UNIVERSITI TEKNOLOGI MARA

# MOLECULAR ANALYSIS OF WEAK D AMONG NON-CHINESE BLOOD DONORS USING SPECIFIC SEQUENCE PRIMER- POLYMERASE CHAIN REACTION (SSP-PCR)


**By**

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# ABSTRACT

## Molecular Analysis Of Weak D Among Non-Chinese Blood Donors Using Specific Sequence Primer-Polymerase Chain Reaction (SSP-PCR)

Determination of weak D types may reduce the risk of unnecessary alloimmunization and also beneficial for proper utilization of Rh negative blood units. Present study aimed to determine the weak D type among non-Chinese blood donors from National Blood Centre (NBC) Kuala Lumpur by using Polymerase chain reaction with specific sequence primer (PCR-SSP). Out of 104 Rh negative blood donors, only one turn out to be positive for weak D, but undefined to be detected by selected kits. The phenotype for the India sample were dCce. Rh phenotyping of all sample consist of 96% dce, 2.9% dCce, and 0.9% dCcEe. In conclusion, due to time constrained for sample collection and the use of kits, the weak D type undetermined. Hence, it is recommended to prolong the duration of the samples collection and to use the different weak D variant kits or using real time PCR.



**Keywords:** Weak D Types, Rh negative blood, Non-Chinese blood donor, specific sequence primer- polymerase chain reaction (SSP-PCR)

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of study

Rhesus is originally known as the second most clinically significant antibody in blood transfusion, next to ABO blood group. There are 5 principal antigens (D,C,c,E,e) and their corresponding antibodies that account for more than 99 percent of clinical problems such as haemolytic transfusion reaction (HTR) and haemolytic disease of foetus and newborns (HDFN) (Kim et al., 2005). Two highly homologous genes are responsible to produce the aberrant antigens, which are *RHD* and *RHCE* which both comprise approximately 60 kb, in a flank position that exist face to face by their tail ends, and only separated by a DNA segment of 30,000 kb (Flegel & Wagner, 2002). These two genes, *RHD* and *RHCE* encodes for RhD and RhCE protein comprise 417 amino acids each, forming 12 transmembranous domains with 6 extracellular loops (Marini *et al.,* 2000; Avent, 2001). RhD and RhCE proteins differ by 32 to 35 amino acids that arise *via* gene duplication of two highly homologous about 97% identical and both dispersed throughout the protein (Westhoff, 2007). These differences are the factor resulting in a potent immune response in D-negative individuals (Westhoff, 2004).

According to Kim *et al.,* (2005), different race may exhibit unique molecular mechanism of Rh-D negative thus specific molecular diagnostic approach is necessary. In Europeans, the deletion of RHD gene entirely was the most prevalent in RhD negative phenotype (Colin *et al.*, 1991; Wagner *et al.*, 2000), while in Africans and Asians are caused by silent or inactive RHD genes due to the presence of various alleles (Westhoff, 2004). Alternatively, there is presence of D antigen with a red cell that does not agglutinate by IgM Anti-D but reacts with IgG Anti-D in an agglutination test, which particularly termed as $D^u$. Previously, most red cells with $D^u$ are considered