



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكرو فيلم

بسم الله الرحمن الرحيم



HANAA ALY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



HANAA ALY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HANAA ALY



Use of PCR as a Tool for RBCs Molecular Genotyping in Repeatedly Transfused Patients

Thesis

*Submitted For Partial Fulfillment of M.D. Degree in
Clinical and Chemical Pathology*

By

Doaa Magdy Mohamed Mostafa

*M.B., B.Ch. and M. Sc Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University*

Under Supervision of

Professor / Deena Mohamed Mohamed Habashy

*Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University*

Doctor/ Mona Fathey Abdelfattah

*Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University*

Doctor/ Dalia Ahmed Diaa El Dine Salem

*Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University*

Doctor/ Heba Mohamed Atif

*Lecturer of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University*

*Faculty of Medicine
Ain Shams University*

2020

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سببنا أنك لا تعلم لنا
إلا ما علمتنا أنك أنت
العليم العظيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Acknowledgment

*First and foremost, I feel always indebted to **ALLAH**,
the Most Kind and Most Merciful.*

*I'd like to express my respectful thanks and profound gratitude to **Prof. Dr. Deena Mohamed Mohamed Habashy**, Professor of Clinical Pathology Faculty of Medicine, Ain Shams University for her keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

*I am also delighted to express my deepest gratitude and thanks to **Dr. Mona Fathey Abdelfattah**, Assistant Professor of Clinical Pathology Faculty of Medicine, Ain Shams University, for her kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

*I am deeply thankful to **Dr. Dalia Ahmed Dina El Dine Salem**, Assistant Professor of Clinical Pathology Faculty of Medicine, Ain Shams University, for her great help, active participation and guidance.*

*I wish to introduce my deep respect and thanks to **Dr. Heba Mohamed Atif**, Lecturer of Clinical Pathology Faculty of Medicine, Ain Shams University, for her kindness, supervision and cooperation in this work.*

Doaa Magdy

List of Contents

Title	Page No.
List of Tables	i
List of Figures	iv
List of Abbreviations.....	vi
Introduction	1
Aim of the Work.....	4
Review of Literature	
Red Blood Cell Antigens and Antibodies	5
Blood Group Typing	39
RBC Sensitization	70
Subjects and Methods.....	83
Results	112
Discussion	141
Summary	153
Conclusion	156
Recommendations	157
References	158
Arabic Summary	—

List of Tables

Table No.	Title	Page No.
Table (1):	Blood group antigens by the ISBT	11
Table (2):	Interpretation of forward and reverse ABO group.	42
Table (3):	Advantages, disadvantages & limitations of different serological techniques for blood group typing.	46
Table (4):	Categories of ABO discrepancy	49
Table (5):	Different techniques used in blood group genotyping.....	52
Table (6):	Applications of red cell genotyping in transfusion medicine.	67
Table (7):	Advantages &disadvantages of serological & molecular methods of blood group typing	69
Table (8):	Alloimmunization rates reported in various disease states.....	71
Table (9):	A package insert that determines which cells have the specific antigens of the screening panels.	96
Table (10):	A package insert that determines which cells have the specific antigens of the identification panels.	99
Table (11):	Primer sequences for RFLP-PCR of Kell, Kidd and Duffy.	106
Table (12):	Demographic and clinical data of the studied patients.....	113
Table (13):	Frequencies of major and Rh blood groups of the studied patients.....	114

List of Tables Cont...

