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DETECTION OF DEL PHENOTYPE IN RHD-NEGATIVE BLOOD USING HEAT ELUTION TECHNIQUE AND SEQUENCE SPECIFIC PRIMER-POLYMERASE CHAIN REACTION

By

NOR ELIZATUL IZZATI BINTI MASWAN

Thesis Submitted in Partial Fulfillment for the Degree of Bachelor of Medical Laboratory Technology (Hons),
Faculty of Health Sciences, Universiti Teknologi MARA

2015

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.

(Nor Elizatul Izzati Binti Maswan)

2011884188

July 2015

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ABSTRACT

Detection of DEL phenotype in RhD-negative blood using heat elution technique and Sequence Specific Primer-Polymerase Chain Reaction

DEL is the weakest RhD-positive phenotype that commonly mistyped as RhD-negative. It may induce alloimmunization when transfused to RhD-negative recipients. Serologically, it can only be detected via adsorption-elution test. In Malaysia, few data exist regarding DEL phenotype. The objective of this study was to detect DEL phenotype in RhD-negative blood using adsorption-elution technique and Sequence Specific Primer–Polymerase Chain Reaction (SSP-PCR). A total of 43 RhD-negative blood samples were collected from Pusat Darah Negara. Rh phenotype for each sample was tested. Heat elution by incubation at 56°C for 10 minutes were implemented. Indirect Antiglobulin Test against Rh(+) cells and Rh(-) cells were completed on the eluates and last wash supernatant. Then, identification of DEL carrying RHD1227A was performed via SSP-PCR. Rh-phenotype identified were ccee with 79.07%, Ccee with 13.95%, 4.65% of ccEe phenotype and CCee phenotype with only 2.33%. One (2.33%) out of 43 samples was identified as DEL phenotype carrying RHD1227A allele when tested using SSP-PCR but none was identified from adsorption-elution. SSP-PCR was more sensitive and specific compared to adsorption-elution test. Hence, implementation of SSP-PCR for efficient DEL phenotypes typing is highly recommended.

Key words: DEL phenotype, Rh negative, heat elution, RHD1227A, Sequence Specific Primer–Polymerase Chain Reaction (SSP-PCR).

CHAPTER 1

INTRODUCTION

1.1. Background

A person blood groups are genetically inherited from their parents and are classified depending on the presence of certain antigens expressed on the red blood cells. Every blood group system is genetically distinct from each other (St-Louis, 2014). 33 characterized blood group systems and over 300 blood group antigens had been acknowledged by International Society of Blood Transfusion (ISBT) for red blood cells only.

The most polymorphic blood group among human is Rhesus blood group (Rh) that is clinically significant in blood transfusion field (F. Wagner, Frohmajer, & Flegel, 2001). Rh antigens have a complex genetic basis due to their unusual large number (Flegel, 2007) with 45 well-defined antigens so far (Daniels, 2005). RHCE and RHD are two genes that encode Rh system antigens (Daniels, 2005). Absence or presence of antigen D on red blood cell determine a person's status as RhD negative or RhD positive. The probability of rhesus D to induce immunization is higher compared to any blood group system antigen that sometimes triggers formation of irregular antibodies. D antigen immunogenicity affected by the amount of D antigen sites on red blood cells (RBCs) surfaces. Weak D RBCs expressed less amount of D thus have low immunogenicity (Yasuda, Ohto, Sakuma, & Ishikawa, 2005).

DEL is the weakest D positive phenotype. Express weak D antigen that can solely be detected using adsorption-elution methods followed by testing the eluate using indirect antiglobulin test (IAT) (Scott *et al.*, 2014). Elution is a process of dissociating antibodies from red blood cells (Judd, 1999). DEL phenotype cannot be differentiate with D-negative by routine serologic typing, therefore DEL