

# ABO chimerism as a limitation of routine genotyping methods

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## Background

A chimera is a single organism composed of cells with distinct genotypes. Several different types of chimeras are described: artificial, twin and dispermic. The artificial chimerism can be seen following hematopoietic stem cell transplantation, or more transiently following blood transfusion. The second type may also be acquired most commonly through blood exchange *in utero* between twins. Dispermic chimerism is induced by the fertilization of two maternal eggs with two spermatozoa and their fusion into one body. This one is also called tetra-gametic chimerism. In transfusion medicine, chimeras are often detected when mixed field reactivity is observed in ABO/D typing or, less commonly, when phenotyping for other blood group antigens.

## Methods

Routine blood grouping was performed by column agglutination. Separation of the double cell populations was performed by differential agglutination with IgM anti-D (immuClone, anti-D fast IgM, clone: D175-2, Immucor). Initial ABO genotyping was performed by PCR-SSP (FluoGene; Inno-train Diagnostik GmbH); further resolution was performed using in-house PCR-ASP and PCR-RFLP methods. Next generation sequencing (Monotype ABO; Omixon using Illumina sequencing platform) and Sanger sequencing analysis were also performed. Identification of reference alleles was investigated by fragment analysis of Short Tandem Repeats (STR) polymorphisms.

## Aims

This investigation was prompted by finding a double population of erythrocytes in a surgery patient with no transfusion history. Our aim was to investigate the chimera and determine the underlying ABO genotype of this patient.

