91	10.37	1

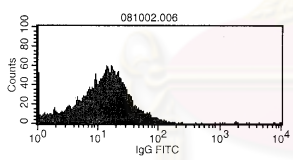


Histogram Statistics

File: 081002.005
Sample ID: Ovaltine BM Blank
Tube: CD11c
Acquisition Date: 03-Oct-08
Gated Events: 9981
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 081002
Panel: 11c,123
Gate: G2
Total Events: 50213

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9981	100.00	19.88	12.11	8.33	103.20	8.98	1

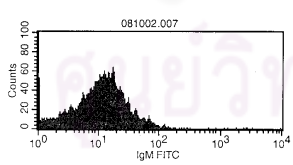


Histogram Statistics

File: 081002.006
Sample ID: Ovaltine BM Blank
Tube: CD123
Acquisition Date: 03-Oct-08
Gated Events: 9959
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 081002
Panel: 11c,123
Gate: G2
Total Events: 119026

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9959	100.00	8.37	24.76	11.68	932.18	13.22	1



Histogram Statistics

File: 081002.007
Sample ID: Ovaltine BM Blank
Tube: CD11c
Acquisition Date: 03-Oct-08
Gated Events: 9952
X Parameter: IgM FITC (Log)

Log Data Units: Linear Values
Patient ID: 081002
Panel: 11c,123
Gate: G2
Total Events: 80699

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9952	100.00	12.33	18.43	10.06	787.85	11.14	1

Fig. 17 control cat number 4

Appendix 19

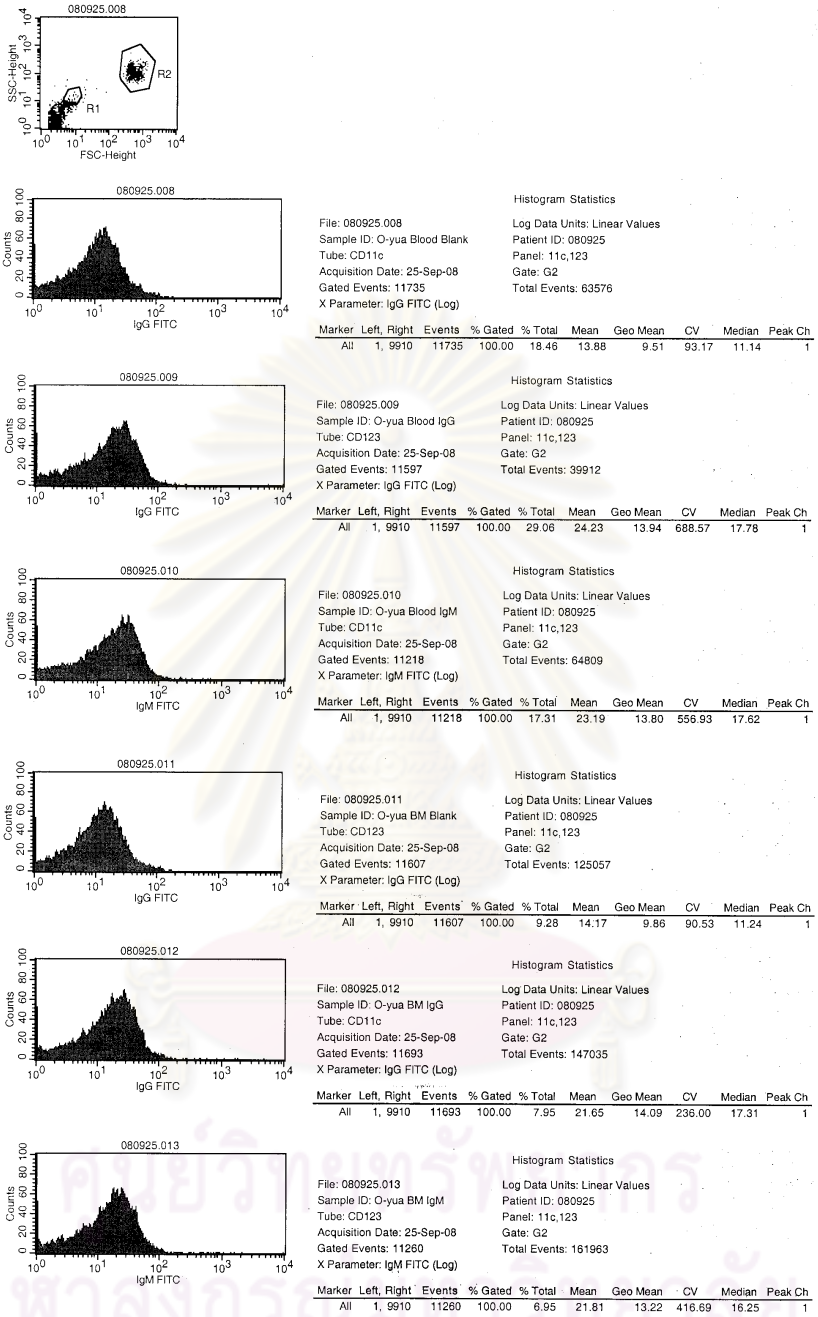


Fig. 18 control cat number 5

Appendix 20

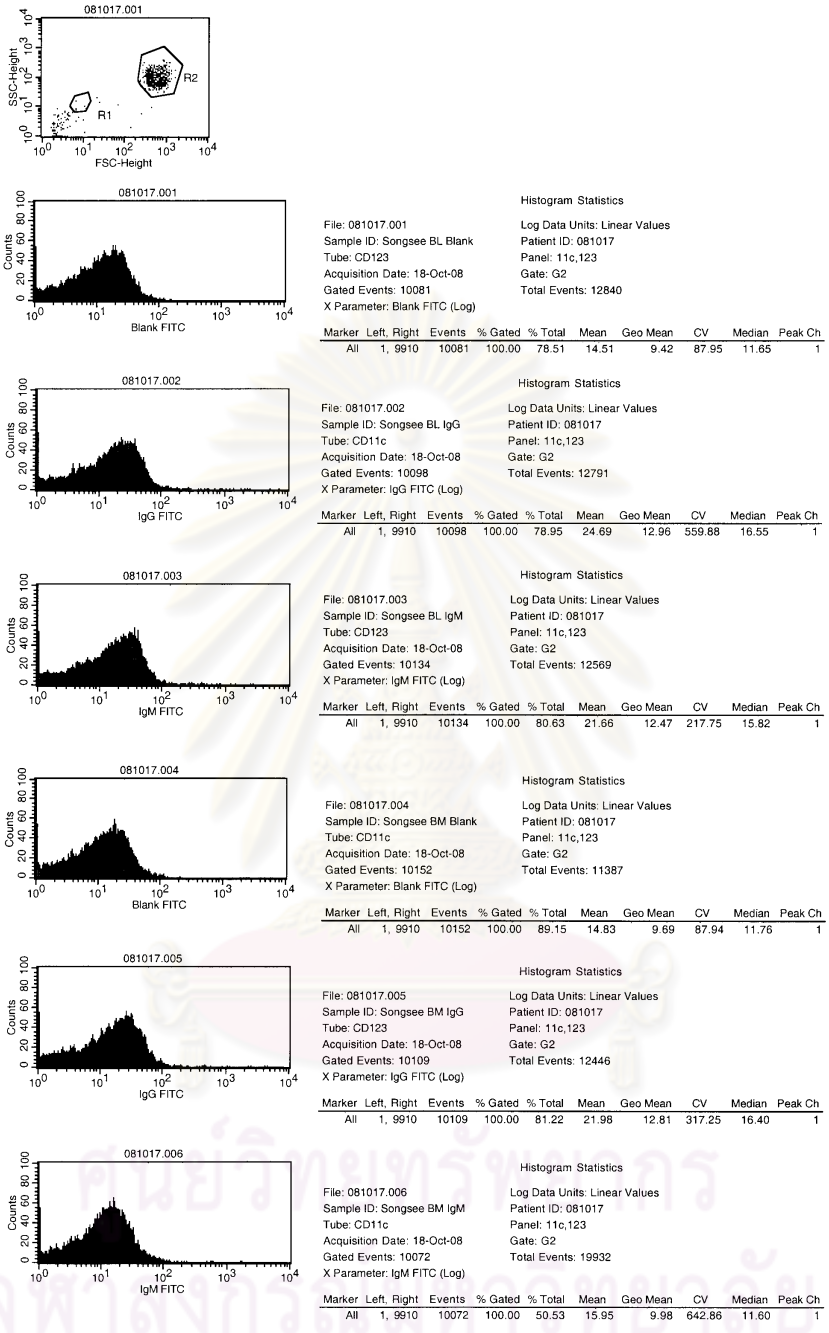
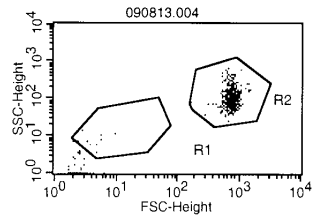
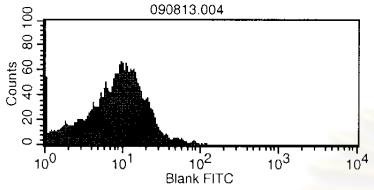


