BLACK AFRICAN VS+ ANTIGEN IS DEFINED BY C733 AND MAY CROSS-REACT WITH *RHCE*E/e* GENOTYPE

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Background

Genotyping for the antigens RhE and Rhe requires specific discrimination of Cytosine (C) versus Guanosine (G) at coding nucleotide 676 in exon 5 of the *RHCE* gene, respectively. Additional discrimination is mandatory for all *RHCE*E/e* genotyping approaches in order to exclude detection of G676 in the highly homologous *RHD* gene. This can be achieved by exclusive amplification of *RHCE*, "anchored" on at least one nucleotide specific for *RHCE* and discriminating *RHD*. However, "anchors" need to be selected carefully, in order to avoid unwanted typing errors, as exemplified here by the variant *RHCE* alleles with a weakened Rhe phenotype and VS positivity, encoded by 733C>G.

Methods

RHCE specific amplification was "anchored" on the two "Caucasian" *RHCE* specific nucleotides G667 and C733, followed by C (*RHCE*E*) versus G (*RHCE*e*) typing at position 676 by single base extension and Matrix-Assisted Laser Desorption/Ionisation, Time-of-Flight Mass Spectrometry (MALDI-TOF MS) analysis. Genotyping of 5,347 blood donors of the Zurich area of Switzerland with known RhE/e phenotypes was done (standard serological techniques). Discrepancies were investigated by PCR-SSP (Inno-Train, Kronberg i.T, Germany) and DNA sequencing.

***RH* genes / alleles**	**exon 1**			**exon 5**									**exon 6**		**exon 7**		
	48	106	340	**667**	**676**	**697**	**712**	**733**	**744**	**748**	**787**	**800**	916	**932**	941	1006	1025
RHD	G	G	C	T	G	G	G	**G**	C	G	G	A	G	A	G	G	T
RHCE*C	C	G	C	G		C	A	C	T	G	A	T	A	G	T	G	C
RHCE*c	G	G	C	G		C	A	C	T	G	A	T	A	G	T	G	C
RHCE*E		G	C	G	C	C	A	C	T	G	A	T	A	G	T	G	C
RHCE*e		G	C	G	G	C	A	C	T	G	A	T	A	G	T	G	C

sample ID	**phenotp.**	**res. MALDI**			**results of *RHCE* specific sequencing in exon 5**								**res. MALDI**		**res. MALDI**		
MU-00063	ccDDEe	G/C	n.a.	n.a.	**G**	**G/C**	**C**	**A**	**G/C**	**T/C**	**G**	**A**	**T**	AG (2D)	n.a.	n.a.	G	n.a.
MO-00067	ccDDEe	G	n.a.	n.a.	**G**	**G/C**	**C**	**A**	**G/C**	**T**	**G**	**A**	**T**	AG (2D)	n.a.	n.a.	G	n.a.
MO-00068	ccDDEe	G/C	n.a.	n.a.	**G**	**G/C**	**C**	**A**	**G/C**	**T**	**G**	**A**	**T**	AG (2D)	n.a.	n.a.	G	n.a.
MO-00069	ccDDEe	G	n.a.	n.a.	**G**	**G/C**	**C**	**A**	**G/C**	**T**	**G**	**A**	**T**	AG (2D)	n.a.	n.a.	G	n.a.
described *RHCE*01.20* alleles (VS+)																	
*RHCE*01.04*		C						G	**G**			G	A		G		
RHCE*01.20.01									**G**								
RHCE*01.20.02		**C**							**G**								
*RHCE*01.20.03*		C							**G**							T	
(RHCE*01.20.04)		**C**							**G**								**T**
*RHCE*01.20.05*									**G**							T	
*RHCE*01.20.06*		C					G		**G**								
*RHCE*01.20.07*				T					**G**								
*RHCE*01.20.08*		C							**G**		A						
*RHCE*01.22*				T		G	G		**G**	C		G	A				
*RHCE*02.04*				T		G	G		**G**	C		G	A				
RHCE ce(W16C,A36T,L245V)(CX,VS)		C	A						**G**								
Rhce ces744									**G**	**C**							
Döscher et al				T		G	G		**G**	C		G					

Fig.1: Nucleotide changes characteristic for all various *RH* alleles, results of the 4 samples analyzed, and specificities of all *RHCE*01.20* alleles described so far.