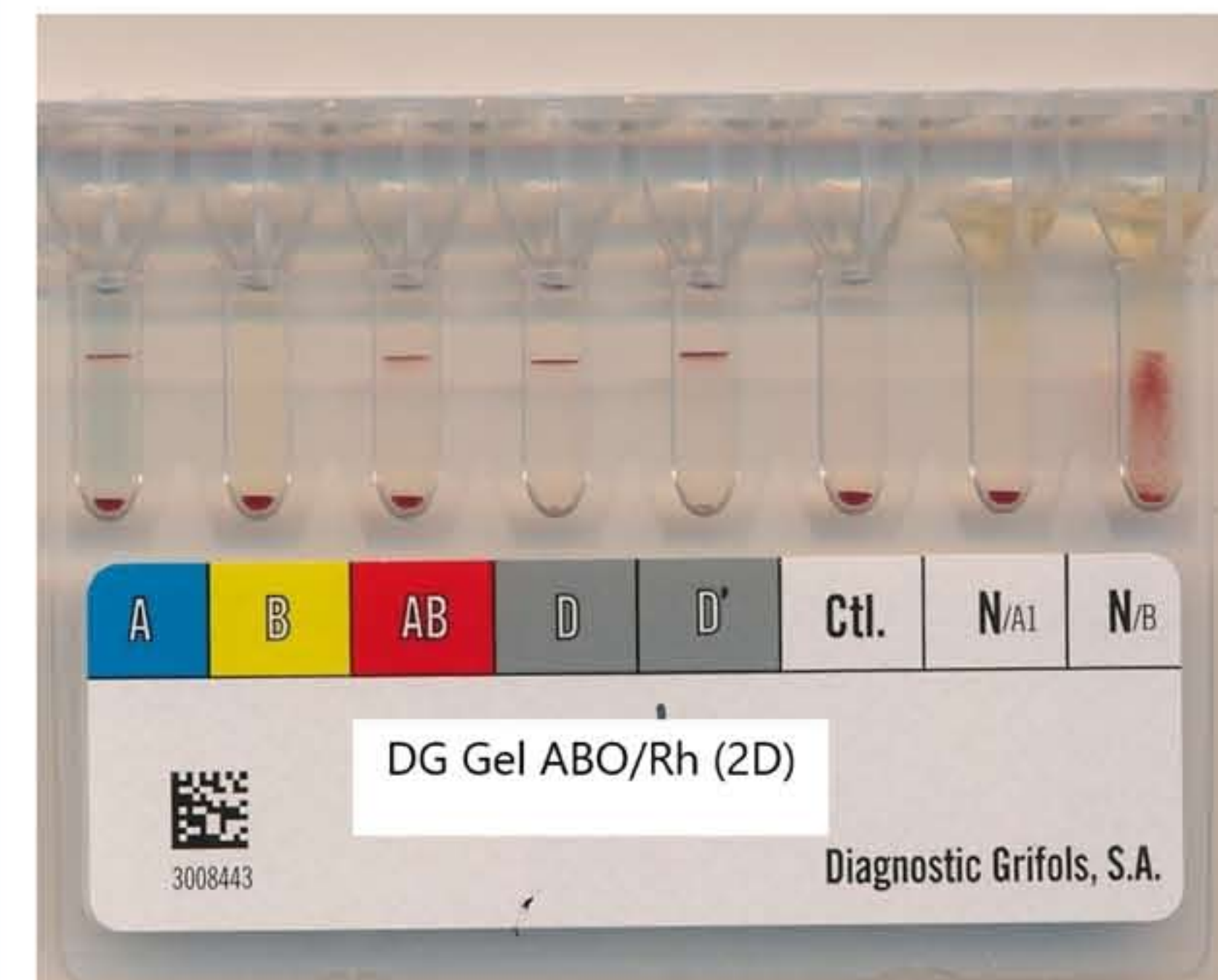


Fig 1: Blood group typing shows the double population of the RBCs in tests with anti-A, anti-AB and weakly with anti-D. /Column agglutination, DG Gel ABO/Rh (2D)/