Fig. 19 control cat number 6

Appendix 21



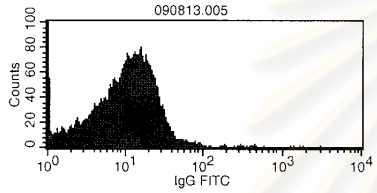


Histogram Statistics

File: 090813.004
Sample ID: DUM-BL-BLANK
Tube: CD11c
Acquisition Date: 13-Aug-09
Gated Events: 10046
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090813
Panel: 11c,123
Gate: G2
Total Events: 11503

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10046	100.00	87.33	10.66	7.89	80.59	8.98	1

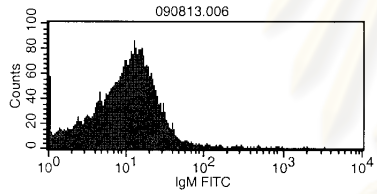


Histogram Statistics

File: 090813.005
Sample ID: DUM-BL-IgG
Tube: CD123
Acquisition Date: 13-Aug-09
Gated Events: 13070
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090813
Panel: 11c,123
Gate: G2
Total Events: 16770

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	13070	100.00	77.94	14.76	9.35	647.56	10.75	1



Histogram Statistics

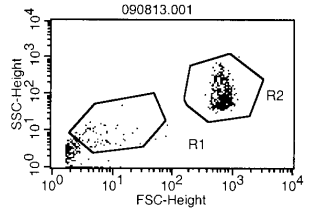
File: 090813.006
Sample ID: DUM-BL-IgM
Tube: CD11c
Acquisition Date: 13-Aug-09
Gated Events: 12969
X Parameter: IgM FITC (Log)

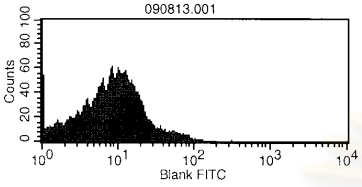
Log Data Units: Linear Values
Patient ID: 090813
Panel: 11c,123
Gate: G2
Total Events: 19185

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	12969	100.00	67.60	13.68	8.94	300.87	10.37	1

Fig. 20 control cat number 7

Appendix 22



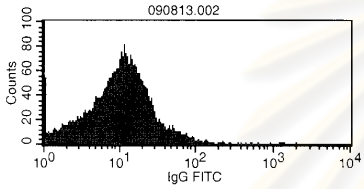


Histogram Statistics

File: 090813.001
Sample ID: DHANG-BL-Blank
Tube: CD123
Acquisition Date: 13-Aug-09
Gated Events: 10081
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090813
Panel: 11c,123
Gate: G2
Total Events: 20036

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10081	100.00	50.31	11.52	7.86	105.07	8.58	1

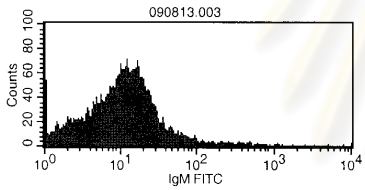


Histogram Statistics

File: 090813.002
Sample ID: DHANG-BL-IgG
Tube: CD11c
Acquisition Date: 13-Aug-09
Gated Events: 12071
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090813
Panel: 11c,123
Gate: G2
Total Events: 23048

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	12071	100.00	52.37	14.54	9.32	234.39	10.27	1



Histogram Statistics

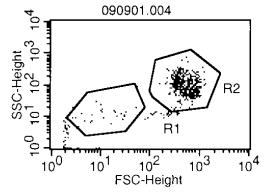
File: 090813.003
Sample ID: DHANG-BL-IgM
Tube: CD123
Acquisition Date: 13-Aug-09
Gated Events: 11981
X Parameter: IgM FITC (Log)

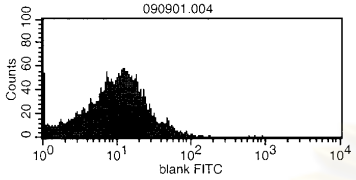
Log Data Units: Linear Values
Patient ID: 090813
Panel: 11c,123
Gate: G2
Total Events: 43602

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	11981	100.00	27.48	23.55	9.66	967.68	10.55	1

Fig. 21 control cat number 8

Appendix 23



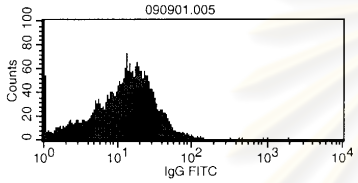


Histogram Statistics

File: 090901.004
Sample ID: POP BL blank
Tube: CD123
Acquisition Date: 01-Sep-09
Gated Events: 9673
X Parameter: blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090901
Panel: 11c,123
Gate: G2
Total Events: 12205

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9673	100.00	79.25	13.36	9.07	130.90	10.18	1

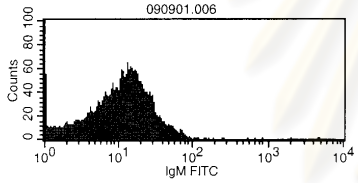


Histogram Statistics

File: 090901.005
Sample ID: POP BLIgG
Tube: CD11c
Acquisition Date: 01-Sep-09
Gated Events: 9958
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090901
Panel: 11c,123
Gate: G2
Total Events: 13399

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9958	100.00	74.32	17.58	11.43	583.66	13.22	1



Histogram Statistics

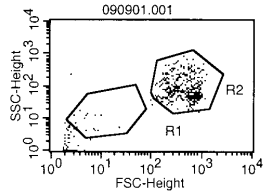
File: 090901.006
Sample ID: POP BLIgM
Tube: CD123
Acquisition Date: 01-Sep-09
Gated Events: 9980
X Parameter: IgM FITC (Log)

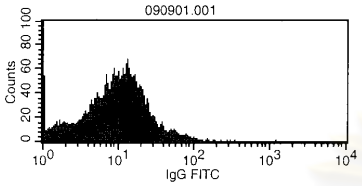
Log Data Units: Linear Values
Patient ID: 090901
Panel: 11c,123
Gate: G2
Total Events: 13610

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9980	100.00	73.33	16.11	9.69	737.11	11.24	1

Fig. 22 control cat number 9

Appendix 24



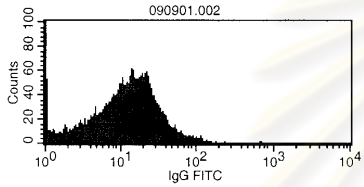


Histogram Statistics

File: 090901.001
Sample ID: boonrod BL BLANK
Tube: CD11c
Acquisition Date: 01-Sep-09
Gated Events: 9935
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090901
Panel: 11c,123
Gate: G2
Total Events: 11675

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9935	100.00	85.10	13.30	9.03	134.41	10.09	1

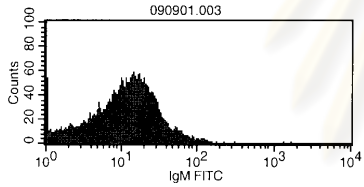


Histogram Statistics

File: 090901.002
Sample ID: boonrod BL IgG
Tube: CD123
Acquisition Date: 01-Sep-09
Gated Events: 9960
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090901
Panel: 11c,123
Gate: G2
Total Events: 10955

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9960	100.00	90.92	16.04	10.97	106.08	12.52	1



Histogram Statistics

File: 090901.003
Sample ID: boonrod BL IgM
Tube: CD11c
Acquisition Date: 01-Sep-09
Gated Events: 9903
X Parameter: IgM FITC (Log)

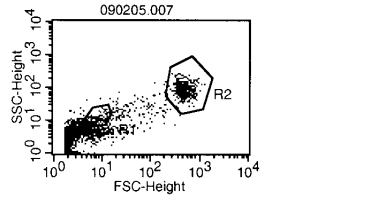
Log Data Units: Linear Values
Patient ID: 090901
Panel: 11c,123
Gate: G2
Total Events: 11183

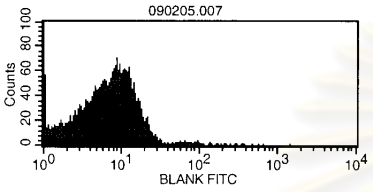
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9903	100.00	88.55	15.41	10.29	158.46	11.86	1

Fig. 23 control cat number 10

Appendix 25

Flow cytometry results of IMHA cat



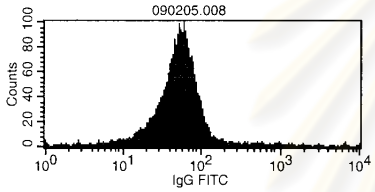


Histogram Statistics

File: 090205.007
Sample ID: TUA-TUNK BL BLANK
Tube: CD123
Acquisition Date: 05-Feb-09
Gated Events: 9863
X Parameter: BLANK FITC (Log)

Log Data Units: Linear Values
Patient ID: 090205
Panel: 11c,123
Gate: G2
Total Events: 185682

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9863	100.00	5.31	9.03	6.19	230.53	6.98	1

