Optimization and validation of RHD and KELL genotyping for non-invasive prenatal diagnostics

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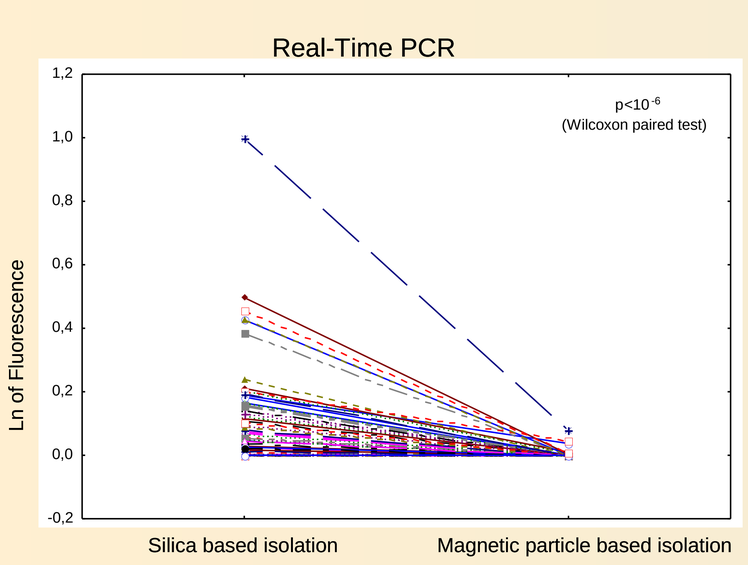
Introduction: There are two reasons for establishing a methodology for non-invasive determination of RHD and KELL genotypes in early pregnancy.
1) To identify fetuses which are at risk of hemolytic disease of fetus and newborn by alloimmunized pregnant women.
2) To prevent alloimmunization during pregnancy.
There is no method validation on a representative number of samples in the Czech Republic, which would allow to introduce methodology into clinical practice.

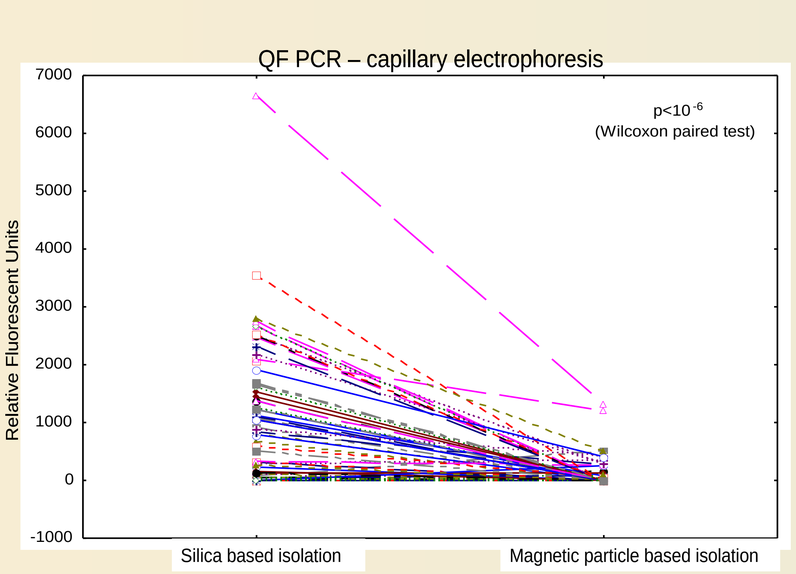
Aim: Evaluate two different cell free fetal (cff) DNA separation procedures based on adsorption on the surface of silica gel and on the separation on magnetic particles. Optimize and evaluate RHD and KELL genotyping.

Material and methods: We tested both isolation procedures in 76 cffDNA samples by Real-Time and capillary electrophoresis.
Together 200 control samples were used for genotype assessment. Optimization and calibration of RHD and KELL genotyping (KEL1/KEL2 – positive/negative) was done using Real-Time PCR and by capillary electrophoresis minisequencing .