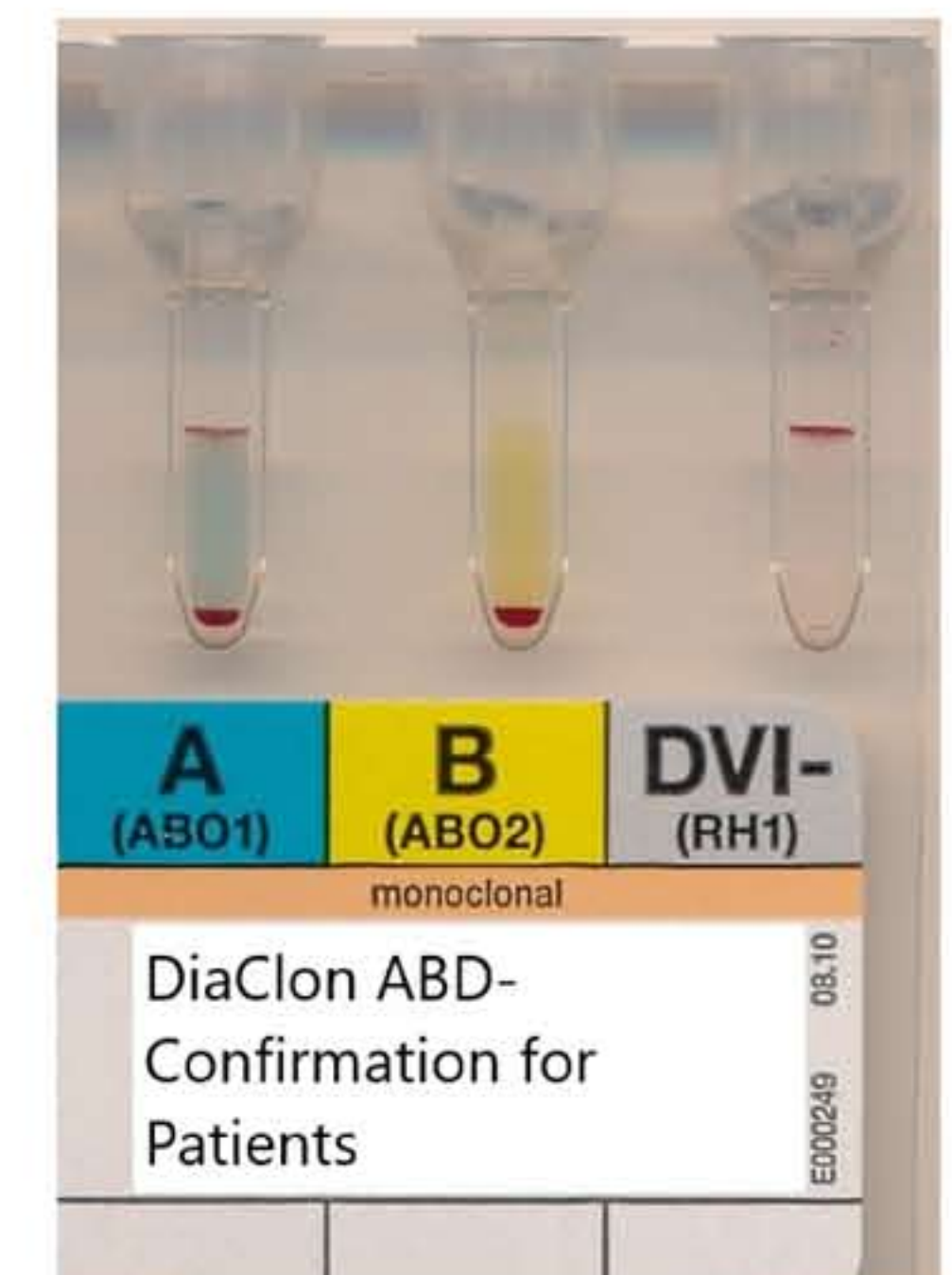


Fig 2: The double population of the RBCs with anti-A and weakly with anti-D. /Column agglutination, DiaClon ABD-Confirmation for Patients/

## Results

Double population was found in column agglutination in tests with anti-A and anti-AB [Fig 1 and 2], and subsequently when typing for D and C antigens, with approximately 85-90% of O D+C+ cells. Extremely weak reactions with anti-A and anti-AB were detected when using microplate technique [Galileo Echo - Fig 3]. Separation of the cell population was performed by agglutination with IgM anti-D. The minority RhD negative population was typed after removal of agglutinated RhD positive population using column agglutination technique [Fig. 4] The patient's genotype was identified as ABO\*O.01/\*A by CE-certified PCR-SSP kit (FluoGene). Routine PCR-ASP and PCR-RFLP could not resolve the patient's genotype. Possible ABO\*A1/\*O1 genotype was detected by PCR-RFLP, but the PCR-ASP analysis gave an apparent ABO\*A1 homozygote result.

Sanger sequencing of ABO exons 6 and 7 also gave anomalous reactions: no ABO\*A allele was detected. Homozygosity for c.261delG was observed as well as heterozygosity for c768C/A. This result therefore suggests the patient's genotype is ABO\*O.01/\*O.01.26. Next generation sequencing (Omixon) revealed the same result [Fig 5].

However, when PCR amplification of the CBF/NF-Y enhancer VNTR 3'-region was performed, possible heterozygosity was observed, i.e. a weak band representing a single copy, and one representing 4 copies of the enhancer region were present. Presence of a single copy of the 43-bp CBF/NF-Y enhancer VNTR region is unique to the ABO\*A.01 and ABO.O.02 alleles, while all other alleles carry 4 copies.

The multiplex analysis of 15 Short Tandem Repeats (STR) polymorphisms and a sex-specific locus detected the presence of chimeric alleles at approximately 10%. The confirmation was performed by monoplex analysis of selected polymorphisms.

## Test Results



UHK - U nemocnice 1, Praha 2

Instrument ID: ECHO 00077 Instrument name: Galileo 00077

Report generated: Friday, May 12, 2017 08:30

Test name	ABOD Full	Software version	1.4.1.9.0
Operator	kralova	Assay version	1.3E
Batch number	9549	Instrument ID	ECHO 00077
Profile name	ABOD Full	Instrument name	Galileo 00077

### Results - ABOD Full

Sample ID	Interp.	Flags	Rh Ctl	Anti-A	Anti-B	Anti-AB	Anti-D1	Anti-D2	A1 Cells	B Cells
	NTD Pos	*	0	0	0	?	3+	3+	0	3+