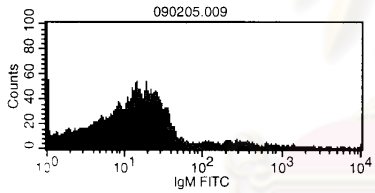


Histogram Statistics

File: 090205.008
Sample ID: TUA-TUNK BL IgG
Tube: CD11c
Acquisition Date: 05-Feb-09
Gated Events: 9712
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090205
Panel: 11c,123
Gate: G2
Total Events: 210488

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9712	100.00	4.61	79.53	48.93	419.25	50.48	57



Histogram Statistics

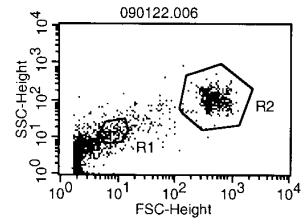
File: 090205.009
Sample ID: TUA-TUNK BL IgM
Tube: CD123
Acquisition Date: 05-Feb-09
Gated Events: 9540
X Parameter: IgM FITC (Log)

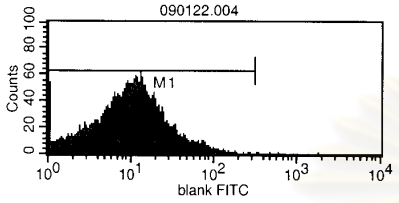
Log Data Units: Linear Values
Patient ID: 090205
Panel: 11c,123
Gate: G2
Total Events: 283431

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9540	100.00	3.37	52.73	11.79	754.85	12.98	1

Fig. 24 cat number 1

Appendix 26



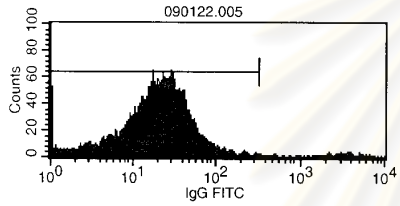


Histogram Statistics

File: 090122.004
Sample ID: Tualek BL Blank
Tube: CD11c
Acquisition Date: 23-Jan-09
Gated Events: 10266
X Parameter: blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090122
Panel: 11c,123
Gate: G2
Total Events: 43138

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10266	100.00	23.80	14.80	9.36	167.89	10.00	1
M1	1, 313	10261	99.95	23.79	14.48	9.34	115.82	10.00	1

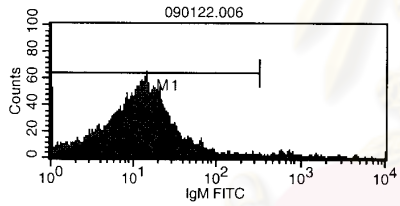


Histogram Statistics

File: 090122.005
Sample ID: Tualek BL IgG
Tube: CD123
Acquisition Date: 23-Jan-09
Gated Events: 10310
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090122
Panel: 11c,123
Gate: G2
Total Events: 121010

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10310	100.00	8.52	69.94	19.61	633.91	20.91	1
M1	1, 313	10157	98.52	8.39	26.23	18.28	99.92	20.54	1



Histogram Statistics

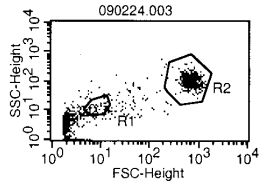
File: 090122.006
Sample ID: Tualek BL IgM
Tube: CD11c
Acquisition Date: 23-Jan-09
Gated Events: 10278
X Parameter: IgM FITC (Log)

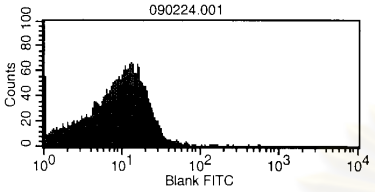
Log Data Units: Linear Values
Patient ID: 090122
Panel: 11c,123
Gate: G2
Total Events: 248951

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10278	100.00	4.13	48.71	11.86	735.32	11.97	1
M1	1, 313	10053	97.81	4.04	17.55	10.75	140.03	11.76	1

Fig. 25 cat number 2

Appendix 27



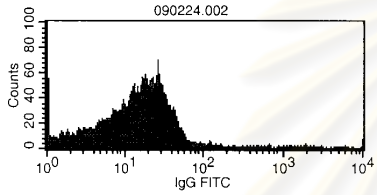


Histogram Statistics

File: 090224.001
Sample ID: TATOE BL blank
Tube: CD123
Acquisition Date: 24-Feb-09
Gated Events: 10115
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090224
Panel: 11c,123
Gate: G2
Total Events: 25459

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10115	100.00	39.73	10.96	7.89	113.68	9.22	1

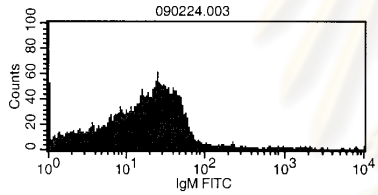


Histogram Statistics

File: 090224.002
Sample ID: TATOE BL IgG
Tube: CD11c
Acquisition Date: 24-Feb-09
Gated Events: 10119
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090224
Panel: 11c,123
Gate: G2
Total Events: 24385

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10119	100.00	41.50	57.25	13.07	836.47	14.99	1



Histogram Statistics

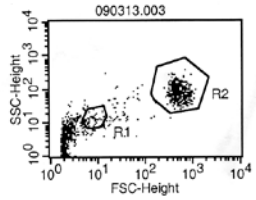
File: 090224.003
Sample ID: TATOE BL IgM
Tube: CD123
Acquisition Date: 24-Feb-09
Gated Events: 10146
X Parameter: IgM FITC (Log)

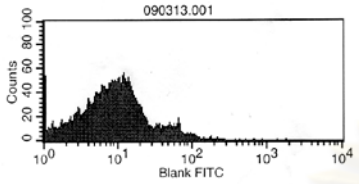
Log Data Units: Linear Values
Patient ID: 090224
Panel: 11c,123
Gate: G2
Total Events: 25611

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10146	100.00	39.62	37.92	14.14	778.10	17.78	1

Fig. 26 cat number 3

Appendix 28



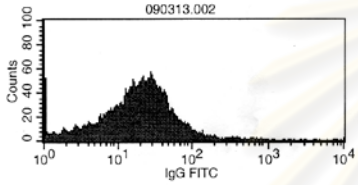


Histogram Statistics

File: 090313.001
Sample ID: Siri BL Blank
Tube: CD123
Acquisition Date: 13-Mar-09
Gated Events: 10114
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090313
Panel: 11c,123
Gate: G2
Total Events: 196550

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10114	100.00	5.15	13.90	8.07	220.26	8.51	1

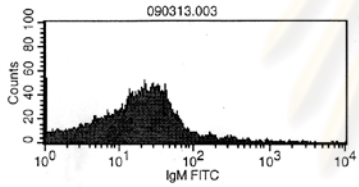


Histogram Statistics

File: 090313.002
Sample ID: Siri BL IgG
Tube: CD11c
Acquisition Date: 13-Mar-09
Gated Events: 10054
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090313
Panel: 11c,123
Gate: G2
Total Events: 59245

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10054	100.00	16.97	43.46	17.59	691.09	19.63	1



Histogram Statistics

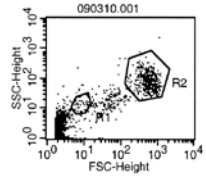
File: 090313.003
Sample ID: Siri BL IgM
Tube: CD123
Acquisition Date: 13-Mar-09
Gated Events: 10064
X Parameter: IgM FITC (Log)

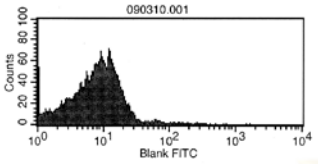
Log Data Units: Linear Values
Patient ID: 090313
Panel: 11c,123
Gate: G2
Total Events: 58766

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10064	100.00	17.13	45.28	16.17	570.43	18.60	1

Fig. 27 cat number 4

Appendix 29



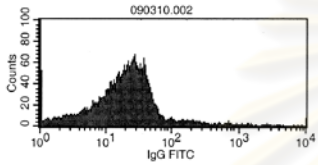


Histogram Statistics

File: 090310.001
Sample ID: MOKKY BL blank
Tube: CD123
Acquisition Date: 10-Mar-09
Gated Events: 10233
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090310
Panel: 11c,123
Gate: G2
Total Events: 443625

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10233	100.00	2.31	10.87	7.20	256.44	8.13	1

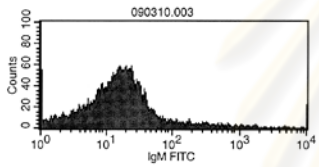


Histogram Statistics

File: 090310.002
Sample ID: MOKKY BL IgG
Tube: CD11c
Acquisition Date: 10-Mar-09
Gated Events: 10278
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090310
Panel: 11c,123
Gate: G2
Total Events: 181669

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10278	100.00	5.66	54.88	17.77	781.72	19.81	1



Histogram Statistics

File: 090310.003
Sample ID: MOKKY BL IgM
Tube: CD123
Acquisition Date: 10-Mar-09
Gated Events: 10649
X Parameter: IgM FITC (Log)

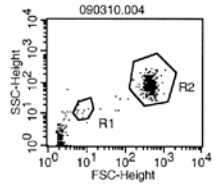
Log Data Units: Linear Values
Patient ID: 090310
Panel: 11c,123
Gate: G2
Total Events: 586555