Table No.	Title	Page No.
Table (14):	Phenotyping of minor blood groups Kell, Kidd& Duffy.	116
Table (15):	Percentage of autoantibodies and alloantibodies in the studied patients.	117
Table (16):	Types of alloantibodies in alloimmunized patients.	117
Table (17):	Association between alloimmunization and demographic data.	118
Table (18):	Association between kidd blood group genotyping and autoantibodies & alloantibodies.	120
Table (19):	Association between Kidd blood group serology and autoantibodies and alloantibodies.	121
Table (20):	Association between Duffy blood group genotyping and autoantibodis & alloantibodies.	122
Table (21):	Association between Duffy blood group serology and autoantibodies and alloantibodies.	122
Table (22):	Association between Kell blood group genotyping and autoantibodies & alloantibodies.	123
Table (23):	Association between Kell 1 serology and autoantibodies and alloantibodies.	124
Table (24):	Association between Kell 2 serology and autoantibodies and alloantibodies.	124
Table (25):	Genotyping of minor blood groups Kell, Kidd &Duffy.	126

List of Tables Cont...

Table No.	Title	Page No.
Table (26):	Typing of Kell, Kidd & Duffy by serology & PCR.	127
Table (27):	Comparison between Duffy blood group genotyping and phenotyping.	127
Table (28):	Comparison between Kidd blood group genotyping and phenotyping.	128
Table (29):	Comparison between Kell blood group genotyping and phenotyping.	128
Table (30):	Coordenance between genotype and phenotype in Kell, Kidd & Duffy blood group systems.	129
Table (31):	Association between Kidd blood group genotyping and demographic & clinical data.....	130
Table (32):	Association between Kidd blood group serology and demographic & clinical data.....	132
Table (33):	Association between Duffy blood group genotyping and demographic & clinical data.....	134
Table (34):	Association between Duffy blood group serology and demographic & clinical data.....	135
Table (35):	Association between Kell genotyping and demographic data of the studied patients.	137
Table (36):	Association between Kell 1 serology and demographic data of the studied patients	139
Table (37):	Association between Kell 2 serology and demographic data of the studied patients.	140

List of Figures

Fig. No.	Title	Page No.
Figure (1):	A schematic model of the organization of the red cell membrane	6
Figure (2):	Kell and Kx proteins	25
Figure (3):	Predicted ten-transmembrane domain structure of the Kidd/urea transporter.....	30
Figure (4):	The Duffy Blood Group antigen.....	36
Figure (5):	A representative blood group analysis using anti A, anti B and anti Rh sera	41
Figure (6):	Forward and reverse ABO/D typing of O+ blood group patient using a gel technique	44
Figure (7):	Microplate Technique for ABO & Rh D Grouping	45
Figure (8):	Basis of the PCR-SSP method.....	53
Figure (9):	Multiplex PCR technique.....	56
Figure (10):	RT-PCR Principle.....	59
Figure (11):	Stages of the Microarray technique, HEA BeadChip™ platform.....	63
Figure (12):	The MALDI-TOF MS biosensor.....	66
Figure (13):	A schematic model of the potential triggers for blood group alloimmunization	73
Figure (14):	Blood grouping cassette showing A positive blood group patient.....	87
Figure (15):	Gel electrophoresis showing Kidd blood group genotyping.....	108
Figure (16):	Gel electrophoresis showing Duffy blood group genotyping.....	109
Figure (17):	Gel electrophoresis showing Kell blood group genotyping.....	110

List of Figures Cont...

Fig. No.	Title	Page No.
Figure (18):	Duffy phenotyping by card method using anti-Fya and anti-Fyb for some patients.....	115
Figure (19):	Kidd phenotyping by card method using anti-Jka and anti-Jkb for some patients	115
Figure (20):	Association between alloimmunization and diagnosis.	119
Figure (21):	Association between kidd blood group genotyping and autoantibody formation.	120
Figure (22):	Association between kidd blood group genotyping and alloantibody formation.....	121
Figure (23):	Association between Duffy blood group serology and alloantibodies.	123
Figure (24):	Gel electrophoresis of patient's Duffy blood group genotyping.....	125
Figure (25):	Gel electrophoresis of patient's Kell blood group genotyping.....	125
Figure (26):	Gel electrophoresis of patient's Kidd blood group genotyping.....	126
Figure (27):	Association between Kidd blood group genotyping & diagnosis.	131
Figure (28):	Association between Kidd blood group serology & rate of transfusion.....	133
Figure (29):	Association between Duffy blood group genotyping & diagnosis.	134
Figure (30):	Association between Duffy blood group serology & splenectomy.	136
Figure (31):	Association between Kell genotyping and age.	138