Fig 3: Weak reactions with anti-A, anti-AB and anti-D detected by the microplate technique /Galileo Echo/

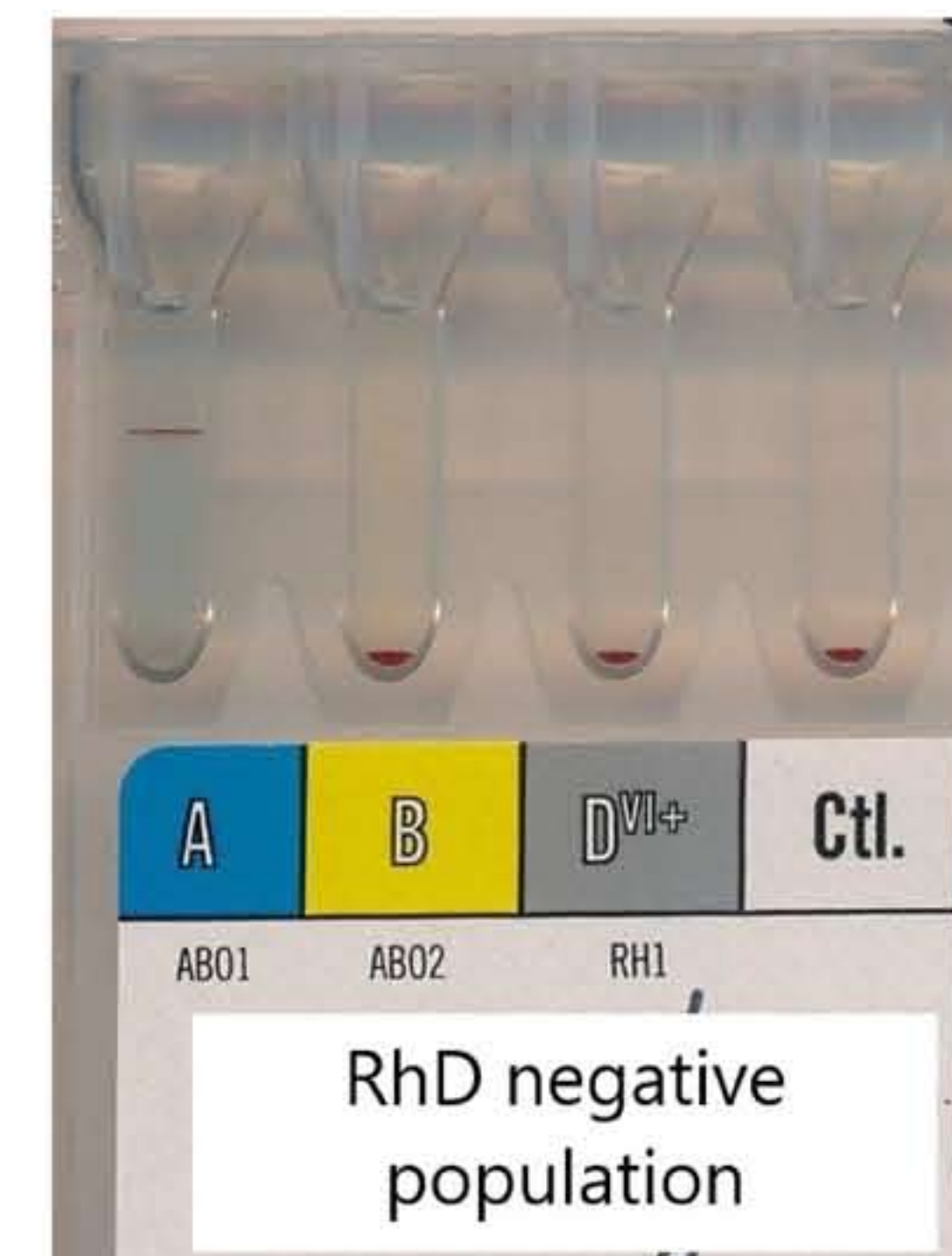


Fig 4: Blood group typing of the RBC population after removal of population agglutinated with anti-D (IgM, clone: D175-2) typed as A RhD negative /Column agglutination, DG Gel ABO Confirm/



