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 phenotype was done (standard serological techniques). Discrepancies were investigated by PCR-SSP (Inno-Train, Kronberg I,T, Germany) and DNA sequencing.

Results
Concordant genotyping results were found for 4,047 Rhe, 1,093 RhE 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.

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.