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# Rapid clarification of suitable blood supply in an anemic patient with inconclusive serologic and genotypic results for the RhCE blood group

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**Background**
In rare cases, standard methodology in routine diagnostics fails to unequivocally determine matched blood for patients in need of blood transfusion. If this happens in a time-sensitive setting, the most parsimonious transfusion recommendation to avoid alloimmunization is usually selected. We present here a case of an anemic patient with unknown transfusion history whose erythrocytes showed strong reactions (4+) with anti-D, anti-C, anti-c and anti-e, as well as a positive direct antiglobulin test (DAT). The detection of an anti-C antibody in the serum and eluate led to further investigations. Molecular analysis for important *RHD* and *RHCE* variants showed, beside the expected heterozygosity for *RHCE*C/c* and homozygosity for *RHCE*e*, the variant c.667G>T in exon 5. In the absence of knowledge on the allelic background of this variant, allele frequencies as well as the observed phenotype suggest that the allele *RHCE*01.07* (also known as *RHCE*ceMO*), reported as being associated with partial c, is the more likely genetic set-up than the allele *RHCE*02.22* associated with weak C.

**Aim**
Here, we evaluated a newly developed Nanopore sequencing approach designed for constructing full *RHD* and *RHCE* haplotypes through co-amplification of both genes in generic long-range PCRs. In particular, we assessed its suitability to resolve the present case within one working day.

**Methods**
The patient was serologically typed for D, C, c, E, e, $C^w$ and K by hemagglutination in gel cards. Standard serological methods such as DAT and indirect antiglobulin tests on test cells with and without papain treatment were used for antibody specification. Molecular genotyping was done by commercial PCR-SSP (sequence-specific priming) kits for *RHD* and *RHCE*. The amplicon sequencing approach was based on long-range PCR with five primer pairs that simultaneously amplify both *RH*-genes. To phase variants in both genes, amplicons (12 – 15.5 kb) were designed to overlap by at least 1 kb. Sequencing library preparation was carried out according to guidelines by Oxford Nanopore Technologies (ONT). Filtered sequences were mapped against a tailored region of HG38 reference including *RHD* and *RHCE*.

**Results**
Protocols for the long-range PCR and ONT library preparation fitted well into half a working day. Sequencing for ~4 hours on a MinION flowcell resulted in more than 20,000 target reads and, despite a relatively unequal distribution among fragments, a coverage above 200x for all PCR-products. Phasing of reads mapping to *RHCE* unexpectedly assigned c.667T to the *RHCE*C* allelic background (*RHCE*02.22*). Consequently, the patient's final transfusion recommendation was blood from a ccD.ee (or ccddee) donor. Regarding the anti-C antibody, its presence in the eluate more than three months after the last transfusion clearly favors an auto-origin. Given the patient's strong serological reactions with anti-C, the previously reported phenotypic information for the *RHCE*02.22* allele (weak C) may be incomplete.

**Summary**
Amplicon-based long-read sequencing by ONT proved a promising tool in urgent situations where optimal blood supply depends on unambiguous genetic allele information. To our knowledge, no other method can accurately elucidate the allelic background of the *RH* locus within approximately one working day, from DNA extraction to entire haplotype sequences.