Fig 5a: Next generation sequencing /Monotype ABO, Omixon/ revealed homozygosity for c.261delG

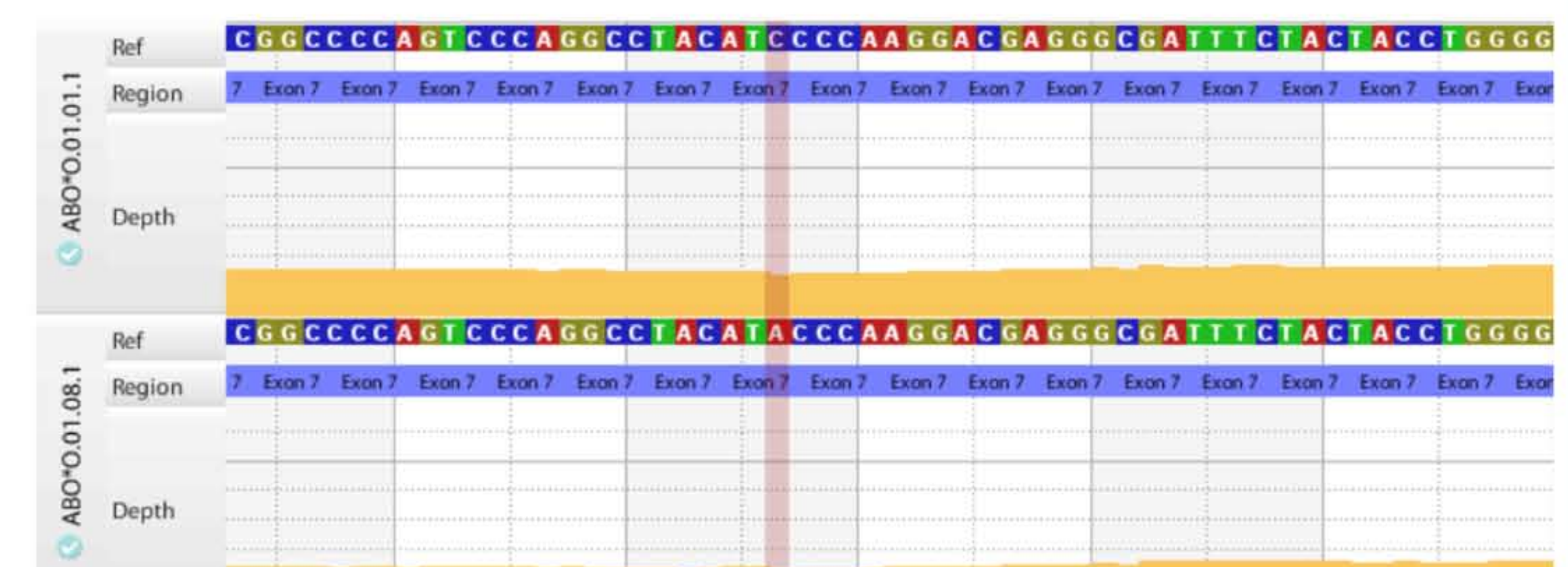


Fig 5b: Next generation sequencing /Monotype ABO, Omixon/ revealed also heterozygosity for c.768C/A

## Summary

A rare case of a low-grade chimera was observed in a patient with no transfusion or transplantation history. Unfortunately, the comparison with the parent DNA samples, or buccal mucosa was not available. The analysis of STR polymorphisms confirmed serological findings which estimated the presence of double population of erythrocytes in a ratio of 85-90% O RhD positive and 10-15% A RhD negative.

The presence of chimeric alleles cannot be detected using only one genotyping method.

However, a combination of multiple methods, including high-resolution techniques (such as NGS), may lead to a conclusion confirming the presence of chimeric alleles and explaining the serological finding.

When detecting double erythrocyte population, a universal O RhD negative transfusion products are administered. If the presence of genetic chimerism is confirmed, the transfusion therapy need not to be restricted. In this case, the patient will also tolerate blood group A RhD positive.