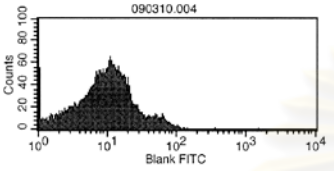
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10649	100.00	1.82	85.09	14.71	807.19	14.99	1

Fig. 28 cat number 5

Appendix 30

Flow cytometry results of non-IMHA cat



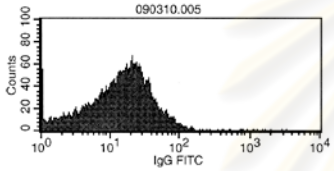


Histogram Statistics

File: 090310.004
Sample ID: Hua-toe BL Blank
Tube: CD11c
Acquisition Date: 10-Mar-09
Gated Events: 10059
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090310
Panel: 11c,123
Gate: G2
Total Events: 32039

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10059	100.00	31.40	13.29	8.85	146.68	9.56	1

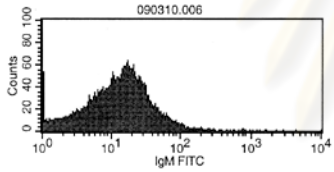


Histogram Statistics

File: 090310.005
Sample ID: Hua-toe BL IgG
Tube: CD123
Acquisition Date: 10-Mar-09
Gated Events: 11323
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090310
Panel: 11c,123
Gate: G2
Total Events: 27195

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	11323	100.00	41.64	21.34	13.12	259.89	15.12	1



Histogram Statistics

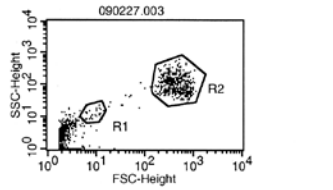
File: 090310.006
Sample ID: Hua-toe BL IgM
Tube: CD11c
Acquisition Date: 10-Mar-09
Gated Events: 11023
X Parameter: IgM FITC (Log)

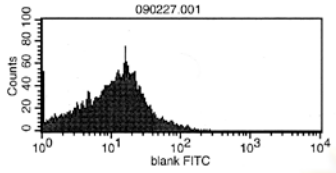
Log Data Units: Linear Values
Patient ID: 090310
Panel: 11c,123
Gate: G2
Total Events: 76801

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	11023	100.00	14.35	22.21	11.69	668.45	13.34	1

Fig. 29 cat number 6

Appendix 31



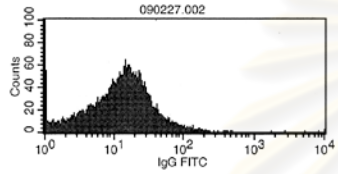


Histogram Statistics

File: 090227.001
Sample ID: Kai-Jeaw BL blank
Tube: CD123
Acquisition Date: 27-Feb-09
Gated Events: 10008
X Parameter: blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090227
Panel: 11c,123
Gate: G2
Total Events: 13332

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10008	100.00	75.07	15.50	9.96	107.27	11.55	1

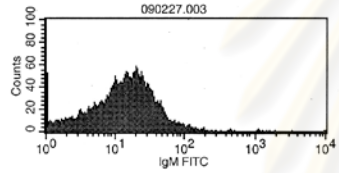


Histogram Statistics

File: 090227.002
Sample ID: Kai-Jeaw BL IgG
Tube: CD11c
Acquisition Date: 27-Feb-09
Gated Events: 10038
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090227
Panel: 11c,123
Gate: G2
Total Events: 48617

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10038	100.00	20.65	24.15	11.47	997.00	12.98	1



Histogram Statistics

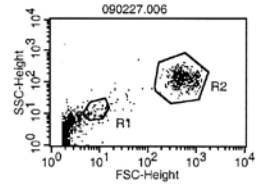
File: 090227.003
Sample ID: Kai-Jeaw BL IgM
Tube: CD123
Acquisition Date: 27-Feb-09
Gated Events: 10032
X Parameter: IgM FITC (Log)

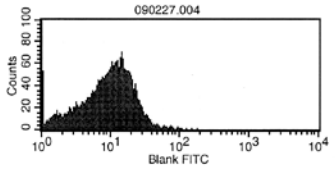
Log Data Units: Linear Values
Patient ID: 090227
Panel: 11c,123
Gate: G2
Total Events: 30490

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10032	100.00	32.90	23.71	11.99	829.50	13.95	1

Fig. 30 cat number 7

Appendix 32



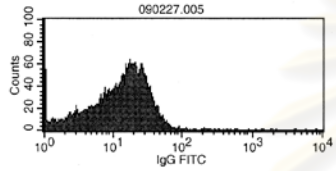


Histogram Statistics

File: 090227.004
Sample ID: MoMo BL blank
Tube: CD11c
Acquisition Date: 27-Feb-09
Gated Events: 9988
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090227
Panel: 11c,123
Gate: G2
Total Events: 21378

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9988	100.00	46.72	11.45	8.60	76.01	10.00	1

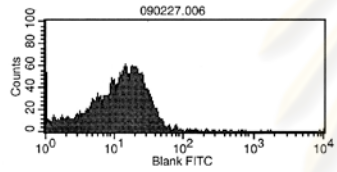


Histogram Statistics

File: 090227.005
Sample ID: MoMo BL IgG
Tube: CD123
Acquisition Date: 27-Feb-09
Gated Events: 10005
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090227
Panel: 11c,123
Gate: G2
Total Events: 25603

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10005	100.00	39.08	21.84	11.67	770.38	14.33	1



Histogram Statistics

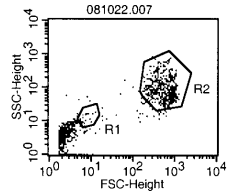
File: 090227.006
Sample ID: MoMo BL IgM
Tube: CD11c
Acquisition Date: 27-Feb-09
Gated Events: 9991
X Parameter: Blank FITC (Log)

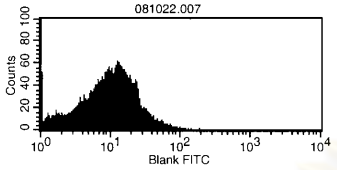
Log Data Units: Linear Values
Patient ID: 090227
Panel: 11c,123
Gate: G2
Total Events: 44555

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9991	100.00	22.42	20.80	10.70	1037.57	12.86	1

Fig. 31 cat number 8

Appendix 33



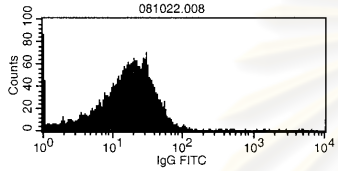


Histogram Statistics

File: 081022.007
Sample ID: Tongtae BL Blank
Tube: CD123
Acquisition Date: 23-Oct-08
Gated Events: 10195
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 081022
Panel: 11c,123
Gate: G2
Total Events: 26230

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10195	100.00	38.87	12.70	9.03	88.63	10.09	1

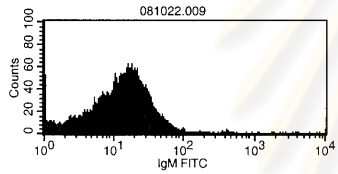


Histogram Statistics

File: 081022.008
Sample ID: Tongtae BL IgG
Tube: CD11c
Acquisition Date: 23-Oct-08
Gated Events: 10127
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 081022
Panel: 11c,123
Gate: G2
Total Events: 44262

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10127	100.00	22.88	25.37	15.20	685.73	17.00	1



Histogram Statistics

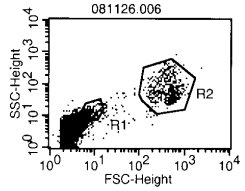
File: 081022.009
Sample ID: Tongtae BL IgM
Tube: CD123
Acquisition Date: 23-Oct-08
Gated Events: 10082
X Parameter: IgM FITC (Log)

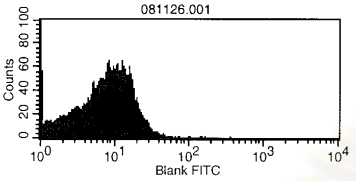
Log Data Units: Linear Values
Patient ID: 081022
Panel: 11c,123
Gate: G2
Total Events: 74215

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10082	100.00	13.58	29.34	12.06	1021.21	13.70	1

Fig. 32 cat number 9

Appendix 34



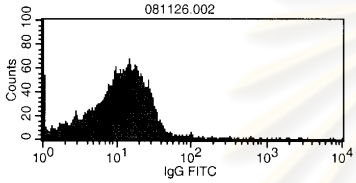


Histogram Statistics

File: 081126.001
Sample ID: Cat-Blank
Tube: CD123
Acquisition Date: 26-Nov-08
Gated Events: 10139
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 081126
Panel: 11c,123
Gate: G2
Total Events: 32834

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10139	100.00	30.88	9.13	6.70	87.62	7.77	1

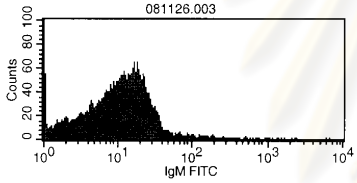


Histogram Statistics

File: 081126.002
Sample ID: Cat-IgG
Tube: CD11c
Acquisition Date: 26-Nov-08
Gated Events: 10158
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 081126
Panel: 11c,123
Gate: G2
Total Events: 369408

