

Analysis of ten years results of an External Quality Assessment program for RHD fetal genotyping

N. Da Silva¹, C. Toly-Ndour¹, S. Huguet-Jacquot¹, J. Beaud¹, E. Maënulein¹, I. Anglard², A. Vassault², M. Vaubourdolle^{2,3}, A. Mailloux¹

1. Service d'Hémostase fœtale et périnatale, Centre National de Référence en Hémostase Périnatale CNRHP, Hôpital Saint-Antoine, 72012 Paris
2. ASQUALAB - Bâtiment Leriche – 8 rue Maria Helena Vieira Da Silva - 75014-PARIS
3. DMU BioGeMH – AP-HP Sorbonne Université - Site Saint-Antoine – 184 rue du Faubourg Saint-Antoine – 75012 - PARIS

BACKGROUND

Following the implementation of RHD fetal genotyping as a pivotal component in the monitoring of D- (RH:-1) pregnant women, there has been a notable surge in the number of laboratories conducting the test. In 2010, the French National Reference Center in Perinatal Hemobiology (CNRHP) determined that it was imperative, as part of its national mandate, to propose an external quality assessment (EQA) to laboratories. This proposal was based on an external quality control (EQC). In 2015, the CNRHP EQC program was transferred to a certified EQA organism. ASQUALAB. The samples provided were meticulously prepared to emulate the amplification of minority RH1-positive fetal DNA in a predominant RH1-negative maternal DNA environment. This was achieved by combining unfiltered plasmas (leukocyte removal filtration) from blood donors with varying concentrations of RH1-negative and RH1-positive components. The objective of this presentation is to provide a comprehensive review of the EQC program a decade following its inception.

METHODS

Three types of control specimens were prepared. The positive EQC was prepared from D- (RH:-1) plasma donors and was spiked with various concentrations of D+ (RH:1) plasma. The purpose of this preparation was to reflect RHD positive fetuses at different gestational ages. The negative EQC is fabricated exclusively from D- (RH:-1) plasma donors. The production of indeterminate EQC was achieved through the use of D- (RH:-1) plasma donors, which were spiked with RHD variant plasma. The samples were subsequently transferred to the participating laboratories, accompanied by a feedback form. This form solicited information regarding the material and methods employed, as well as the results and the clinical biological interpretation in the context of a clinical case.

RESULTS

23 assessments were conducted since 2015, with a notable increase in the number of participating laboratories from 7 to 17 between 2023 and 2025. Annually, a 100% response rate was achieved. The evaluation of results is conducted by assigning grades A, B, C, or D based on the following criteria: the expected qualitative response of the test, the discrepancy between the laboratory's result and the mean of all participating laboratories, the clinical and biological recommendations associated with the results. A score of D corresponds to an incorrect result. The results of the EQC analysis were found to be 89.8% consistent with the expected outcomes, despite the utilization of disparate extraction and amplification protocols by the respective laboratories.

