Performance of a new non-invasive fetal RHD genotyping technology on a French population of pregnant women

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No disclosure

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INTRODUCTION

- Feto-maternal blood group incompatibility is common and may result in hemolytic disease of the fetus and newborn (HDFN) characterized by anemia and hyperbilirubinemia which may lead to fetal hydrops, kernicterus or death
- The maternal anti-D alloimmunization is the most common cause of feto-maternal red blood cells incompatibility resulting in HDFN
- Non-invasive fetal *RHD* genotyping help the practitioners to greatly improve the accuracy follow-up in RH: -1 pregnant women

Non-invasive Fetal *RHD* genotyping

Indications in France - CNGOF (French obstetricians college) 2017 recommendations

Anti-D allo-immunized pregnant women

Risk of fetal anemia in case of *RHD* positive fetus

Results of the test

***RHD*+ FETUS**

Feto-maternal incompatibility

→ monitoring : regular anti-D quantification +/- weekly fetal ultrasound and MCA-PSV measurements

***RHD*- FETUS**

(confirmed on 2 separate samples)

No feto-maternal incompatibility

→ No specific monitoring

INDETERMINATE (discordant exons amplification) **/ UNINTERPRETABLE** (early (=mother) exons amplication) ***RHD* FETUS**

Potential feto-maternal incompatibility

→ same monitoring as for RHD+ fetus

Non-alloimmunized D- pregnant women

Risk of anti-D allo-immunization in case of *RHD* positive fetus

Results of the test

***RHD*+ FETUS**

RhIg Injection

- systematic prophylaxis (28GW)
- targeted prophylaxis
- post partum prophylaxis

***RHD*- FETUS**

(confirmed on 2 separate samples)

No RhIg injection

- no antenatal prophylaxis (systematic / targeted)
- Newborn D phenotype to perform at birth to guide post-partum prophylaxis

INDETERMINATE (discordant exons amplification) **/ UNINTERPRETABLE** (early (=mother) exons amplication) ***RHD* FETUS**

RhIg injection

- systematic and targeted prophylaxis
- Newborn D phenotype to perform at birth to guide post-partum prophylaxis

Principle of non-invasive fetal erythrocyte genotyping = the determination of fetal sequences in maternal plasma

DNA is extracted from maternal plasma/serum (which contains both maternal and fetal circulating DNA)

↓

The identification of *RHD* sequences that are present in the fetus and absent in the mother

Presence of sequences
POSITIVE FETUS

↓

Risk of alloimmunization
Risk of fetomaternal incompatibility

Absence of sequences
NEGATIVE FETUS
(default diagnosis)

↓

No risk of alloimmunization
Fetal-maternal compatibility

Non-invasive Fetal *RHD* genotyping at the CNRHP

- At the CNRHP, the identification of *RHD* positive fetuses is carried out first by real-time PCR on *RHD* exons 10, 7 and 5 using **Free DNA fetal® kit *RHD***
- Approximately **7%** of the patients tested have a *RHD* variants interfering with the test or carry a fetus *RHD* variant.
  - The CNRHP has developed amplifications of ***RHD* exons 4, 6 and 5Ψ** to genotype fetuses from mother having ***RHD**Ψ* or *RHD-CE (4-7)-D* silent allele** or fetuses having themselves the same alleles.

AIM

Since 2020, many commercial kits for fetal RHD genotyping have become available, using different technologies to amplify one, two, or three RHD exons

- The aim of this study was to evaluate the FetoGnost® RHD Kit to determine whether it can be used in clinical practice in the French population
- The kit must have a negative predictive value of 100% and should detect RHD variants in both the fetus and the mother, in order to minimize false-positive fetal RHD genotyping results