Results

Concordant genotyping results were found for 4,047 Rhee, 1,093 RhEe and 203 RhEE phenotypes, respectively (correct: 5,343 of 5,347 = 99,925%). Four samples with ccDEe phenotype however, displayed discrepant *RHCE*EE* genotypes ("erroneous": 4 of 5,347 = 0,075%) using G667 and C733 specific *RHCE*-amplification followed by single base extension and MALDI-TOF MS. PCR-SSP analysis, anchored on C, or G 676 and A787, respectively, resulted in phenotype-concordant *RHCE*Ee* genotypes in all 4 cases. *RHCE* specific sequencing of exons 5 and 3 of the discrepant samples identified three *RHCE*01.20.01*, one of which with an additional C744, and one *RHCE*01.20.02*, or *RHCE*01.20.04*. All alleles are known to encode positivity for the antigen VS.

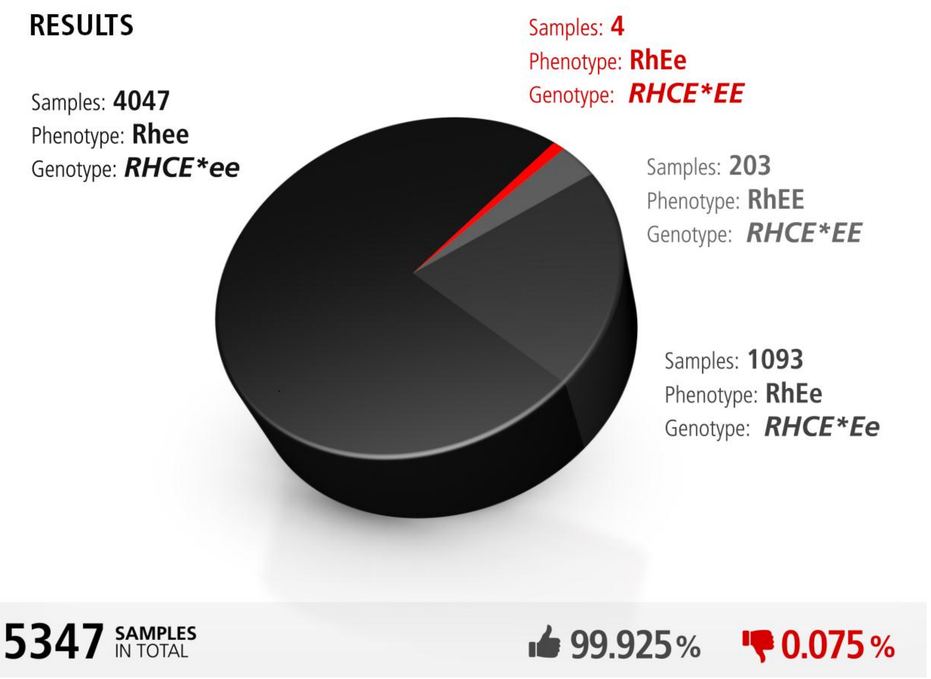

Fig.2: Statistical diagram of correct and erroneous typing for *RHCE*E/e* using C733 as anchor for specific *RHCE* amplification.

Conclusion

As expected among the large study group investigated, *RHCE*01.20* alleles were encountered and delivered "erroneous" *RHCE*e* negative results in samples of non-Caucasian ethnicity. Using different anchors for *RHCE*-specific amplification could avoid this problem, but may result in other discordant results, caused by the pleiority of other variant *RHCE*-alleles. However, considering the high frequency of VS positivity among Africans, C733 seems of limited qualification as anchor for *RHCE*E/e* genotyping.

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