ADSORPTION-ELUTION DEPENDENT CONFIRMATION OF RHD POSITIVITY EXPRESSED BY THE LARGE HYBRID-ALLELE *RHD*-CE(4-9)-D

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Background: ”Small” *RHD*-CE-D hybrid-alleles still code for some RhD epitopes and consequently appear serologically as “partial” RhDs, e.g. RhD VI. In contrast, “large” *RHD*-CE-D hybrid-alleles lack almost all RhD coding nucleotides (nt) and therefore generally appear as RhD negative. Since *RHD*-CE(4-7)-D (ISBT: *RHD*01N.07*) consistently reacts RhD negative by serology, accordingly, the hybrid-allele *RHD*-CE(4-9)-D, with an even larger CE insert, would be expected to be RhD negative as well.

Study design and methods: Screening for *RHD* among RhD negative blood donor samples was done using an in house validated method to detect the presence of *RHD* exon 5 (combined nts 676G with 787G), exon 7 (1048G) and the 3’ UTR after exon 10 (nt 1359A) on single donor DNAs with RhD negativity. Molecular allele discrimination was done with commercially available kits, suitable (1) to scan for the presence of *RHD* gene exons by Sequence-Specific Priming (PCR-SSP), and (2) to detect the type(s) of “Rhesus boxes” (innotrain GmbH, Kronberg i.T., Germany). Phenotypic expression of residual RhD was assessed by adsorption-elution technique using polyclonal Anti-D antiserum.

Results: One single sample of *RHD*-CE(4-9)-D|*RHd*(deletion) genotype was observed among 5’854 RhD negative blood donors. Adsorption-elution of Anti-D onto the red blood cells (RBC) of the propositus repetitively confirmed the expression of weak RhD on his RBC membrane. *RHDCE-,* and Rhesus box analysis-PCR-SSP revealed a heterozygous presence of a *RHD*-CE(4-9)-D large hybrid-allele in combination with a RhD negative *RHd*(deletion) haplotype.

Conclusion: The observed *RHD*-CE(4-9)-D hybrid allele exhibited weak RhD expression on the RBC surface, confirming a similar observation made in China. Therefore, RhD negativity of all large *RHD*-CE-D hybrid alleles may not be concluded in general. In the observed sample, presence of *RHD* specific nucleotides within the exon 4-9 CE-specific sequence hybridized into *RHD*, or on one of the two *RHCE* genes must be postulated, but did not become apparent by PCR-SSP analysis. Large *RHD*-CE-D hybrid-alleles need (1) accurate identification, and/or (2) adsorption-elution driven RhD negativity confirmation, before their RhD negative status may be infered.