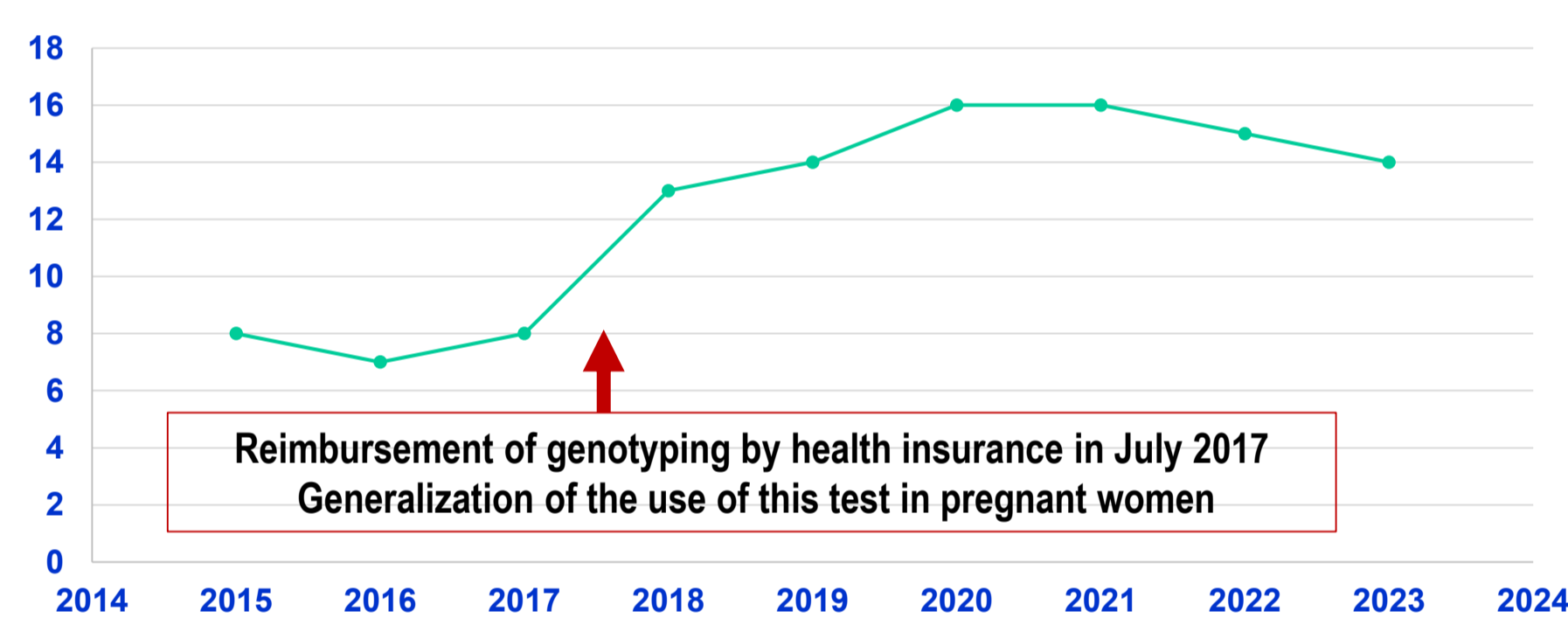


Figure 1 : Number of laboratories

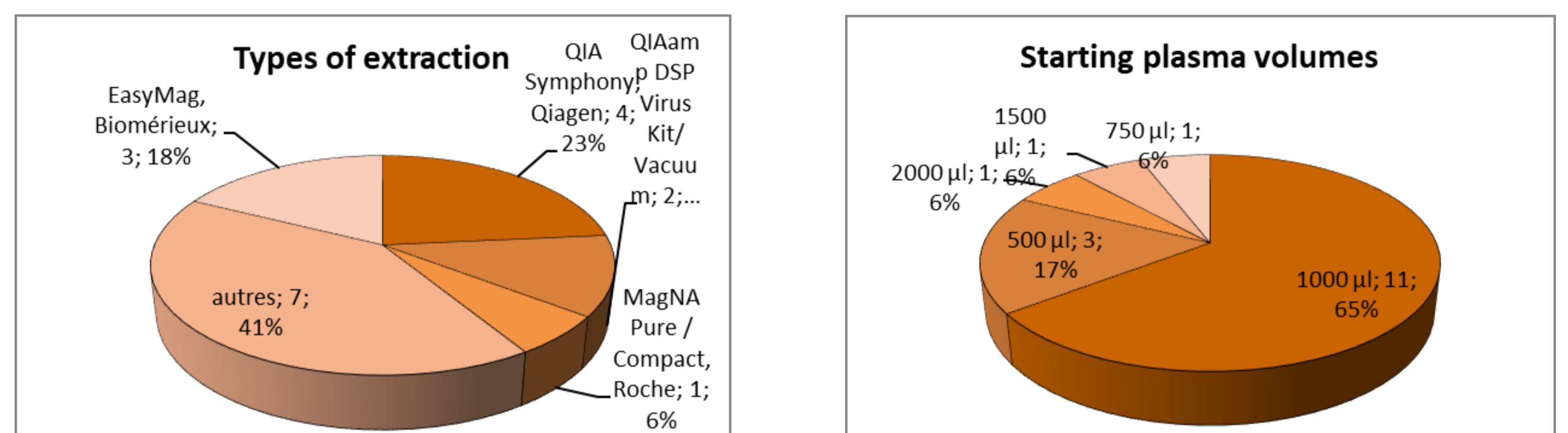


Figure 2 : Different types of extraction used and volumes of plasma extracted

Lab Code	2015				2016				2017				2018				2019				2020				2021				2022				2023				2024				2025							
	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	24P1	24P2	24P3	24P4	25P1	25P2	25P3	25P4				
01	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	A	A	A	A	A	A	A	A	A	A	A	A	A
02	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	A	A	A	A	A	A	A	A	A	A	A	A	A
03	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	A	A	A	A	A	A	A	A	A	A	A	A	A
04a	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	A	A	A	A	A	A	A	A	A	A	A	A	A
04b	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	A	A	A	A	A	A	A	A	A	A	A	A	A
05	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	A	A	A	A	A	A	A	A	A	A	A	A	A
06	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	A	A	A	A	A	A	A	A	A	A	A	A	A
07a	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	A	A	A	A	A	A	A	A	A	A	A	A	A
07b	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	A	A	A	A	A	A	A	A	A	A	A	A	A
08	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	A	A	A	A	A	A	A	A	A	A	A	A	A
09	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	A	A	A	A	A	A	A	A	A	A	A	A	A
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	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
11	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	A	A	A	A	A	A	A	A	A	A	A	A	A
12	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	A	A	A	A	A	A	A	A	A	A	A	A	A
13	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	A	A	A	A	A	A	A	A	A	A	A	A	A
14	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	A	A	A	A	A	A	A	A	A	A	A	A	A