List of Abbreviations

Abb.	Full term
A.....	Adenine
AA.....	Aplastic anaemia
ABOHDN.....	ABO haemolytic disease of the newborn
AC	Auto control
AHTR.....	Acute haemolytic transfusion reaction
AIHA.....	Autoimmune hemolytic anemia
ART4.....	Adenosine diphosphate ribosyltransferase-4
ASPCR.....	Allele-specific primer PCR
BCAM	Basal cell adhesion molecule
BCSH.....	British Committee For Standards in Haematology
Bp	Base pair
C.....	Cytosine
CDA	Congenital dyserythropoietic anaemia
CE-IVD.....	Certification mark-in vitro diagnostic
DARC	Duffy Antigen Chemokine Receptor
DAT	Direct antiglobulin test
dATP.....	Deoxyadenosine triphosphate
DBP	Duffy binding protein
dCTP.....	Deoxycytidine triphosphate
ddNTP.....	Dideoxynucleotides triphosphate
dGTP.....	Deoxyguanosine triphosphate
DHTRs.....	Delayed haemolytic transfusion reactions
DNA.....	Deoxyribonucleic acid
dNTP	Deoxynucleotides triphosphate
DO.....	Dombrock
dTTP	Dexythymidine triphosphate
EDTAK3	Ethylenediaminetetraacetic acid tripotassium
ELN	European Leukemia Network

List of Abbreviations Cont...

Abb.	Full term
ExoSAP.....	Exonuclease I and Recombinant Shrimp Alkaline Phosphatase
FUO	Fever of unknown origin
FUT	Fucosyl transferase
g	Gravity
G	Guanine
GPI.....	Glycosylphosphatidyl inositol
HDFN	Hemolytic disease of the fetus and newborn
HDN	Hemolytic disease of the newborn
HEA	Human-erythrocyte-antigen
HLA	Human leucocyte antigen
HLH.....	Haemophagocytic syndrome
HTRs.....	Hemolytic transfusion reactions
IAT.....	Indirect antiglobulin test
ID.....	Identity
Ig.....	Immunoglobulin
IL8	Interleukin8
IQR	Inter quartile range
ISBT	International Society for Blood Transfusion
KLF1.....	Kruppel Like Factor 1
LISS.....	Diamed system Diluent 2
LU.....	Lutheran
LW	Landsteiner – Wiener
MALDI-TOF-MS	Matrix assisted laser desorption ionization- time of flight- mass spectrometry
MDS.....	Myelodysplastic syndromes
MF	Mixed field
NGS	Next generation sequencing
NTC	No template control

List of Abbreviations Cont...

Abb.	Full term
P.....	Plasmodium
PBS.....	Phosphate buffered saline
PCR RSSO.....	Polymerase Chain Reaction –Reverse Sequence Specific Oligonucleotide
PCR SSP.....	Polymerase chain reaction sequence specific primer
PCR.....	Polymerase chain reaction
PE	Phycoerythrin
PRBCs	Packed RBCs
RANTES.....	Regulated on activation, normal T-expressed and secreted
RBC	Red blood cell
RFLP	Restriction fragment length polymorphism
RhD.....	Rhesus D
RPM.....	Revolutions per minute
SAP	Shrimp Alkaline Phosphatase
SAPE	Streptavidin-phycoerythrin
SCA	Sickle cell anaemia
SCD.....	Sickle cell disease
SLE.....	Systemic lupus erythromatosis
SNPs.....	Single nucleotide polymorphisms
SPSS.....	Statistical Package for Social Science
ssDNA.....	Single-stranded DNA
T	Thymine
TBE.....	Tris/Borate/EDTA
TE	Tris/EDTA