Detection of Red Blood Cell Bound Immunoglobulin by the Use of Gel Test and Flow Cytometry and Its Application in the Diagnosis of Coombs' Negative Autoimmune Hemolytic Anemia

#### Thesis

Submitted for Partial Fulfillment of M.Sc. Degree in Clinical and Chemical Pathology

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#### List of Abbreviations

Antibody **Ab.....** Antibody-dependent cell-mediated cytotoxicity ADCC..... **Ag**..... Antigen AHG..... Antihuman globulin AHTRs..... Acute Hemolytic Transfusion Reactions AIHA..... Autoimmune hemolytic anemia Acute leukemia **AL**..... Acute myelocytic leukemia **AML**..... Anti-C3..... Anti-complement AHG..... Anti-human globulin **AUC**..... Area under the curve **BM**..... Bone marrow **BFU-E....** Burst-forming unit-erythroid CFU-E..... Colony-forming unit-erythroid Colony forming unit granulocyte, erythroid, CFU<sub>GEMM</sub>..... monocyte and megakaryocyte **CMV**..... Cytomegalovirus **CLL**..... Chronic lymphocytic leukemia Cold agglutinin disease **CAD**..... CAHA..... Cold-Reactive Autoimmune Hemolytic Anemia Cold agglutinin syndrome **CAS** ..... Column Agglutination Technique **CAT**..... **CBC**..... Complete blood count **CLD**..... Chronic liver disease **CR**..... Complement receptor **CT**..... Computerized tomography **CTT**..... Conventional tube technique Direct Antiglobulin Test **DAT**..... DHTRs..... **Delayed Hemolytic Transfusion Reactions** DIHA..... Drug-Induced hemolytic anemia Drug-Induced Immune HA DIIHA..... **D-L Ab.....** Donath-Landsteiner antibody **DPT**..... Diphtheria-pertussis-tetanus **EBV**..... Epstein-Barr virus

**ELAT**..... Enzyme-linked antiglobulin test

**ELISA** ...... Enzyme-linked immunosorbent assay

**EPO**..... Erythropoietin hormone

Fc R..... Fc receptor

**FITC**..... Fluorescein isothiocyanate

FACS..... Fluorescent-activated Cell Sorter

**FC**..... Flow cytometry

**FS**..... Forward angle scatter

**G**..... Grades of agglutination reactions

**GT**..... Gel Test

**Hb**..... Haemoglobin

**HA**..... Hemolytic Anemia

**HDN**..... Hemolytic disease of Newborn

**HBV**..... Hepatitis B virus

**HCC**..... Hepatocellular carcinoma

HCT.... Hematocrit

**HCV**..... Hepatitis C virus

**HLA**...... Human leukocyte antigen

**HIV**...... Human immunodeficiency virus

**HP**..... Haptoglobin

**HTR**..... Hemolytic Transfusion Reaction

IAT ..... Indirect Antiglobulin Test

IDBil..... Indirect bilirubin
Ig.... Immunoglobulin

**IMHA**...... Immune-Mediated Hemolytic Anemia

**IMN**..... Infectious mononucleosis

**IL-2**..... Interleukin-2 **IL-10**..... Interleukin-10

IRMA..... Immunoradiometric assay
IVIG..... Intravenous immune globulin

**K2-EDTA....** Dipotassium ethylenediamine tetra-acetic acid

**LDH**..... Lactate dehydrogenase enzyme

LISS..... low ionic strength saline

**LS**..... Light scatter

**LPD**..... Lymphoproliferative disease

MCH..... Mean corpuscular Hb

**MCV**..... Mean corpuscular volume **MAC**..... Membrane attack complex MDS..... Myelodysplastic syndrome Mean Fluorescence Intensity MFI..... MHC..... Major histocompatibility complex

MnI X..... Mean intensity of reactive RBCs

MM..... Multiple myeloma

Monocyte monolayer assay **MMA**..... MoAbs..... Monoclonal Antibodies MRI..... Magnetic resonance image Mitogen-stimulated-DAT MS-DAT.... NHL.... Non Hodgkin's lymphoma

NIPA..... Non-immune protein adsorption

**OC**..... ovarian cyst Peripheral blood **PB**.....

**PBS**..... Phosphate buffered saline

**PCH**..... Paroxysmal cold hemoglobinuria

PE..... Phycoerythrin

**PEG.....** Polyethylene glycol

Paroxysmal nocturnal haemoglobinuria PNH.....

**PVP**..... Polyvinylpyrrolidone Rheumatoid arthritis **RA** .....

RBCs..... Red blood cells

**RES**..... Reticuloendothelial system

**Rh D....** Rh Disease

**ROC** curve... Receiver-operator characteristic curve

Reticulocyte production index **RPI**.....

**SS**..... Side scatter

Tr cells..... Regulatory T cells

**SCT**..... Stem cell transplantation

Systemic lupus erythematosus **SLE....** TGF- ...... transforming growth factor-

**US**..... Ultrasound WL..... wavelength

# Introduction and Aim of the Work,