15	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	A	A	A	A	A	A	A	A	A	A	A	A	A
16	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	A	A	A	A	A	A	A	A	A	A	A	A	A
17	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	A	A	A	A	A	A	A	A	A	A	A	A	A
18	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	A	A	A	A	A	A	A	A	A	A	A	A	A
19	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	A	A	A	A	A	A	A	A	A	A	A	A	A
20	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	A	A	A	A	A	A	A	A	A	A	A	A	A
21	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	A	A	A	A	A	A	A	A	A	A	A	A	A
22	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	A	A	A	A	A	A	A	A	A	A	A	A	A
23	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	A	A	A	A	A	A	A	A	A	A	A	A	A
Total attendees	8	8	6	6	6	6	7	7	8	8	8	8	11	11	12	12	13	13	14	14	15	15	15	15	13	13	13	13	14	14	14	14	14	14	14	14	15	15	15	15	15	15	15	15	15	15	14	14
Total registered % participation	100%	100%	75%	75%	86%	86%	100%	100%	100%	100%	100%	100%	85%	85%	92%	92%	93%	93%	100%	100%	94%	94%	94%	94%	81%	81%	81%	81%	93%	93%	93%	93%	93%	93%	93%	93%	100%	100%	100%	100%	100%	100%	100%	100%	93%	93%	93%	93%
A notes	5	7	4	6	6	6	7	7	7	8	8	8	8	11	12	11	11	12	14	14	14	15	12	13	13	14	11	10	13	13	11	14	14	14	12	15	12	17	13	11	17	14	11	13				
B notes	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C notes	3	1	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D notes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table I : Evaluation of results

The letters A, B, C or D are assigned according to:

- the expected qualitative response of the test
- the difference between the laboratory result and the average of all the participating laboratories clinical -biological advice