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10158	100.00	2.75	20.65	10.02	898.80	11.44	1



Histogram Statistics

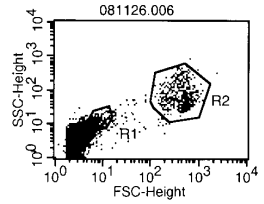
File: 081126.003
Sample ID: Cat-IgM
Tube: CD123
Acquisition Date: 26-Nov-08
Gated Events: 10274
X Parameter: IgM FITC (Log)

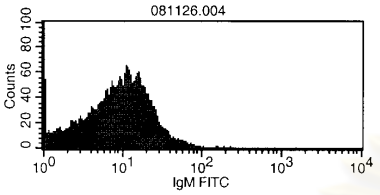
Log Data Units: Linear Values
Patient ID: 081126
Panel: 11c,123
Gate: G2
Total Events: 420112

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10274	100.00	2.45	17.70	9.24	563.52	10.65	1

Fig. 33 cat number 10

Appendix 35



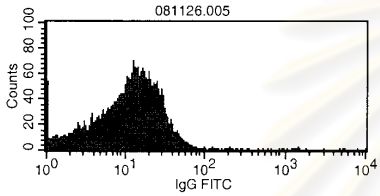


Histogram Statistics

File: 081126.004
Sample ID: Dang-Blank
Tube: CD123
Acquisition Date: 26-Nov-08
Gated Events: 10669
X Parameter: IgM FITC (Log)

Log Data Units: Linear Values
Patient ID: 081126
Panel: 11c,123
Gate: G2
Total Events: 51585

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10669	100.00	20.68	11.55	8.10	88.07	9.31	1

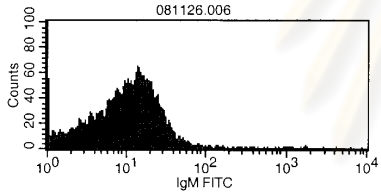


Histogram Statistics

File: 081126.005
Sample ID: Dang-IgG
Tube: CD11c
Acquisition Date: 26-Nov-08
Gated Events: 10572
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 081126
Panel: 11c,123
Gate: G2
Total Events: 131683

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10572	100.00	8.03	20.74	10.25	895.14	11.97	1



Histogram Statistics

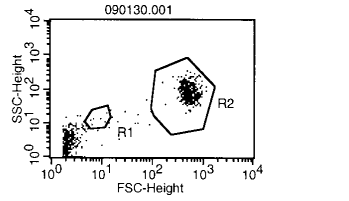
File: 081126.006
Sample ID: Dang-IgM
Tube: CD123
Acquisition Date: 26-Nov-08
Gated Events: 10688
X Parameter: IgM FITC (Log)

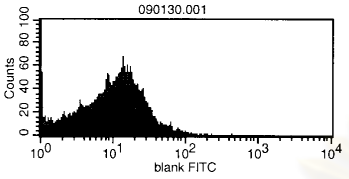
Log Data Units: Linear Values
Patient ID: 081126
Panel: 11c,123
Gate: G2
Total Events: 223009

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10688	100.00	4.79	16.85	9.11	671.97	10.55	1

Fig. 34 cat number 11

Appendix 36



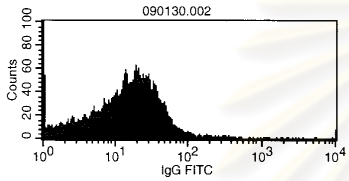


Histogram Statistics

File: 090130.001
Sample ID: MiMi BL blank
Tube: CD123
Acquisition Date: 31-Jan-09
Gated Events: 10202
X Parameter: blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090130
Panel: 11c,123
Gate: G2
Total Events: 20116

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10202	100.00	50.72	13.67	9.06	102.64	10.55	1

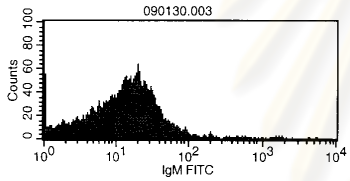


Histogram Statistics

File: 090130.002
Sample ID: MiMi BL IgG
Tube: CD11c
Acquisition Date: 31-Jan-09
Gated Events: 10635
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090130
Panel: 11c,123
Gate: G2
Total Events: 75415

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10635	100.00	14.10	38.63	12.94	992.92	15.26	1



Histogram Statistics

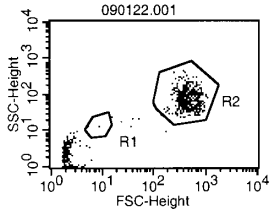
File: 090130.003
Sample ID: MiMi BL IgM
Tube: CD123
Acquisition Date: 31-Jan-09
Gated Events: 10292
X Parameter: IgM FITC (Log)

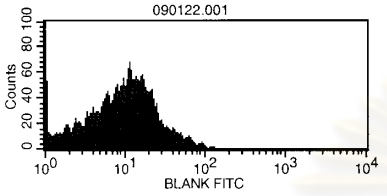
Log Data Units: Linear Values
Patient ID: 090130
Panel: 11c,123
Gate: G2
Total Events: 162218

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10292	100.00	6.34	24.33	11.82	465.13	13.46	1

Fig. 35 cat number 12

Appendix 37



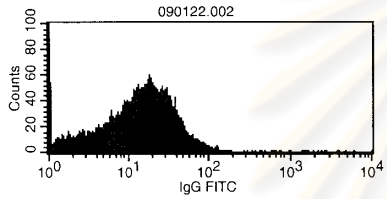


Histogram Statistics

File: 090122.001
Sample ID: Khai-DOWN BL BLANK
Tube: CD123
Acquisition Date: 23-Jan-09
Gated Events: 10425
X Parameter: BLANK FITC (Log)

Log Data Units: Linear Values
Patient ID: 090122
Panel: 11c,123
Gate: G2
Total Events: 28061

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10425	100.00	37.15	12.85	8.73	94.43	10.00	1

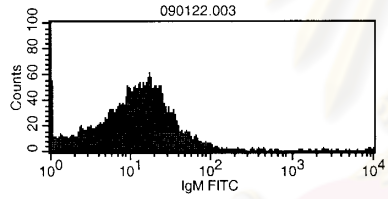


Histogram Statistics

File: 090122.002
Sample ID: Khai-DOWN BL IgG
Tube: CD11c
Acquisition Date: 23-Jan-09
Gated Events: 10272
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090122
Panel: 11c,123
Gate: G2
Total Events: 67267

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10272	100.00	15.27	25.47	12.27	786.21	14.46	1



Histogram Statistics

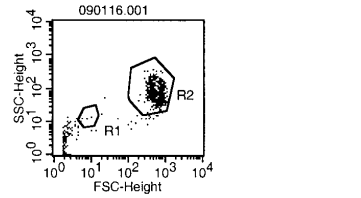
File: 090122.003
Sample ID: Khai-DOWN BL IgM
Tube: CD123
Acquisition Date: 23-Jan-09
Gated Events: 10311
X Parameter: IgM FITC (Log)

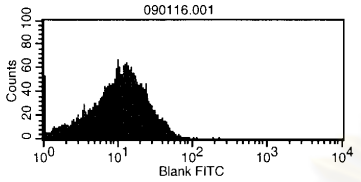
Log Data Units: Linear Values
Patient ID: 090122
Panel: 11c,123
Gate: G2
Total Events: 167345

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10311	100.00	6.16	30.18	10.72	1000.31	11.97	1

Fig. 36 cat number 13

Appendix 38



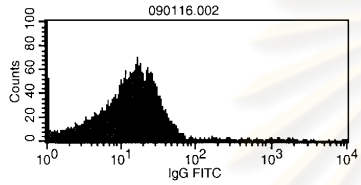


Histogram Statistics

File: 090116.001
Sample ID: Petai Noi BL Blank
Tube: CD123
Acquisition Date: 17-Jan-09
Gated Events: 10159
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090116
Panel: 11c,123
Gate: G2
Total Events: 13869

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10159	100.00	73.25	13.28	9.86	78.95	10.94	1

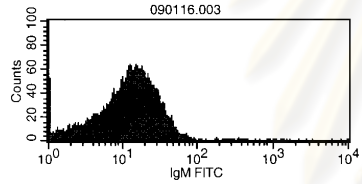


Histogram Statistics

File: 090116.002
Sample ID: Petai Noi BL IgG
Tube: CD11c
Acquisition Date: 17-Jan-09
Gated Events: 10167
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090116
Panel: 11c,123
Gate: G2
Total Events: 21758

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10167	100.00	46.73	32.02	12.65	785.71	14.20	1



Histogram Statistics

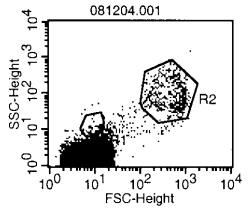
File: 090116.003
Sample ID: Petai Noi BL IgM
Tube: CD123
Acquisition Date: 17-Jan-09
Gated Events: 10393
X Parameter: IgM FITC (Log)

