Long experience and methodological progress of non-invasive cffDNA genotyping for assessment of clinically most significant maternal and fetal blood groups incompatibilities

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Background: Molecular pathology of hemolytic disease of the fetus and newborn (HDFN) is determined by different RHD, RHCE, and KEL genotypes and by blood group incompatibility between the mother and fetus. In Czech Republic, clinically significant antierythrocyte alloantibodies include anti-D, anti-K, anti C/c, and anti-E. In this contribution, we present an overview of the methodological progress of NIPT in pregnancies with possible development of HDFN. In close cooperation with the Department of Fetal Medicine and the Transfusion Department of the University Hospital Olomouc, we have established a unique centre in the Czech Republic, which routinely and continuously performs the whole spectrum of the clinically most important blood group incompatibilities. This particularly includes the testing of the deletion of the *RHD* gene and 3 SNPs in the *RHCE* and *KEL* genes (rs676785, rs609320, and rs8176058). The difficulty of the examination is due to the high homology of *RH (DCE)* genes in addition to the limited amount of cff DNA. Over the years, 4 methodological approaches have been tested and successfully implemented.

Methods and results: The overview of tested and/or implemented methodological approaches is shown below:
RHD: QF PCR (Tested/implemented); Real-Time PCR (Tested/implemented); ddPCR(Tested/implemented)
RHC: Real-Time PCR(Tested); Minisequencing (Tested); ddPCR (Tested/implemented)
RHE: Real-Time PCR (Tested); Minisequencing (Tested); ddPCR (Tested/implemented)
KEL: Real-Time PCR(Tested); Minisequencing (Tested/implemented); ddPCR (Tested/implemented).

Our methods and results are described in detail in the publications:
VODICKA R, et al 2021:doi:10.3390/diagnostics11050803
BÖHMOVA J, et al 2020:doi:10.3390/diagnostics10080564
DURDOVÁ V, et al 2020: PMID:33562967
KRATOCHVÍLOVÁ T, et al 2020: PMID:33562966
BÖHMOVA J, et al 2016:doi:10.1159/000441296
BOHMOVÁ J, et al 2013: PMID:23607381
VODICKA R, et al 2010: PMID:20925229

Conclusion: In current routine practice, the ddPCR is the methodology of first choice and can fully replace the reliable but more time-consuming methods of minisequencing and real-time/QF PCR. Accurate and rapid noninvasive fetal genotyping minimizes the possibility of HDFN development.