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DETECTION OF DEL PHENOTYPE IN RHD NEGATIVE BLOOD BY FREEZE-THAW ELUTION TECHNIQUE AND SEQUENCE-SPECIFIC PRIME POLYMERASE CHAIN REACTION

By

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DECLARATION

I hereby declare that this thesis is my original work and has not been submitted previously or currently for any other degree at UiTM or any other institutions.

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ABSTRACT

Detection of DEL phenotype in RhD negative blood by freeze-thaw elution technique and Sequence-Specific Polymerase Chain Reaction (SSP-PCR)

The weakest weak D called DEL phenotype was able to be detected via serological adsorption elution and molecular genotyping. In this study, the freeze-thaw elution and SSP-PCR were used to detect the DEL phenotype. A total of 43 RhD negative samples collected from the National Blood Centre were serologically tested for Rh phenotyping and screened for DEL phenotype by both serological freeze-thaw elution and SSP-PCR. Based on the Rh phenotyping, the 43 RhD negative samples consisted of 34 ccee phenotype, 6 Ccee phenotype, 2 ccEe phenotype and 1 CCee phenotype. No DEL phenotype was detected by serological freeze-thaw elution but 2.33% (1/43) from the samples were detected for DEL phenotype that carried the RHD1227A allele by SSP-PCR. As a conclusion, the aim of this study was accomplished by the ability to detect DEL phenotype via molecular genotyping technique in distinguishing the DEL phenotype from truly D negative but not for freeze thaw elution technique.

Keywords: RhD negative, DEL phenotype, Adsorption elution method, RHD1227A allele, weak D, Malaysian population

CHAPTER 1

INTRODUCTION

1.1 Background of the study

In 1940, Karl Landsteiner and Alexander Wiener accidently discovered the Rhesus blood group in one of their experiments. They concluded that the agglutination between antisera and the red cell categorized individual either as Rhesus positive or Rhesus negative. Genetically, Rh positive person could be either homozygous dominant alleles for Rhesus (DD) or heterozygous alleles (Dd) and Rh negative person has homozygous recessive alleles (dd).

Rhesus antigens were acylated red cell transmembrane protein with a molecular weight of 30-32 kDa and encoded by two highly homologous genes, RhD and RhCE which located on 1p34.3-1p36.1 (Musa, Hassan, Ayob, & Yusoff, 2014; Wagner, 2002). The RhD and RhCE protein were encoded by two genes differ by 32-35 amino acids (Srijinda, Suwanasophon, Visawapoka, & Pongsavee, 2012). The RHD encoded the D polypeptide while the RHCE encoded for CE antigens in various combinations of CE, cE, Ce and ce (Li Qinwei, L. Hou, Zhonghui Guo, Ye Luyi, Danqi Q, Yue & Ziyan Zhu, 2009).

As the RhD and RhCE proteins were encoded by two genes differ by 32 to 35 amino acids, the degree of immunogenic of antigen D resulted from the different of a large number of amino acids between RhD and RhCE. Reduced RHD expression on the red blood cells compared to the D positive individuals was the characteristic of weak D phenotype.The DEL phenotype serologically defined as a quantitative variant of D antigen and known as the weakest RhD-positive phenotype in the Rh blood group system (Srijinda *et al.*, 2012).