METHODS

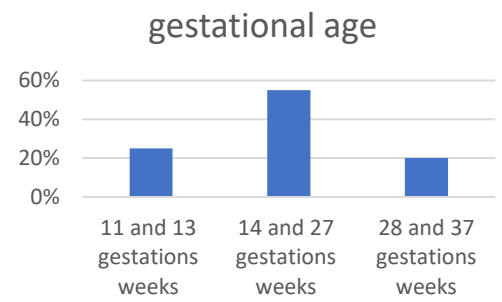
FetoGnost® Kit RHD Ingenetix

The test is based on multiplex real-time PCR by TaqMan technology done in triplicate

Amplification of ***RHD*** **exon 5, exon 7, exon 10**

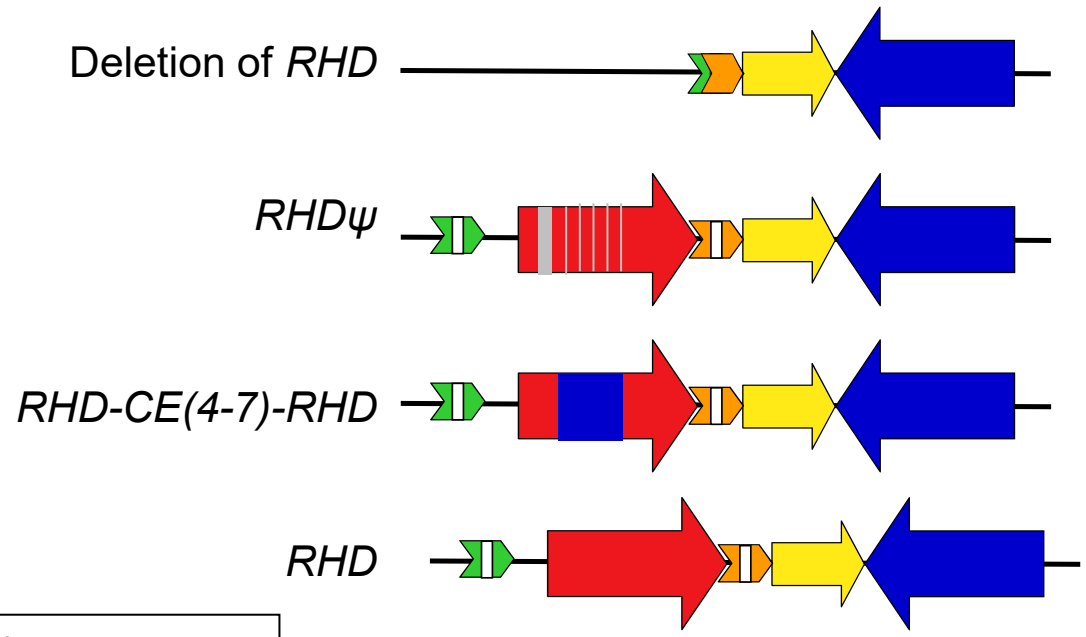
Results obtained with **the FetoGnost® Kit RHD,** with additional amplification of RHD exons 4, 6 and 5Ψ if necessary, were compared with those previously found by the CNRHP or with new born phenotype or predicted genotype.

METHODS



- The study included 105 plasma samples of pregnant women and 10 external quality assessment samples
- 74 % of patients non alloimmunized / 26 % patients anti-D alloimmunized

- Maternal *RHD* variant



Frequency	Study population
>99% of RH-1 caucasians 17% of RH-1 sub-Saharan Africa population	66%
66% of RH-1 sub-Saharan Africa population	16%
17% of RH-1 sub-Saharan Africa population	9%

9 % of patients possessed *RHD* gene sequences in their genome that could interfere with the technique

- Fetal *RHD* variant

- 13 % of patient carried a fetus with a probable silent or partial fetal variant

RESULTS
ACCURACY OF THE TEST

Sample tested

10 external quality assessment samples for non-invasive *RHD fetal genotyping* provided by Asqualab from the campaigns of 2022 to 2025 were tested

Results

100% agreement was observed with the results obtained

	RhD- negative fetus	RhD Positive Fetus	Total
RHD test negative	5	0	5
Positive RHD test	0	5	5
RHD Test Undetermined	0	0	0
Total	5	5	10

Conclusion : The method is accurate.