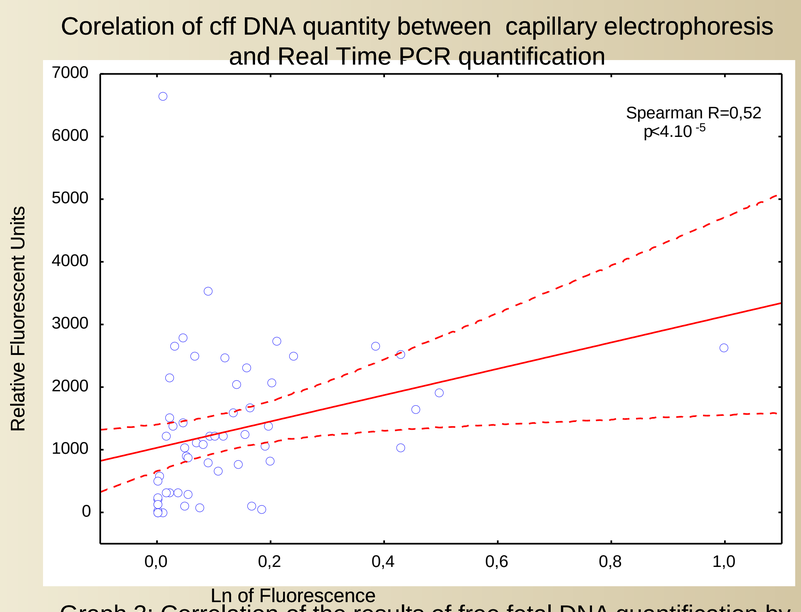
Results: There were found significant differences in the yield of cell free fetal DNA between the tested cffDNA isolation methods (Graph 1, 2). Low efficiency of magnetic particles can be caused by stronger bond fragmented fetal DNA to magnetic particles or conversely by weaker binding to magnetic particles and washing out during the isolation procedure. Silicagel membrane based method for isolation of cffDNA shorter molecules is more suitable than the magnetic particle one. If cffDNA was successfully detected by both methods, the amount of DNA has correlated to each other (Graph 3).
TaqMan based Real Time PCR was able to detect 0,22% of artificial RHD +/- and KEL1 admixture (Graphs 4, 5, 6). Disadvantage of this approach is fluorescent background particularly in KELL negative samples (Graph 6).
Minisequencing (SNaPshot) based quantification was able to detect 0,22% of artificial RHD +/- and 1,7% of artificial KEL1 admixtures (Graphs 7, 9; Pictures 1, 3).
In addition, SNaPshot assay is suitable for heterozygozity from homozygozity recognition (Graph 8, Pictures 2, 3).
Both methods are able to clearly distinguish fetal genotype.



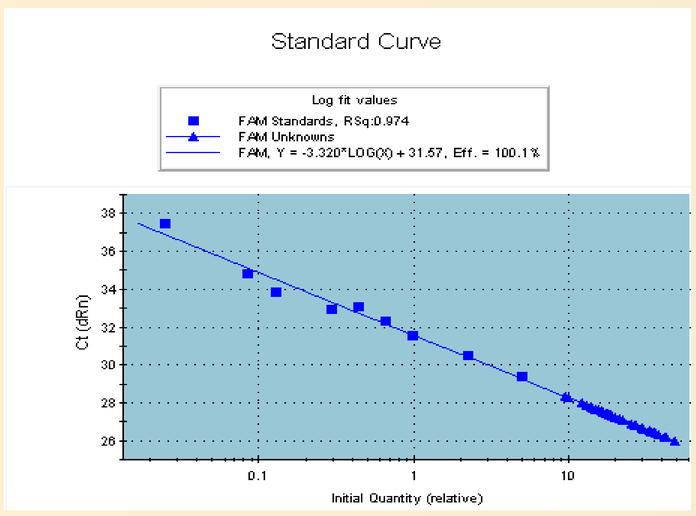
Graph 1: Comparison of silica gel and magnetic particles isolation effectiveness by Real-time PCR quantification.



