AN EIGHT-YEAR EXPERIENCE OF EXTERNAL QUALITY ASSESSMENT PROGRAM FOR FETAL *RHD* GENOTYPING

N. Da Silva[1], C. Toly-Ndour[1], S. Huguet-Jacquot[1], H. Delaby[1], J. Beaud[1], J. Babinet[1], E. Maenulein[1], I. Anglard[2], A. Vassault[2], M. Vaubourdolle[3] and A. Mailloux[1]

1. Service d'Hémobiologie fœtale et périnatale - Centre National de Référence en Hémobiologie Périnatale (CNRHP), AP-HP.SorbonneUniversité - Site Saint-Antoine – 184 rue du Faubourg St Antoine, 75012 Paris, France
2. ASQUALAB - Bâtiment Leriche – 8 rue Maria Helena Vieira Da Silva, 75014 Paris, FRANCE
3. DMU BioGeMH – AP-HP.SorbonneUniversité - Site Saint-Antoine – 184 rue du Faubourg St Antoine, 75012 Paris, France

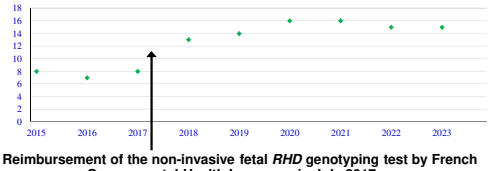
Context : Non-invasive fetal *RHD* genotyping helps the practitioners to improve the monitoring of D (RH1) negative pregnant women. In a context of anti-D (anti-RH1) alloimmunization, a positive *RHD* fetal genotyping allows the diagnosis of feto-maternal incompatibility. For non-immunized women, a negative test prevents prophylactic injections of anti-D immunoglobulin (RhIg) during pregnancy. Since fetal *RHD* genotyping became a key element to monitor D negative pregnant women, an increasing number of laboratories has implemented this test routinely. In 2010, it appeared essential for the CNRHP, as part of its missions, to propose an external quality assessment, based on external quality controls (EQC). The CNRHP can rely on more than twenty years' experience in fetal *RHD* genotyping from maternal blood to establish such controls. The laboratory is accredited according to EN ISO 15189 for this test since 2012. In 2015, the CNRHP transferred its EQC program to a certified EQA organism: ASQUALAB.

The aim of this presentation is to review the results of the EQC program eight years after its launch by ASQUALAB.

Method : 3 types of specimen were prepared : positive, negative and uninterpretable. Positive control specimen were prepared from D negative plasma donors with a *RHD* deleted allele spiked with various concentration of D positive plasma in order to reflect *RHD* positive fetuses at different gestational ages. Negative control specimen, made also from D negative plasma donors with a *RHD* deleted allele, remained unspiked. Uninterpretable control specimen were prepared from D positive plasma in order to mimic the presence of maternal *RHD* sequences or from D negative plasma donors with a *RHD* deleted allele spiked with D variant plasma. After the initial CNRHP analysis, the samples were conveyed to the participating laboratories with a feedback form where they had to state 1) the material and methods used and 2) the results and the clinical biological interpretation in the context of a clinical case. The control samples were sent twice a year.

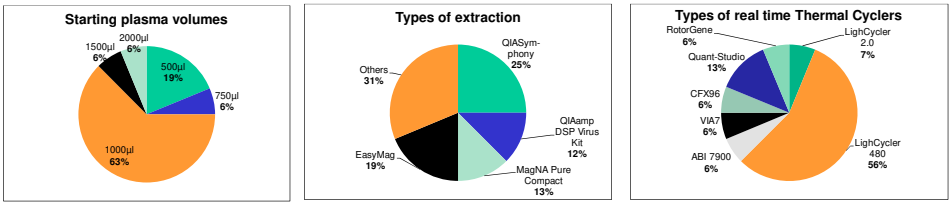
Results : 18 assessments were conducted since 2015 with an increasing number of laboratories participating from 7 to 15 in 2023 (figure 1). During this period 21 laboratories participated but never more than 16 laboratories at the same time (table 1 and 2). Each year, we achieved a 100 % response rate. EQC results were most of the time conform to those expected (table 1) with 89,8% of the EQC reaching A score (table 2) although the laboratories use different protocols (figure 2). All laboratories used real time PCR with TaqMan technology excepted one that used HRM technology. All laboratories targeted at least 2 *RHD* exons to conclude the fetal *RHD* genotype, using home made or CE IVD labelled reagents.

Figure1 : Number of laboratories performing non-invasive fetal *RHD* genotyping per year



Reimbursement of the non-invasive fetal *RHD* genotyping test by French Governmental Health Insurance in July 2017 →
Generalization of the use of this test for pregnant women

Figure 2 : Materials and Methods used by laboratories performing non-invasive fetal *RHD* genotyping