RESULTS - PERFORMANCE OF THE TEST

Using CNRHP strategy

Performance was evaluated in 33 patients between 12 and 37 weeks of gestation, for whom neonatal phenotype or fetal genotype was known (from amniotic fluid or paternal genotyping).

	RhD- negative fetus	RhD Positive Fetus	Total
RHD test negative	15	0	15
Positive RHD test	0	14	14
RHD Test indeterminate	1	3	4
Total	16	17	33

Genotype/expected phenotype concordance table *RHD* genotype with the FetoGnost RHD kit (Ingenetix)

- Sensitivity = **93.75%**
- Specificity = **100%**
- NPV = **100%**
- PPV = **100%**

The analysis of discrepancies showed that 4 samples were classified as indeterminate due to complementary amplification: (1 contamination of 1 exon and 3 by discordance of exons)

The technique using the **FetoGnost RHD kit** gave no false negatives and complies with the requirements. The use of the results of **complementary PCRs targeting *RHD* exons 4, 6 and 5 Ψ** allowed **10 fetuses to be classified as negative instead of indeterminate** (1 RHD-CE(4-7)-D fetus and 9 *RHD*Ψ fetuses*).

RESULTS BY COMPARISON (1)
FetoGnost® Kit RHD / Free DNA fetal® kit *RHD*

- The study showed a concordance of 97.4 % between results obtained with the FetoGnost® Kit RHD and those obtained with Free DNA fetal® kit RHD.

FreeDNA fetal Kit / FetoGnost Kit	RHD Negative	RHD Positive	RHD undetermined	Total
RHD Negative	50	0	0	50
RHD Positive	0	48	0	48
RHD undetermined	0	**3**	14	17
Total	50	51	14	115

3 fetuses were found to be indeterminate due to the absence of exon 5 *RHD amplification* with the FetoGnost RHD kit (Ingenetix), whereas the Free DNA Fetal Kit RhD-Duplex (IBJB) reported these three fetuses as positive. **These three fetuses are likely partial.**

RESULTS BY COMPARISON (2)

FetoGnost® Kit RHD / Free DNA fetal® kit *RHD*

Contribution of additional amplification of RHD exons 4, 6 and 5Ψ

- All fetuses and all mothers having *RHD*Ψ* or *RHD-CE (4-7)-D* silent allele were revealed using the **FetoGnost® Kit RHD** and the use of additional amplification of RHD exons 4, 6 and 5Ψ allowed to determine fetal *RHD* genotype.
  - As a result, **16 fetuses could be classified as negative instead of indeterminate** (4 RHD-CE(4-7)-D fetuses and 12 *RHD*Ψ fetuses*).
- **FetoGnost RHD kit** identified *RHD variant* that had not been identified by the CNRHP technique. The kit brought to light mother carrying *RHD variant* of type 4.0 or 4.3, so identification of this variant can also be expected in the fetus
- All mothers with maternal RHD sequences interfering with the method were detected allowing new born phenotype determination at birth.

Conclusion

- Non-invasive RHD genotype using FetoGnost®Kit RHD demonstrated a negative predictive value of 100%.
- It detects RHD variants in both the fetus and the mother significantly reducing the risk of false-positive results for the fetus and ensuring appropriate clinical-biological advice for both non-alloimmunized and alloimmunized anti-RH1 women.
- The combination of the kit with additional amplifications of RHD exons 4, 6 and 5Ψ developed at the CNRHP allows the identification of 1) RHD negative fetuses carried by mothers having RHD*Ψ or RHD-CE (4-7)-D silent allele and 2) RHD negative fetuses having these same alleles.

Thank you for your attention

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