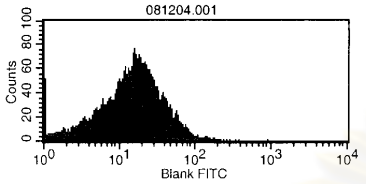
Log Data Units: Linear Values
Patient ID: 090116
Panel: 11c,123
Gate: G2
Total Events: 26683

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10393	100.00	38.95	21.88	11.24	893.12	12.86	1

Fig. 37 cat number 14

Appendix 39



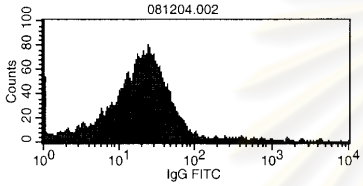


Histogram Statistics

File: 081204.001
Sample ID: Fu BL Blank
Tube: CD123
Acquisition Date: 04-Dec-08
Gated Events: 12028
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 081204
Panel: 11c,123
Gate: G2
Total Events: 497209

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	12028	100.00	2.42	20.25	13.96	101.41	15.54	1

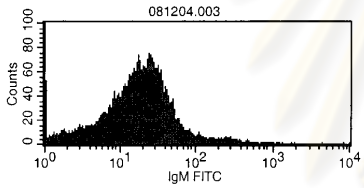


Histogram Statistics

File: 081204.002
Sample ID: Fu BL IgG
Tube: CD11c
Acquisition Date: 04-Dec-08
Gated Events: 12632
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 081204
Panel: 11c,123
Gate: G2
Total Events: 276050

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	12632	100.00	4.58	38.76	17.79	575.51	19.28	1



Histogram Statistics

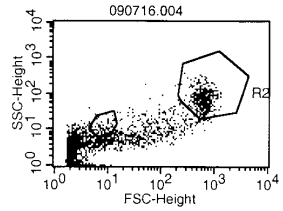
File: 081204.003
Sample ID: Fu BL IgM
Tube: CD123
Acquisition Date: 04-Dec-08
Gated Events: 12358
X Parameter: IgM FITC (Log)

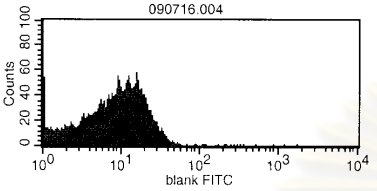
Log Data Units: Linear Values
Patient ID: 081204
Panel: 11c,123
Gate: G2
Total Events: 255816

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch.
All	1, 9910	12358	100.00	4.83	30.67	15.84	592.68	17.62	1

Fig. 38 cat number 15

Appendix 40



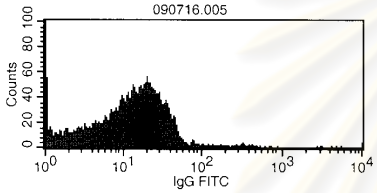


Histogram Statistics

File: 090716.004
Sample ID: Nong-phun-blood-blank
Tube: CD11c
Acquisition Date: 16-Jul-09
Gated Events: 8713
X Parameter: blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090716
Panel: 11c,123
Gate: G2
Total Events: 195557

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	8713	100.00	4.46	11.38	7.80	226.55	9.22	1

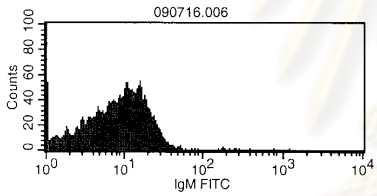


Histogram Statistics

File: 090716.005
Sample ID: Nong-phun-blood-IgG
Tube: CD123
Acquisition Date: 16-Jul-09
Gated Events: 8987
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090716
Panel: 11c,123
Gate: G2
Total Events: 131301

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	8987	100.00	6.84	29.42	11.32	1063.70	13.58	1



Histogram Statistics

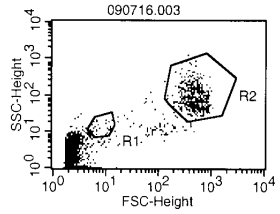
File: 090716.006
Sample ID: Nong-phun-blood-IgM
Tube: CD11c
Acquisition Date: 16-Jul-09
Gated Events: 8319
X Parameter: IgM FITC (Log)

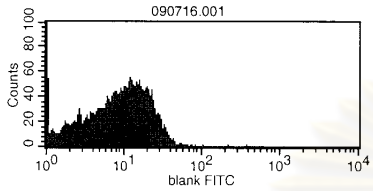
Log Data Units: Linear Values
Patient ID: 090716
Panel: 11c,123
Gate: G2
Total Events: 155784

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	8319	100.00	5.34	11.70	7.46	940.80	8.66	1

Fig. 39 cat number 16

Appendix 41



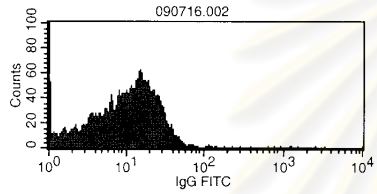


Histogram Statistics

File: 090716.001
Sample ID: KHEW-blood-blank
Tube: CD123
Acquisition Date: 16-Jul-09
Gated Events: 9552
X Parameter: blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090716
Panel: 11c,123
Gate: G2
Total Events: 107205

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9552	100.00	8.91	10.75	7.59	85.47	8.98	1

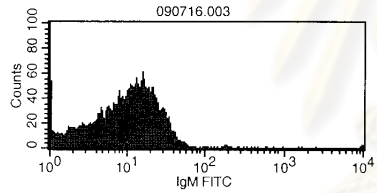


Histogram Statistics

File: 090716.002
Sample ID: KHEW-blood-IgG
Tube: CD11c
Acquisition Date: 16-Jul-09
Gated Events: 9775
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090716
Panel: 11c,123
Gate: G2
Total Events: 55046

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9775	100.00	17.76	15.71	8.87	917.73	10.55	1



Histogram Statistics

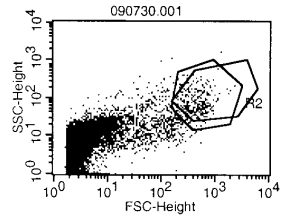
File: 090716.003
Sample ID: KHEW-blood-IgM
Tube: CD123
Acquisition Date: 16-Jul-09
Gated Events: 9630
X Parameter: IgM FITC (Log)

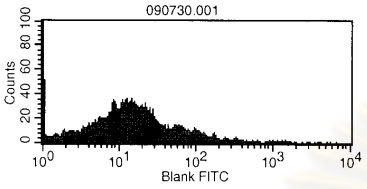
Log Data Units: Linear Values
Patient ID: 090716
Panel: 11c,123
Gate: G2
Total Events: 106001

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	9630	100.00	9.08	19.74	8.78	1224.94	10.46	1

Fig. 40 cat number 17

Appendix 42



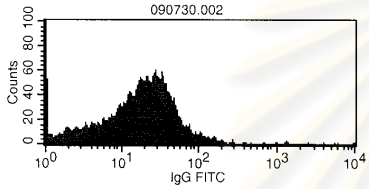


Histogram Statistics

File: 090730.001
Sample ID: SLIP BL Blank
Tube: CD123
Acquisition Date: 30-Jul-09
Gated Events: 6924
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090730
Panel: 11c,123
Gate: G2
Total Events: 720000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	6924	100.00	0.96	39.51	14.03	474.48	13.34	1

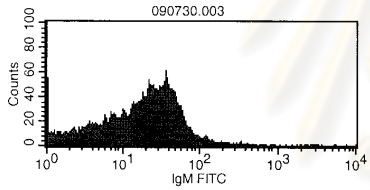


Histogram Statistics

File: 090730.002
Sample ID: SLIP BL IgG
Tube: CD11c
Acquisition Date: 30-Jul-09
Gated Events: 10029
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090730
Panel: 11c,123
Gate: G2
Total Events: 12531

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10029	100.00	80.03	30.63	15.65	762.62	18.43	1



Histogram Statistics

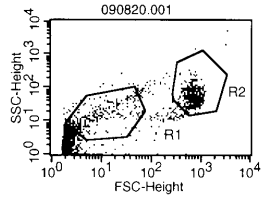
File: 090730.003
Sample ID: SLIP BL IgM
Tube: CD123
Acquisition Date: 30-Jul-09
Gated Events: 10030
X Parameter: IgM FITC (Log)

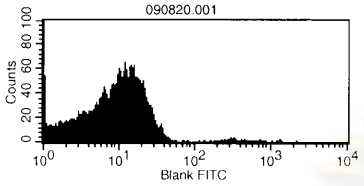
Log Data Units: Linear Values
Patient ID: 090730
Panel: 11c,123
Gate: G2
Total Events: 13126

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10030	100.00	76.41	34.11	15.39	584.72	19.63	1

Fig. 41 cat number 18

Appendix 43



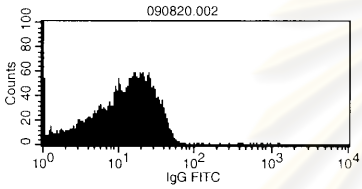


Histogram Statistics

File: 090820.001
Sample ID: Dum 2 Blood Blank
Tube: CD123
Acquisition Date: 20-Aug-09
Gated Events: 10185
X Parameter: Blank FITC (Log)