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

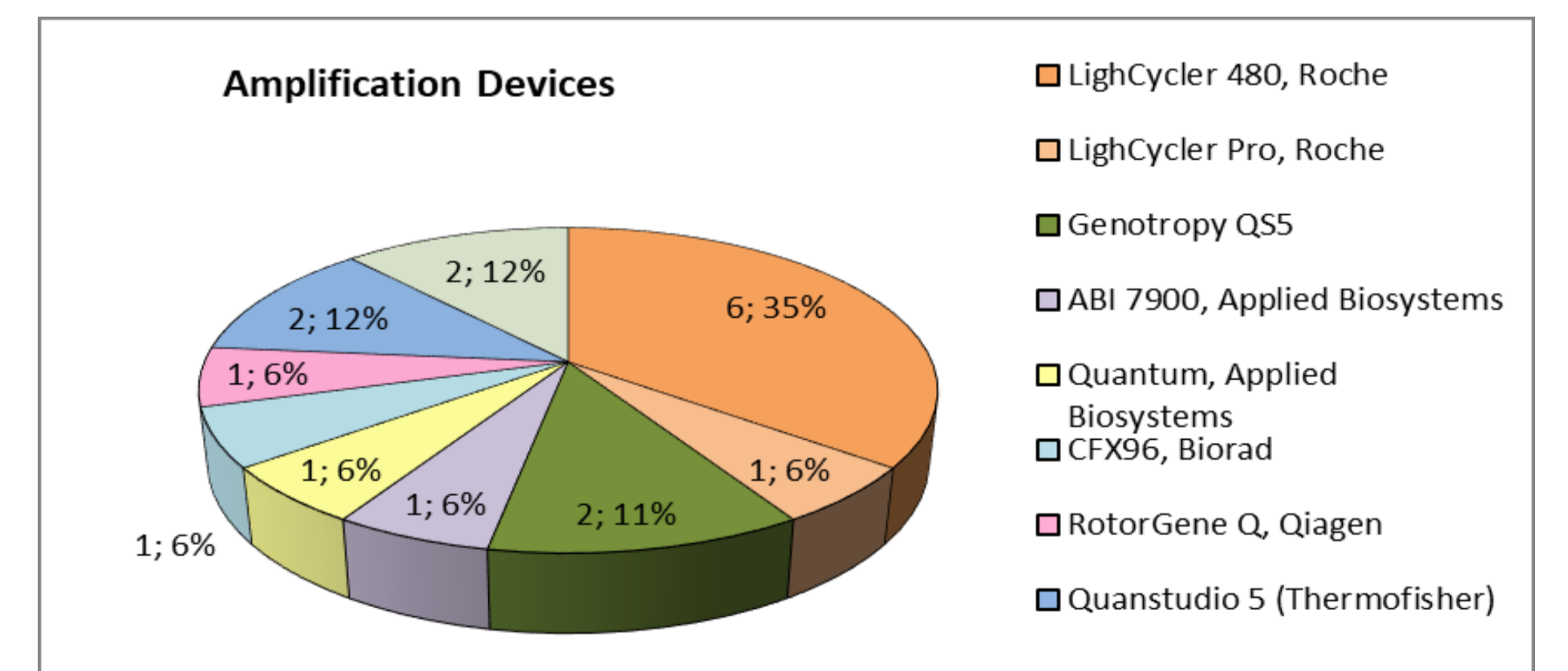


Figure 3: Amplification devices used

A notes	87%
B notes	7%
C notes	1%
D notes	5%

DISCUSSION

Despite the presence of correct analytical results, 5% of laboratories made erroneous clinical interpretations. The D score is observed for uninterpretable RHD genotyping due to the presence of maternal or fetal variants. In this particular instance, the false positive results from two laboratories can be attributed to the fact that the utilized techniques are not capable of detecting RHD variants. This phenomenon is not exclusive to EQAs; these laboratories also generate false positive results for patients.

CONCLUSION

The EQC that has been presented meets the criteria necessary to evaluate the practices of laboratories performing noninvasive fetal RHD genotyping. The inclusion of results interpretation and biological advice for physicians in this evaluation was imperative to enhance national harmonization of the results. This enhancement was undertaken to assist in the prevention of fetal or newborn anemia.