UNIVERSITI TEKNOLOGI MARA

DETECTION OF DEL PHENOTYPE IN RH NEGATIVE BLOOD BY USING CHLOROFORM ELUTION TECHNIQUE AND SPECIFIC SEQUENCE PRIMER-POLYMERASE CHAIN REACTION (SSP-PCR)

By

NOR ARINA BINTI CHE MOHD NOOR

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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 institution"

NOR ARINA BINTI CHE MOHD NOOR
920212115064
2011453602

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// ABSTRACT

Detection of DEL Phenotype in Rh Negative Blood by Using Chloroform Elution Technique and Specific Sequence Primer-Polymerase Chain Reaction (SSP-PCR)

DEL phenotype is known as the weakest D antigen in the Rh blood group system. Serologically, DEL phenotype commonly mistyped as true Rh negative. An anti-D immunization may occurred in Rh negative recipient who received blood from the DEL phenotype donor. The goals of the present study were to detect DEL phenotype in Rh negative blood by using chloroform elution technique and specific sequence primer-polymerase chain reaction (SSP-PCR). A total of 43 Rh negative blood donor's samples from National Blood Centre were examined for the Rh phenotyping and the presence of DEL phenotype by using chloroform elution technique and SSP-PCR. The Rh negative phenotypes consisted of 79.07% dce, 13.95% dCce, 4.65% dcEe and 2.33% dCe. Chloroform elution technique failed to detect DEL phenotype, but only 2.33% (1/43) Rh negative blood donor's sample was being detected as DEL phenotype by using SSP-PCR. In conclusion, based on these findings, chloroform elution technique might not be an effective method in detection of DEL phenotype. Sensitivity and specificity of molecular typing method can overcome the limitation of serological method in the clinical laboratory. Hence, it is highly recommended to use SSP-PCR in detection of DEL phenotype in Rh negative blood, thus preventing the anti-D immunization.

Key words: DEL phenotype, Rh negative blood, chloroform elution technique, RHD 1227A allele, specific sequence primer-polymerase chain reaction (SPP-PCR).

CHAPTER 1

INTRODUCTION

1.1 Background of the study

RhD antigen is known as one of the highly immunogenic blood group antigens. Severe hemolytic transfusion reaction and hemolytic disease of the newborn commonly caused by anti-D alloantibodies (Daniels, 2002). Individual's Rh status as Rh positive or Rh negative is determined by the presence or absence of antigen D on red blood cell membrane (Srijinda, Suwanasophon, Visawapoka, & Pongsavee, 2012). Rh system is encoded by RHD gene and RHCE gene (Wang *et al.,* 2005). Recently, 51 antigens and more than 200 alleles have been recognized within the Rh blood group system (Flegel, 2011).

In 1980, DEL phenotype is first reported and it is identified as the weakest D antigen in Rh blood group system (Li *et al.,* 2009). A normal Rh positive person has about 30000 per cell of antigen D sites but for the DEL phenotype, the sites of antigen D is about 20 to 40 per cell only on the RBC (Gassner, Shao, Uchikawa, & Legler, 2005). DEL phenotype is caused by several mechanisms includes splice site mutation, missense mutation, frame shift mutation and a long deletion of RHD gene (Gu *et al.,* 2014). Exchange of single or a few amino acid in intracellular parts of D site causing the reduction of D antigen density (Wagner *et al.,* 1999).

Serologically, detection of DEL phenotype can only be done by using adsorption elution technique (Okuda *et al.,* n.d.). Elution technique is used to remove antibody molecule that unbound from the red blood cell membrane by disrupting the antigen or separation of antibody from the antigen. One of the elution technique is chloroform elution technique that has been used in order to replace ether and xylene (Roberts, 2006).