Log Data Units: Linear Values
Patient ID: 090820
Panel: 11c,123
Gate: G2
Total Events: 65094

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10185	100.00	15.65	14.69	8.16	343.22	9.56	1

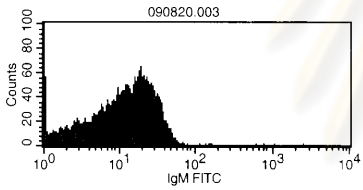


Histogram Statistics

File: 090820.002
Sample ID: Dum 2 Blood IgG
Tube: CD11c
Acquisition Date: 20-Aug-09
Gated Events: 10062
X Parameter: IgG FITC (Log)

Log Data Units: Linear Values
Patient ID: 090820
Panel: 11c,123
Gate: G2
Total Events: 20842

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10062	100.00	48.28	16.99	10.76	604.46	13.10	1



Histogram Statistics

File: 090820.003
Sample ID: Dum 2 Blood IgM
Tube: CD123
Acquisition Date: 20-Aug-09
Gated Events: 10012
X Parameter: IgM FITC (Log)

Log Data Units: Linear Values
Patient ID: 090820
Panel: 11c,123
Gate: G2
Total Events: 37982

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10012	100.00	26.36	14.94	10.10	191.52	12.30	1

Fig. 42 cat number 19

Appendix 44 Table 22 Follow up and treatment of IMHA cat number 2

Date	Prednisolone Dose	PCV (%)	Red blood cell count (cell/µl)	Platelet count (cell/µl)	White blood cell count(cell/µl)	ALT (U/L)	Creatinine (mg/dl)	TP (g/dl)
4 Apr 2009	1 mg/kg/day	18	3.55×10^6	0.8×10^5	33,400	ND	ND	8.2
10 Apr 2009	1 mg/kg/day	20	4.27×10^6	1.64×10^5	8,600	94	0.5	8.6
17 Apr 2009	0.5 mg/kg/day	21	4.26×10^6	2.31×10^5	8,400	93	ND	8.2
30 Apr 2009	0.5 mg/kg/EOD	25	5.15×10^6	2.12×10^5	7,400	117	ND	7.9
15 May 2009	Discontinued	25	5.03×10^6	1.45×10^5	8,800	137	0.6	8.4
12 Jun 2009	Discontinued	19	3.79×10^6	0.96×10^5	6,400	71	0.5	7.4
19 Jun 2009	Discontinued	15	3.04×10^6	0.61×10^5	5,000	ND	ND	7.8
23 Jul 2009	2 mg/kg/day	24	4.73×10^6	1.41×10^5	6,500	283	0.7	8.6
31Jul 2009	1mg/kg/EOD	22	4.00×10^6	1.42×10^5	4,500	220	ND	7.4
6 Aug 2009	0.5 mg/kg/EOD	24	4.47×10^6	1.40×10^5	5,100	ND	ND	7.4

Note: PCV = Packed cell volume ALT = Alanine aminotransferase

Appendix 45 Table 23 Follow up and treatment of IMHA cat number 4

Date	Prednisolone Dose	PCV (%)	Red blood cell count (cell/μl)	Platelet count (cell/μl)	White blood cell count(cell/μl)	ALT (U/L)	Creatinine (mg/dl)
13 Mar 2009	1 mg/kg/day	17	3.55×10^{6}	0.49×10^{5}	6,603	42	0.7
20 Mar 2009	1 mg/kg/day	19	3.62×10^{6}	0.95×10^{5}	31,700	ND	ND
27 Mar 2009	0.5 mg/kg/day	21	4.01×10^{6}	2.24×10^{5}	3,706	55	1.2
3 Apr 2009	Discontinued	27	4.66×10^{6}	2.59×10^{5}	5,300	ND	ND
10 Apr 2009	Discontinued	30	6.25×10^{6}	2.16×10^{5}	3,700	ND	ND
7 Aug 2009	Discontinued	31	7.01×10^{6}	2.72×10^{5}	6,900	ND	ND

Note: Patient also treated with cyclophosphamide and vincristine for lymphoma

PCV = Packed cell volume ALT = Alanine aminotransferase

Appendix 46 Table 24 Follow up and treatment of IMHA cat number 5

Date	Prednisolone Dose	PCV (%)	Red blood cell count (cell/µl)	Platelet count (cell/µl)	White blood cell count(cell/µl)	ALT (U/L)	Creatinine (mg/dl)
12 Mar 2009	4 mg/kg/day	12	1.49×10^6	1.19×10^5	24,500	33	1.74
19 Mar 2009	2 mg/kg/day	26	3.9×10^6	1.52×10^5	13,400	153	1.3
26 Mar 2009	1 mg/kg/day	31	5.32×10^6	2.28×10^5	12,400	183	1.1
3 Apr 2009	1 mg/kg/day	37	5.97×10^6	2.14×10^5	12,700	192	1.2
16 Apr 2009	0.5 mg/kg/day	37	6.37×10^6	1.85×10^5	19,200	63	1.1
1 May 2009	0.5 mg/kg/EOD	34	6.10×10^6	1.95×10^5	13,400	31	1.2
21 May 2009	Discontinued	31	5.7×10^6	1.34×10^5	13,100	26	1.3
1 Jun 2009	2 mg/kg/day	ND	ND	ND	ND	ND	ND
18 Jun 2009	2 mg/kg/day	35	6.26×10^6	1.74×10^5	15,600	50	1.6