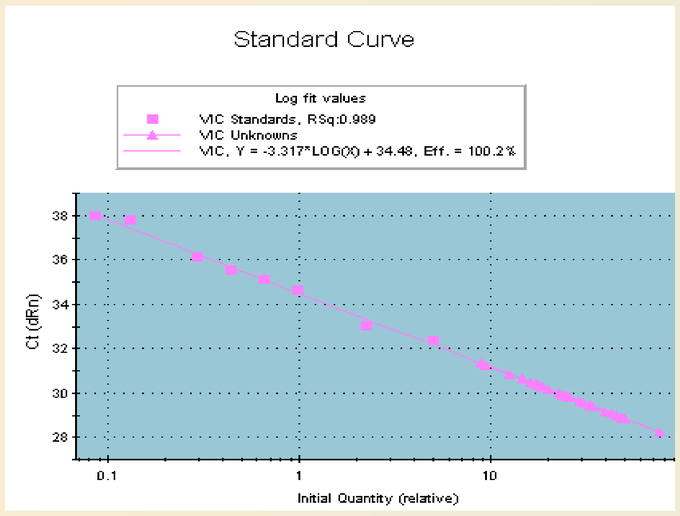
Graph 2: Comparison of silica gel and magnetic particles isolation effectiveness by capillary electrophoresis quantification



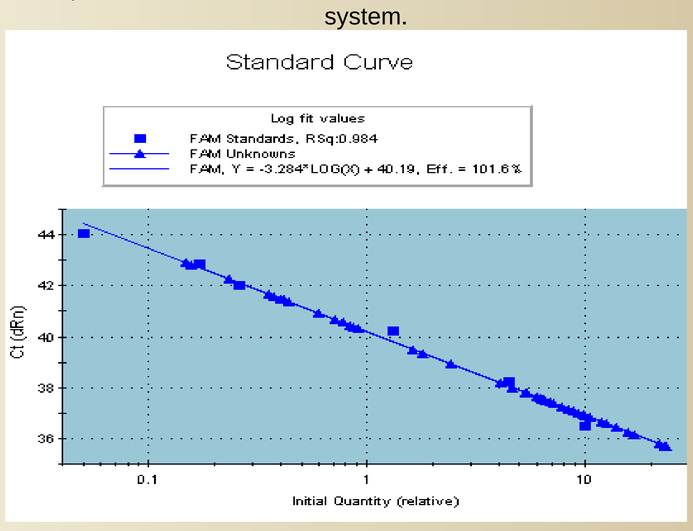
Graph 3: Correlation of the results of free fetal DNA quantification by capillary electrophoresis and real-time PCR (RT-PCR). The Y axis shows the Relative fluorescence units obtained from capillary electrophoresis, X axis shows the fluorescence scanned in Real time system.



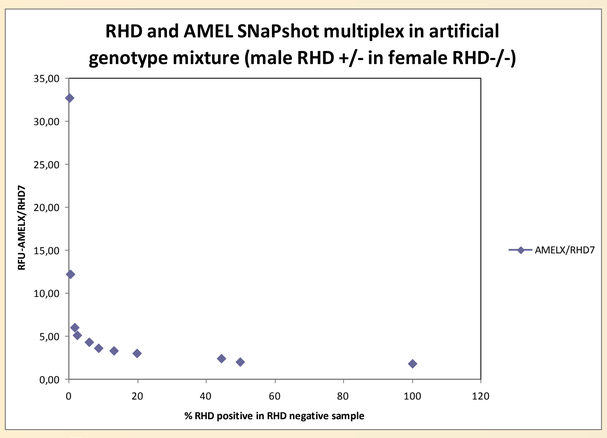
Graph 4: RHD comparative quantification using calibration curve by RHD exon 7



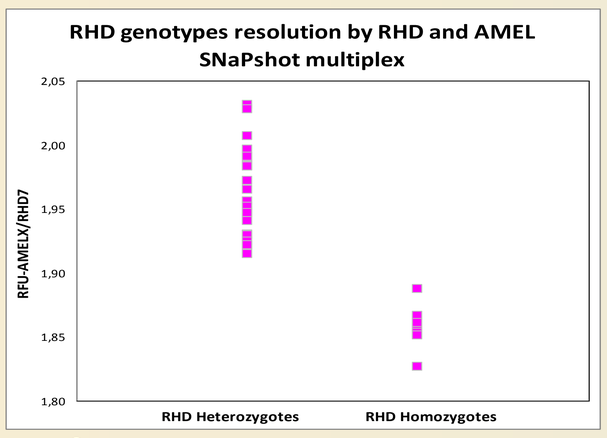
Graph 5: RHD comparative quantification using calibration curve by RHD exon 10



