The Utility of Multiplex PCR in Simultaneous Detection of HCV, HBV and HIV Infections in Sero-Negative Blood Donors

Thesis

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Mohamed Sakr

Table of Contents

List of Tables

List of Figures

List of Images

List of Abbreviations

Abb.	Mean
µL	Micro-liter
Abs	Antibodies
Ags	Antigens
AIDS	Acquired immune deficiency syndrome
Anti-HBs	Antibodies to HBsAg
ART	Antiretroviral therapy
cDNA	Complementary DNA
CLIAs	Chemiluminescent immunoassays
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
EIAs	Enzyme immunoassays
ELISA	Enzyme-linked immunosorbent assay
ELISA-3	Third-generation ELISA
FDA	Food and Drug Administration
GRC BTS	German Red Cross Blood transfusion Service
HA	Haemagglutination
HBc IgM	IgM antibody against Hepatitis B core antigen
HBIG	Hepatitis B immunoglobulin
HbsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HCV-4	HCV genotype 4
HD	Hemodialysis
HIV	Human immune deficiency Virus
HLA	Human leucocyte antigen
IAs	Immunoassays

Abb.	Mean
IC	Internal control
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-28B	Interleukin-28B
IU/ml	International unit/milliliter
JPAC	Joint UKBTS/NIBSC Professional Advisory Committee's
LED	Light-emitting diode
LFT	lateral flow technology
LOD	Lower limit of detection
Mn2	Manganese
NAT	Nucleic acid testing
ORF	Open reading frame
PA	Particle agglutination
PAT	Parenteral antischistosomal-therapy
PCR	Polymerase chain reaction
PDM	Pooling and Data Management
PT	Proficiency testing
RDTs	Rapid diagnostic tests
RIBA	Recombinant immunoblot assay
RLSs	Resource limited settings
RNA	Ribonucleic acid
RR	Repeatedly reactive
SARS	Severe acute respiratory syndrome
SAS	Signal amplification system
SIV gor	Gorilla Simian Immunodeficiency virus
SIVcpz	Chimpanzee Simian Immunodeficiency virus
SPU	Sample processing unit
SSA	Sub-Saharan Africa
Th1	T-helper 1

Abb.	Mean
TTIs	Transfusion transmitted infections
U-DNA	Uracil-containing DNA
UNG	Uracil N-Glycosylase
US	United Sates
v2.0	Version 2.0
WHO	World Health Organization

Introduction

Every second, someone in the world needs blood. In every country, surgery, trauma, severe anemias, and complications of pregnancy are among clinical conditions that demand blood transfusion. Whatever the degree of development of a health care system, transfusion is the only option for survival for many patients **(Nansseu et al., 2013).**

Though transfusion of blood and its components is life saving, it has also life threatening hazards. With every unit of blood there is a 1% chance of transfusion associated problems including transfusion transmitted diseases. Preventing the transmission of infectious diseases through blood transfusion in developing countries is difficult given that the resources required are not always available. Even when effective policies and strategies are in place, transmission of diseases still occurs **(Fernandes et al., 2010).**

Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) infections and Human immune deficiency virus (HIV) are prevalent transfusion transmitted infections (TTIs) among multiple blood transfused patients as thalassemia, sickle cell anemia, hemophilia, aplastic anemia and patients on chronic hemo-dialysis. The problem of TTI is directly proportional to the

prevalence of the infection in the blood donor community **(Vidja et al., 2011).**

Sensitivity and specificity of the currently used fourth generation enzyme-linked immunosorbent assays (ELISAs) are much better than the earlier versions but it is clear that they fail to detect donors in the window period and with occult infections without detectable levels of circulating antigens and antibodies **(Ismail et al., 2012)**.

Transfusion services all over the world are constantly trying to improve blood safety and to reduce the residual risk of TTIs. Studies from Europe and America suggest that the addition of nucleic acid testing (NAT) will increase the detection rate of the transmissible viral infections, thereby increasing safety of blood transfusion **(Mathan, 2013).**

The aim of the current study is to determine the utility of Cobas® TaqScreen Multiplex polymerase chain reaction (PCR) Test, version 2.0 in simultaneous detection of HCV, HBV and HIV nucleic acids among sero-negative blood donors.

Chapter (1):

Blood transfusion

All patients requiring transfusion should have reliable access to safe blood products, including whole blood, labile blood components and plasma-derived medicinal products appropriate to their clinical needs, provided in time and safely administered **(World Health Organization [WHO], 2014a).**

Systems of blood transfusion:

Two systems, centralized and hospital-based, exist in low income countries for managing blood supply:

A. Centralized system: Voluntary blood donors are recruited, screened and bled by regional centers and the blood collected is distributed to peripheral hospitals. Strategies for recruiting blood donors have to provide blood for all who need it in a timely manner while ensuring that the blood is as safe as possible. The safest type of blood donor is the one who donates regularly (i.e. repeat donors) **(Bloch et al., 2012 and Erhabor et al., 2015).**

B. Hospital-based systems: These are the predominant source of blood across sub-Saharan Africa. Hospital-based systems obtain blood predominantly from relatives of patients, and

blood is screened and used within the local vicinity **(Bates et al., 2007).**

Blood unit from the centralized system costs at least three times as much as that from a hospital-based system. Although centralized systems can save costs through batching and bulk purchasing, the quality assurance processes and donor recruitment components are expensive and difficult to maintain without dependence on external funds. In hospital-based transfusion services, testing quality is variable and the families of patients bear the cost of finding blood donors **(Lara et al., 2007).**

Transfusion-transmitted Infections:

Transfusion of infected blood causes morbidity and mortality in the recipients, and has an economic and emotional impact on their families and communities. Those who become infected through blood transfusion are infectious to others and contribute to the spread of disease, thereby increasing the burden on health services and reducing productive labor **(Farrar et al., 2014)**.