Determining the Frequency of *RH* Blood Group System in the Maltese Population

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Introduction

The Rh blood group system does not only refer to the RhD antigen, but it is a complex protein based system.
The 5 most clinically significant antigens from this blood group system are the D,C,c,E and e since they are the cause of most alloimmunisations.

RHD and RHCE genes are distributed on chromosome 1p34.1-1p36. They are very similar, closely linked loci (figure 1). The 2 RH genes have opposite orientation, face each other with their 38 ends, and are separated by about 30 000 bp. A third gene, SMP1, has the same orientation as RHD and is positioned between RHD and RHCE. The RHD gene is flanked on both sides by the 2 highly homologous Rhesus boxes.

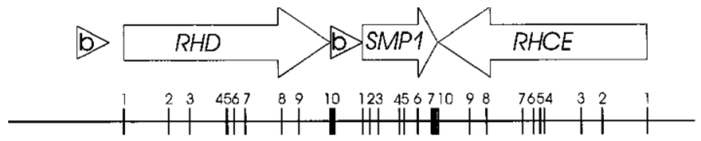

Figure 1: Schematic structure of the RH gene locus.
The positions and orientations of the genes and the Rhesus boxes are indicated by open arrows and triangles, respectively. The exons are shown as vertical bars, and their exon numbers are indicated., which are noted by (b)

Methods and Materials

400 blood donor samples and 397 neonatal blood samples were enrolled in this study. An allele-specific polymerase chain reaction (AS-PCR) method was used to determine the presence of *RHD*, *RHCE*E* and *RHCE*e*, while multiplex PCR was used to test for *RHCE*C/c*. The PCR products were analysed by Agarose-Gel electrophoresis (Figure 2,3,4,5).

81 from these 400 blood donor samples were tested by serology for the RhD, C,c,E and e antigens, and the results were used for comparison with the results obtained by genotyping.

M= ladder

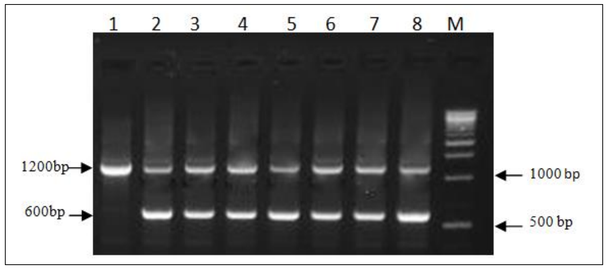

Figure 2. *RHD* products on a 1% agarose gel after electrophoresis
Samples from lane 2 to lane 8 are *RHD* positive samples,. Sample 1 is an *RHD* negative sample since only the control fragment was obtained.

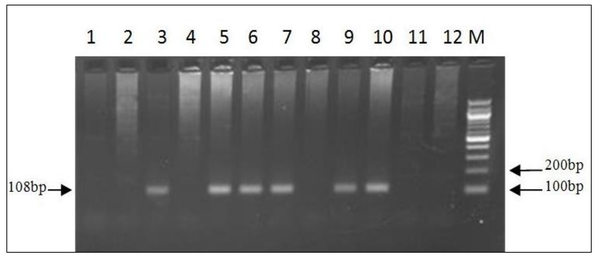

***Figure 4: RHCE*E* product on a 2.7% agarose gel after electrophoresis**
Samples 1, 2, 4,8,11 and 12 are *RHCE*E* negative, while the rest are RHCE*E neg ative.

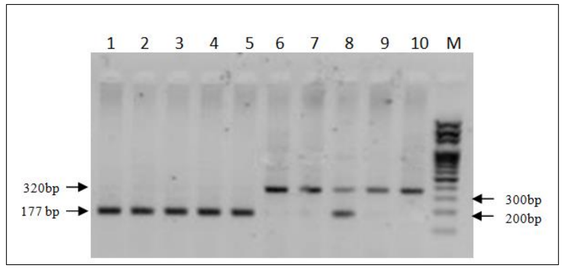

Figure 3*: RHC* products on a 2% agarose gel after electrophoresis
Samples 1 to 5 are recessive homozygotes (cc), samples 6, 7, 9 and 10 are dominant homozygous (CC) whilst sample 8 is heterozygote (Cc).

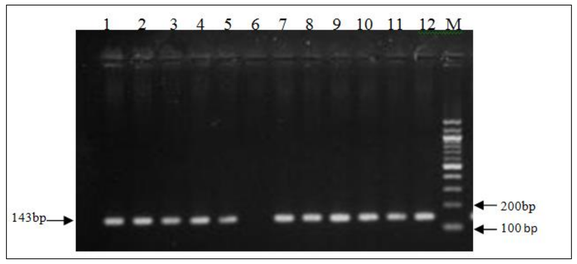

Figure 5: *RHCE*e* product on a 2.7% agarose gel after electrophoresis
Sample 6 is an *RHCE*e* negative while the rest are *RHCE*e* positive.

Results

Rh D Positive				Rh D Negative			
Genotype	**Cases**	**Percentage**	**Frequency**	**Genotype**	**Cases**	**Percentage**	**Frequency**
DCCEE	0	00.00	00.00	**dCCEE**	0	00.00	00.00
DCcEE	2	00.25	0.003	**dCcEE**	0	00.00	00.00
DccEE	12	01.50	0.015	**dccEE**	0	00.00	00.00
DCCEe	11	01.38	0.014	**dCCEe**	0	00.00	00.00
DCCee	195	24.46	0.245	**dCCee**	0	00.00	00.00
DCcEe	93	11.67	0.117	**dCcEe**	0	00.00	00.00
DccEe	53	06.64	0.064	**dccEe**	3	00.38	0.004
DCcee	305	38.27	0.383	**dCcee**	5	00.63	0.006
Dccee	62	07.78	0.078	**dccee**	55	06.90	0.069

Table 1: The *RHDCE* genotype frequencies in the population

Discussion and Conclusion

Out of 797 samples, the most common genotype was Dccee (38.27%) followed by DCCee (24.46%). In RHD negative samples dccee was the most frequents (6.9%) (Table 1).

The molecular techniques used, offer a fast in-house testing system to obtain the *RHD* and *RHCE* genotype status.

However this may merit further development to be used in a clinical setting

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References

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