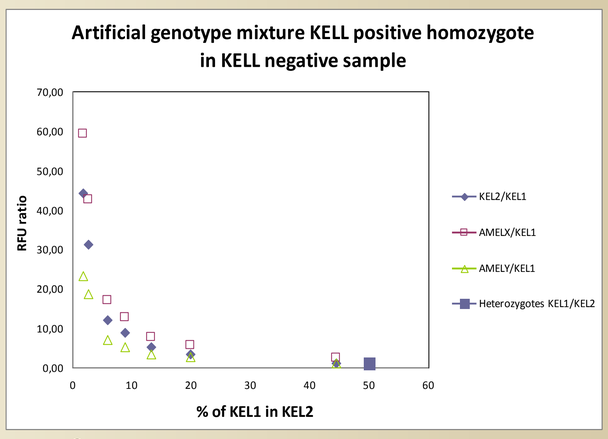
Graph 6: KELL comparative quantification using calibration curve prepared from KEL1 and KEL2 samples



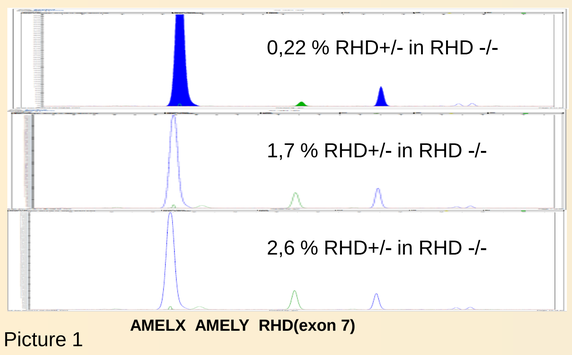
Graph 7



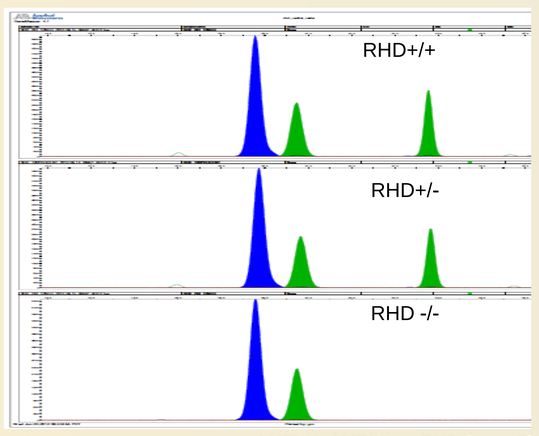
Graph 8



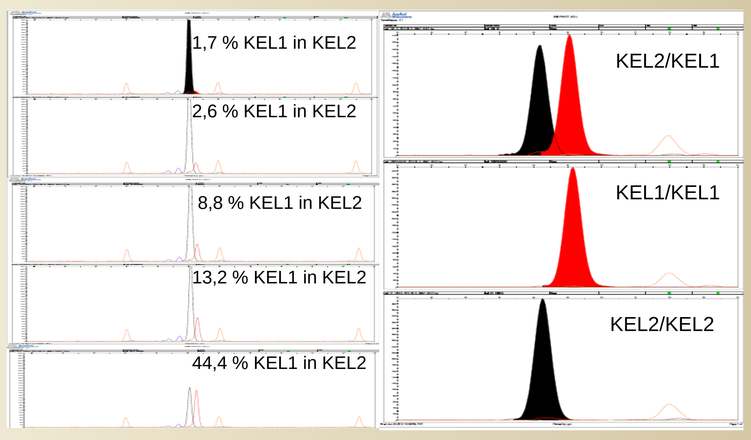
Graph 9



Picture 1



Picture 2



Picture 3