



## RHCE Discrepancies in Apparent R1R1 Individuals: Identification of Novel Null Alleles

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### BACKGROUND/CASE STUDIES

New alleles are often uncovered following investigation of antigen typing discrepancies, weak antigen expression, or identification of an unexpected antibody.

DNA assays have become routinely used to aid patient antibody investigation and to obtain extended antigen profiles for donors.

Rh discordances between serology and DNA results have led to the discovery of novel alleles.

Here we investigate two apparent R1R1 samples with discordant antigen and DNA results for:

- c antigen in a donor sample
- c and E antigens in a Black Hispanic patient

### MATERIALS AND METHODS

#### Serologic testing

- RBCs typing for was performed by standard tube testing
- Donor typing was initially by PK7300

#### DNA testing

- Genomic DNA was isolated from WBCs.
- *RHCE* BeadChip (Immucor) was performed according to manufacturer's instructions.
- Laboratory developed tests (LDTs) for *RHCE* c.254C>G, c.577A>G, and c.907 deleted C changes were performed.
- Sanger sequencing of *RHCE* exons 1 to 10 and surrounding splice sites was performed.
- For the patient sample, sequencing with SNP-specific primers was used for determining linkage of the new change to the specific *RHCE* allele.

### RESULTS

#### DONOR SAMPLE

##### Initial results:

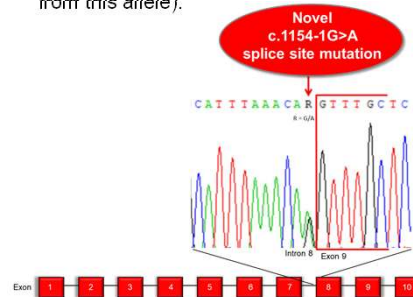
- RBCs typed D+C+E-c-e+ (R1R1) by PK7300 automated testing.
- RBCs were predicted to be C+E-c+e+ by HEA BeadChip analysis.

##### Serology Investigation:

- RBCs were non-reactive with Immucor and Bio-Rad anti-c by tube testing.

##### DNA Investigation:

- *RHCE* BeadChip results: c.48G/C and 109ins, predicting C+E-c+e+ (consistent with HEA).
- No changes in targeted LDTs (M&M)
- Sanger sequencing results:
  - No changes were found in exons and flanking intron regions for 1 to 7 and 9 and 10.
  - Identified a novel -1g>a heterozygous splice site change in intron 8 (c.1154-1A).
  - Predicted to silence expression of c (and e from this allele).



### RESULTS

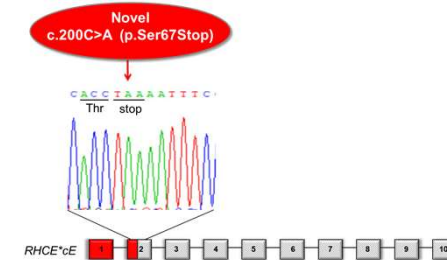
#### PATIENT SAMPLE

##### Initial results:

- RBCs typed D+C+E-c-e+ (R1R1).
- RBCs were predicted to be C+E+c+e+ by HEA BeadChip analysis.
- The patient had made anti-E, suggesting the patient may be lacking the E antigen or have a weak partial E.

##### DNA Investigation:

- *RHCE* BeadChip results: c.48G/C, 109ins, and c.676G/C, predicting C+E+c+e+ (consistent with HEA).
- Negative for *RHCE\*cEN.02* (c.907 deleted C) associated with silenced c and E antigens.
- Sanger sequencing results:
  - Changes in exons 1, 2, and 5 were consistent with *RHCE\*c*, \*c, \*E, and \*e.
  - Identified a novel heterozygous c.200C>A change in exon 2, encoding a premature stop codon (p.Ser67Stop).
  - The c.200A was linked to *RHCE\*cE* by sequencing with a c-specific primer.



### CONCLUSIONS

- We report two novel null *RHCE* alleles:
  - *RHCE\*cE(1154-1A)* is a splice site change predicted to abolish expression of c and by association e. (The impact on e antigen could not be confirmed with *RHCE\*cE* in *trans*.)
  - *RHCE\*cE(200A)* causes loss of expression of c and E, supported by the RBC typing and the presence of anti-E in the plasma.
  - Neither of the changes were found on any SNP database, suggesting they are rare.