Year	2015				2016				2017				2018				2019				2020				2021				2022				2023			
CQE number	15P1	15P2	15P3	15P4	16P1	16P2	16P3	16P4	17P1	17P2	17P3	17P4	18P1	18P2	18P3	18P4	19P1	19P2	19P3	19P4	20P1	20P2	20P3	20P4	21P1	21P2	21P3	21P4	22P1	22P2	22P3	22P4	23P1	23P2	23P3	23P4
RHD fœtal variant	D-CE[4-7]-D	deletion	positive	positive	deletion	deletion	positive	positive		positive	deletion	positive	deletion	deletion	positive	deletion		positive	positive	positive	Dpsi	deletion		positive	deletion	positive	deletion	positive	positive	positive	Dpsi	deletion	positive	deletion	deletion	positive
RHD maternal variant	deletion	deletion	deletion	deletion	deletion	deletion	deletion	deletion	positive	deletion	deletion	deletion	deletion	deletion	deletion	deletion	positive	deletion	deletion	deletion	deletion	deletion	positive	deletion	deletion	deletion	deletion	deletion	Dpsi	deletion	deletion	deletion	deletion	deletion	deletion	deletion
Expected Result	Inint ou RHD -	RHD -	RHD +	RHD +	RHD -	RHD -	RHD +	RHD +	Inint.	RHD +	RHD -	RHD +	RHD -	RHD +	RHD +	RHD -	Inint.	RHD +	RHD +	RHD +	Inint. ou RHD -	RHD -	Inint.	RHD +	RHD -	RHD +	RHD -	RHD +	Inint. ou RHD +	RHD +	Inint. ou RHD -	RHD -	RHD +	RHD -	RHD -	RHD +
Laboratory 1	A		A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Laboratory 2	A	B							A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	A	B	A	A	A	A	A	A
Laboratory 3																					A	A	D	A												
Laboratory 4															A	B	A	A	A	A	A	A	D	A												
Laboratory 5													A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Laboratory 6																			A	A	A	A	A	A	A	A	A	A	A	A	D	A	A	A	B	A
Laboratory 7																					A	B	D	A	A	A	B	B	A	B	A	A	B	A*	B	A
Laboratory 8	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Laboratory 9	A	A	B	A	A	A	A	A	A	A	A	A	B	A	A	A	D	B	A	A	D	A	A	B	A	A	A	A								
Laboratory 10	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A
Laboratory 11	B	A																																		
Laboratory 12													A	A	A	A	A	A	A	A	A	A	A	A												
Laboratory 13																									A	A	A	A	A	A	A	A	A	B	B	A*
Laboratory 14													B	A																						
Laboratory 15																	A	A	A	A	A	A	A	A	A	A	A	D	A	A	A	A	A	A*	A	A
Laboratory 16																													A	A	A	A	A	A	A	A
Laboratory 17																													A	A	D	D	A	A*	A	A
Laboratory 18	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A
Laboratory 19															A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A*	A	A
Laboratory 20	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	A	B	A	A	A	D	A	A	A	A	A
Laboratory 21							A	A	C	A	A	A	B	A	A	A	D	A	A	A	D	A	D	B	A	A	B	A	D	A	D	A	A	A	A	A
Score A	62,5%	87,5%	66,7%	100%	100%	100%	100%	100%	87,5%	100%	100%	100%	72,7%	100%	100%	91,7%	84,6%	92,3%	100%	100%	87,5%	93,8%	75,0%	81,3%	92,9%	100%	78,6%	71,4%	86,7%	86,7%	73,3%	93,3%	93,3%	93,3%	80,0%	100,0%
Score B	37,5%	12,5%	33,3%	0%	0%	0%	0%	0%	0%	0%	0%	0%	27,3%	0%	0%	8,3%	0%	7,7%	0%	0%	0%	6,3%	0%	18,8%	0%	0%	21,4%	14,3%	6,7%	13,3%	0%	0%	6,7%	6,7%	20%	0%
Score C	0%	0%	0%	0%	0%	0%	0%	0%	12,5%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	7,1%	0%	0%	0%	0%	0%	0%	0%	0%
Score D	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	15,4%	0%	0%	0%	12,5%	0%	25,0%	0%	7,1%	0%	0%	7,1%	6,7%	0%	26,7%	6,7%	0,0%	0%	0,0%	0,0%

Table 1 : Results evaluation : The A, B, C or D score is assigned according to:
- the expected qualitative response of the test
- the difference between the laboratory result and the average of all the participating laboratories
- The clinical-biological advices given with the results
  - **A : Correct answer, difference between 0-10%, appropriate clinical -biological advice**
  - **B : Correct answer, difference between 0-10%, unsuitable clinical -biological advice**
  - **C : Correct answer, difference > 10%, adapted clinical -biological advice**
  - **D : Wrong answer**

Table 2 : Global results

A Score	89,8%
B Score	6,7%
C Score	0,5%
D Score	3%

6.7% of laboratories made erroneous clinical interpretations despite right analytical results (B score).

3% of the results correspond to wrong answers (D score). More than 81% of wrong answers are observed for uninterpretable *RHD* genotyping specimen due to the presence of maternal or fetal variants. Two laboratories give false positive results in this case because the techniques used do not detect *RHD* variants. This problem is not limited to EQAs and these laboratories also return false positive results for patients.

Conclusion : The presented EQC meets the criteria required to evaluate the practices of laboratories performing non-invasive fetal *RHD* genotyping. The extension of the field from analytical to post-analytical process, including results interpretation and biological advices for physicians, was important to improve national harmonization of the results of this specialized test, and to highlight the importance of giving clinical advices to help prevention of fetal and/or neonatal anemia.