



Non-invasive fetal *RHD* genotyping: validation of the test and implementation of a screening program to guide anti-D prophylaxis

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In his commentary on “Non-invasive fetal RHD-genotyping”, Frederik Banch Clausen referred to our recently published article (1). We agree on the contents and we would like to provide some additional information on our study and on its implementation.

Fetal *RHD* genotyping on cell-free fetal DNA (cf-DNA) from RhD-negative women can be used to guide targeted antenatal prophylaxis for the prevention of RhD immunization: the knowledge of fetal RhD type in fact can direct and restrict the use of prenatal anti-D Ig exclusively to RhD negative women carrying an RhD positive fetus (60% of individuals of European descent). This approach optimizes the use of a plasma-derived medicinal product, which is becoming more and more a limited resource (2-4), also avoiding any unnecessary exposition to plasma-derived medicinal products.

Besides the mandatory validation steps, the implementation of any diagnostic test requires however to adapt the organizational approaches. In our study, in addition to standard validation parameters (analytical sensitivity, lower limit of detection, analytical specificity, assay precision and diagnostic accuracy) we investigated the performance of the diagnostic kit to optimize routine laboratory organization, by assessing the feasibility of automatic DNA extraction, to improve efficiency and standardization of the cf-DNA extraction phase, and the reliability of preserving extracted cf-DNA in frozen state before real-time quantitative PCR assays.

Validation results were very satisfying for all quality

parameters and added evidence to the reliability of a large-scale fetal *RHD* genotyping in clinical setting; in particular, it has been achieved an excellent concordance between the fetal *RHD* genotype predicted by the assay on different gestational week samples and the RhD phenotype assessed after birth by standard cord blood serology, ensuring an evidence-based use of antenatal anti-D Ig prophylaxis in pregnant women at risk of HDFN (*Table 1*).

These results were crucial for planning and implementing the first Italian region-wide screening service for fetal *RHD* genotyping at 22th–24th gestational weeks (gw), taking also as a model the North-European countries experience (5-8). We have demonstrated that it is feasible to obtain the predicted fetal RhD phenotype before the 28th gw (when ante-natal prophylaxis is usually administered in Italy) in all RhD-negative pregnant women previously typed before the 12th gw; therefore, the antenatal screening program has been effectively implemented, by collecting, storing and automatically extracting DNA from maternal plasma to analyze fetal *RHD* gene (exons 5, 7 and 10). In our Region, tests are provided free of charge by the Regional Health System from November 2019 and they have received excellent user satisfaction. The adoption of fetal *RHD* antenatal testing is confirmed to be highly reliable and the centralization of the test in a single laboratory guarantees the quality of the results, the concordance of reports and the sustainability of the costs, representing an excellent guide to targeted use of prophylaxis.

Table 1 Diagnostic accuracy in relation to gestational week

Trimester (gestational week)	N. samples	RHD genotype		RhD phenotype at birth		Diagnostic accuracy
		Pos	Neg	Pos	Neg	
I (11–13)	75	55	20	54	21	100%
II (14–26)	24	19	5	19	5	100%
III (27-term)	34	25	9	25	9	100%
Total	133	99	34	99	34	

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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