Note: PCV = Packed cell volume ALT = Alanine aminotransferase ND = Not determine

Appendix 47

Descriptives

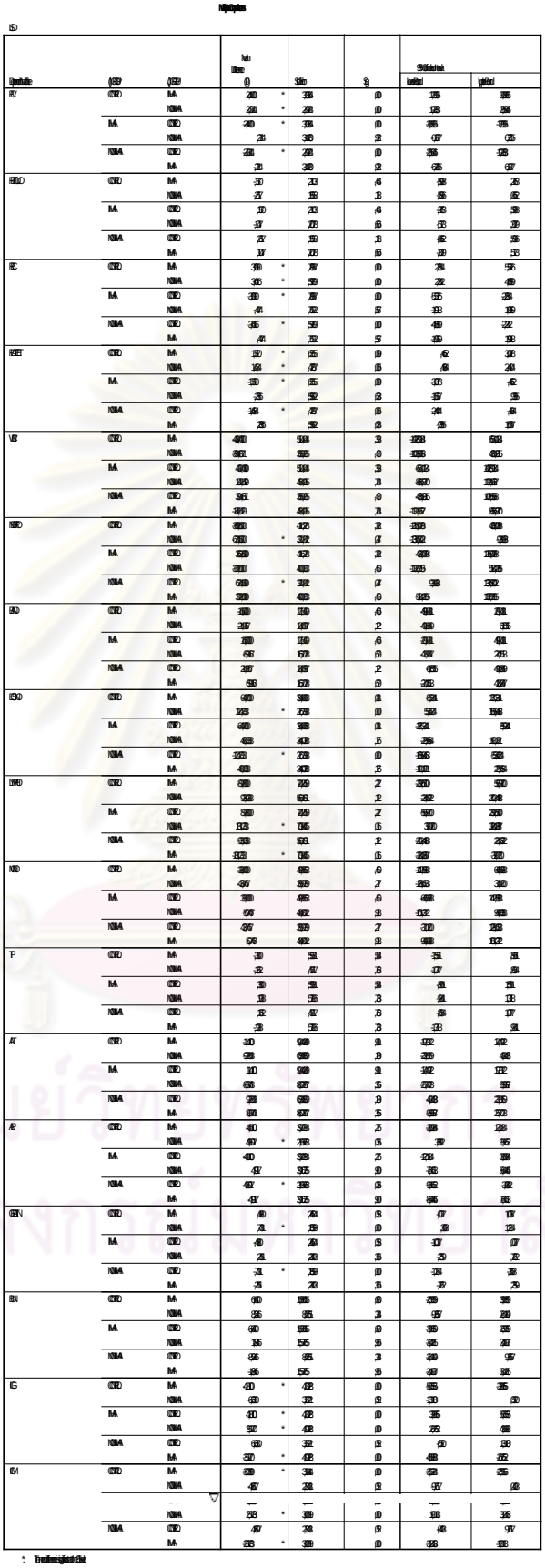
						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
PCV	CONTROL	10	41,3000	6,01941	1,90351	36,9940	45,6060	33,00	52,00
	IMHA	5	17,2000	5,80517	2,59615	9,9919	24,4081	12,00	26,00
	NON IMHA	14	16,9286	6,10755	1,63231	13,4022	20,4550	8,00	26,00
	Total	29	25,3793	13,11328	2,43508	20,3913	30,3673	8,00	52,00
RETICULO	CONTROL	10	,0850	,11306	,03575	,0041	,1659	,00	,28
	IMHA	5	,2420	,31878	,14256	-,1538	,6378	,00	,80
	NON IMHA	14	,3467	,50674	,13543	,0541	,6393	,00	1,95
	Total	29	,2384	,39002	,07243	,0901	,3868	,00	1,95
RBC	CONTROL	10	6,7530	,98822	,31250	6,0461	7,4599	5,50	8,66
	IMHA	5	2,8440	1,25113	,55952	1,2905	4,3975	1,48	4,00
	NON IMHA	14	3,2914	1,73251	,46303	2,2911	4,2917	1,00	6,07
	Total	29	4,4079	2,22603	,41336	3,5612	5,2547	1,00	8,66
PLATELET	CONTROL	10	2,5920	1,38315	,43739	1,6026	3,5814	1,05	5,86
	IMHA	5	,8400	,46357	,20732	,2644	1,4156	,49	1,48
	NON IMHA	14	1,1236	1,10372	,29498	,4863	1,7608	,10	4,00
	Total	29	1,5810	1,33377	,24767	1,0737	2,0884	,10	5,86
WBC	CONTROL	10	10723,00	3402,73762	1076,040	8288,8281	13157,1719	6300,00	16800,00
	IMHA	5	15160,40	8371,24497	3743,735	4766,1265	25554,6735	6000,00	24500,00
	NON IMHA	14	13817,86	12004,03149	3208,212	6886,9357	20748,7786	300,00	40300,00
	Total	29	12982,14	9146,26340	1698,418	9503,0854	16461,1905	300,00	40300,00
NEUTRO	CONTROL	10	6144,500	2069,82030	654,5346	4663,8398	7625,1602	3213,00	9912,00
	IMHA	5	9807,000	6835,88776	3057,102	1319,1243	18294,8757	2340,00	17204,00
	NON IMHA	12	12879,00	10135,55656	2925,883	6439,1746	19318,8254	135,00	33046,00
	Total	27	9815,852	7851,70873	1511,062	6709,8193	12921,8844	135,00	33046,00
BAND	CONTROL	10	15,0000	47,43416	15,00000	-18,9324	48,9324	,00	150,00
	IMHA	5	160,0000	357,77088	160,0000	-284,2312	604,2312	,00	800,00
	NON IMHA	12	229,9167	406,96805	117,4816	-28,6585	488,4918	,00	1227,00
	Total	27	137,3704	316,78592	60,96548	12,0540	262,6867	,00	1227,00
EOSINO	CONTROL	10	1299,700	670,07148	211,8952	820,3597	1779,0403	441,00	2856,00
	IMHA	5	655,0000	1152,20441	515,2815	-775,6507	2085,6507	,00	2695,00
	NON IMHA	12	174,9167	247,60726	71,47806	17,5945	332,2388	,00	646,00
	Total	27	680,4074	806,95002	155,2976	361,1886	999,6262	,00	2856,00
LYMPHO	CONTROL	10	2988,600	799,57227	252,8470	2416,6205	3560,5795	1664,00	3795,00
	IMHA	5	3886,400	2043,63654	913,9420	1348,8901	6423,9099	1122,00	6615,00
	NON IMHA	12	2054,667	1331,53789	384,3819	1208,6479	2900,6855	316,00	4980,00
	Total	27	2739,778	1451,62949	279,3662	2165,5323	3314,0233	316,00	6615,00
MONO	CONTROL	10	268,8000	438,48117	138,6599	-44,8705	582,4705	,00	1355,00
	IMHA	5	651,8000	887,14215	396,7420	-449,7325	1753,3325	,00	2205,00
	NON IMHA	12	702,5467	1168,92079	337,4384	-40,1502	1445,2435	,00	3224,00
	Total	27	532,5022	899,19066	173,0493	176,7942	888,2102	,00	3224,00
TP	CONTROL	10	7,4100	,73098	,23116	6,8871	7,9329	6,20	8,60
	IMHA	5	7,7400	,49800	,22271	7,1217	8,3583	7,20	8,40
	NON IMHA	13	7,5462	1,40630	,39004	6,6963	8,3960	5,70	11,00
	Total	28	7,5321	1,05235	,19888	7,1241	7,9402	5,70	11,00
ALT	CONTROL	10	33,9300	13,40697	4,23966	24,3392	43,5208	20,70	57,80
	IMHA	5	45,3400	30,11275	13,46683	7,9501	82,7299	25,00	98,00
	NON IMHA	14	126,2143	232,65606	62,17995	-8,1173	260,5459	7,00	911,00
	Total	29	80,4483	165,40487	30,71491	17,5316	143,3649	7,00	911,00
ALP	CONTROL	10	89,3600	50,00514	15,81301	53,5885	125,1315	10,30	179,00
	IMHA	2	44,8500	29,91062	21,15000	-223,8862	313,5862	23,70	66,00
	NON IMHA	12	39,8583	53,79054	15,52799	5,6815	74,0352	,00	178,00
	Total	24	60,9000	54,83369	11,19288	37,7458	84,0542	,00	179,00
CREATIN	CONTROL	10	1,7260	,40700	,12870	1,4348	2,0172	1,20	2,21
	IMHA	5	1,2280	,39613	,17716	,7361	1,7199	,70	1,74
	NON IMHA	14	,9829	,49052	,13110	,6996	1,2661	,00	1,80
	Total	29	1,2814	,55049	,10222	1,0720	1,4908	,00	2,21
BUN	CONTROL	10	29,4400	8,32389	2,63224	23,4854	35,3946	18,00	50,00
	IMHA	2	22,8000	12,16224	8,60000	-86,4734	132,0734	14,20	31,40
	NON IMHA	13	20,9154	26,58643	7,37375	4,8494	36,9814	,00	101,50
	Total	25	24,4760	20,07321	4,01464	16,1902	32,7618	,00	101,50
IGG	CONTROL	10	19,2020	4,00257	1,26572	16,3387	22,0653	14,54	24,69
	IMHA	5	61,0120	13,98617	6,25481	43,6459	78,3781	43,46	79,53
	NON IMHA	14	25,8350	7,21924	1,92942	21,6667	30,0033	15,71	38,76
	Total	29	29,6128	16,70903	3,10279	23,2570	35,9685	14,54	79,53
IGM	CONTROL	10	17,8650	3,82830	1,21062	15,1264	20,6036	13,68	23,55
	IMHA	5	47,9040	6,69272	2,99308	39,5939	56,2141	37,92	54,88
	NON IMHA	14	22,7257	6,51118	1,74019	18,9663	26,4852	11,70	34,11
	Total	29	25,3907	12,04493	2,23669	20,8090	29,9723	11,70	54,88

Appendix 48

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PCV	Between Groups	3868,999	2	1934,500	53,178	,000
	Within Groups	945,829	26	36,378		
	Total	4814,828	28			
RETICULO	Between Groups	,400	2	,200	1,346	,278
	Within Groups	3,860	26	,148		
	Total	4,259	28			
RBC	Between Groups	84,675	2	42,337	20,358	,000
	Within Groups	54,071	26	2,080		
	Total	138,746	28			
PLATELET	Between Groups	15,896	2	7,948	6,093	,007
	Within Groups	33,914	26	1,304		
	Total	49,810	28			
WBC	Between Groups	8,5E+07	2	42269571,3	,487	,620
	Within Groups	2,3E+09	26	86837562,1		
	Total	2,3E+09	28			
NEUTRO	Between Groups	2,5E+08	2	123691577	2,190	,134
	Within Groups	1,4E+09	24	56479142,7		
	Total	1,6E+09	26			
BAND	Between Groups	255083,4	2	127541,690	1,300	,291
	Within Groups	2354103	24	98087,622		
	Total	2609186	26			
EOSINO	Between Groups	6904712	2	3452355,751	8,264	,002
	Within Groups	1,0E+07	24	417736,042		
	Total	1,7E+07	26			
LYMPHO	Between Groups	1,3E+07	2	6412682,200	3,668	,041
	Within Groups	4,2E+07	24	1748440,344		
	Total	5,5E+07	26			
MONO	Between Groups	1113530	2	556764,888	,671	,520
	Within Groups	2,0E+07	24	829525,427		
	Total	2,1E+07	26			
TP	Between Groups	,368	2	,184	,156	,857
	Within Groups	29,533	25	1,181		
	Total	29,901	27			
ALT	Between Groups	57125,84	2	28562,921	1,048	,365
	Within Groups	708919,8	26	27266,145		
	Total	766045,6	28			
ALP	Between Groups	13927,94	2	6963,971	2,648	,094
	Within Groups	55226,92	21	2629,853		
	Total	69154,86	23			
CREATIN	Between Groups	3,239	2	1,619	8,025	,002
	Within Groups	5,246	26	,202		
	Total	8,485	28			
BUN	Between Groups	416,845	2	208,422	,496	,616
	Within Groups	9253,561	22	420,616		
	Total	9670,406	24			
IGG	Between Groups	6213,201	2	3106,601	50,351	,000
	Within Groups	1604,163	26	61,699		
	Total	7817,364	28			
IGM	Between Groups	3200,035	2	1600,018	48,248	,000
	Within Groups	862,214	26	33,162		
	Total	4062,249	28			

Appendix 49



BIOGRAPHY

Mr. Parkorn Limlenglert, D.V.M. was born on 1st November 1978 in Chiang Mai, Thailand. He graduated doctor of veterinary medicine in 2001 from Chiang Mai University. He was a practitioner for 4 years then moved to Chulalogkorn University for study.