Implementation of a non-invasive fetal *RHE* genotyping test for the monitoring of anti-RH3 immunised pregnant women

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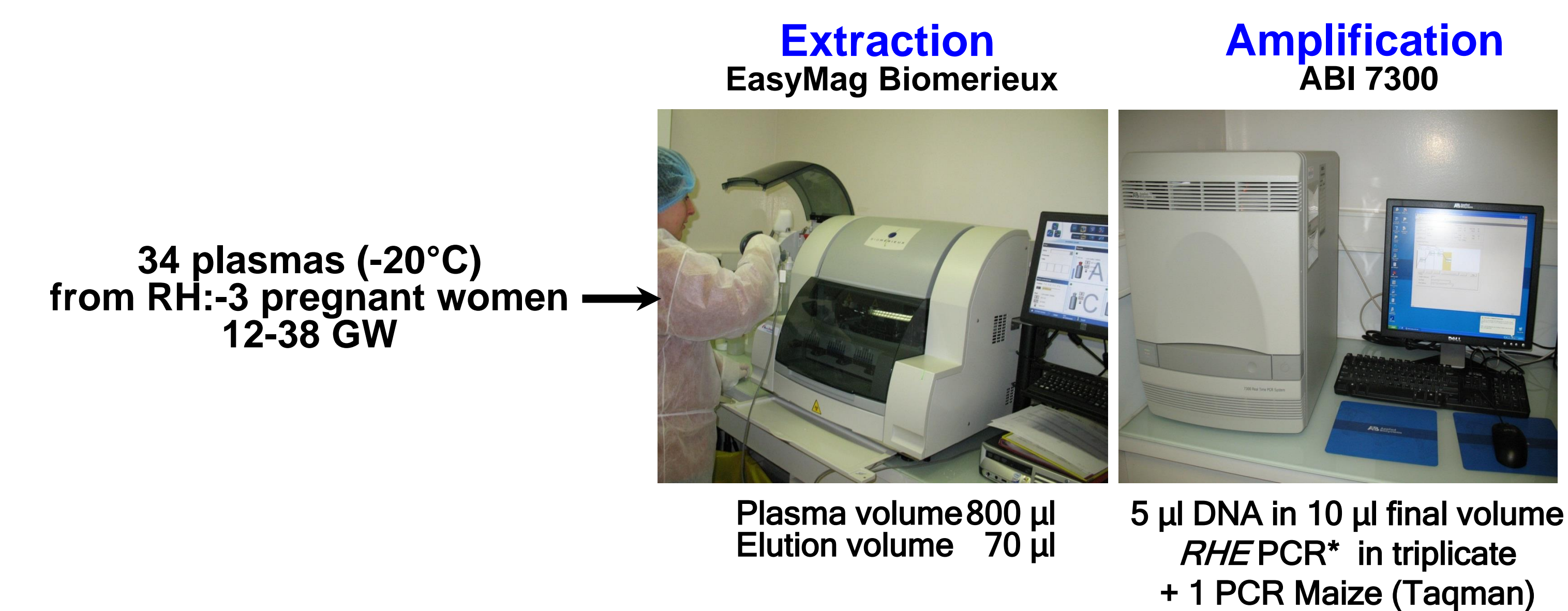
Background

Materno-fetal blood group incompatibilities are responsible for the haemolytic disease of the fetus and new-born. This disease is caused by maternal antibodies crossing the placental barrier and targeting the fetus red blood cells, which leads to haemolytic anaemia and/or hyperbilirubinemia and, in severe forms, entails fetal hydrops, neurological damage and even death. Concerning anti-RH3 antibodies, the complications of the haemolytic disease appear essentially during the 3rd trimester of pregnancy and after the birth. To prevent those complications, pregnancies of RH3-immunized women are highly monitored, clinically and biologically, towards the end of their pregnancy and after delivery.

Fetal genotyping from amniotic liquid requires amniocentesis which is at risk of reactivation of the immunisation and is therefore not indicated in first intention. That's why we aimed at developing a non-invasive fetal genotyping test for the RH3 gene.
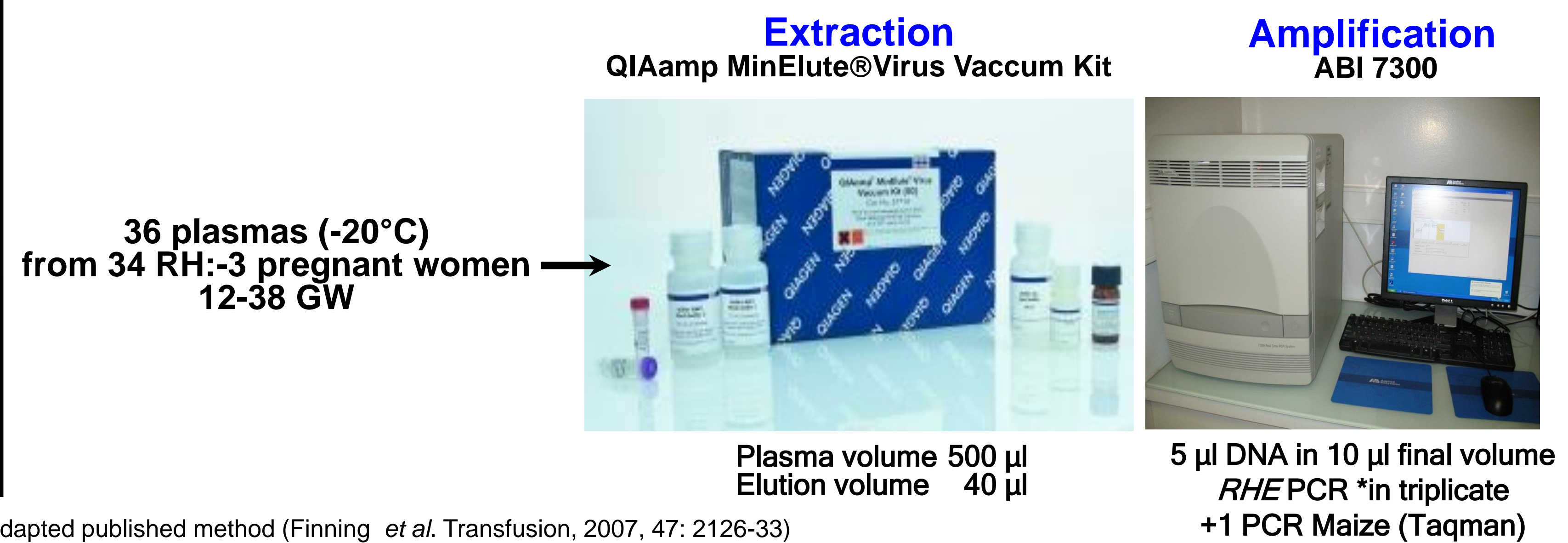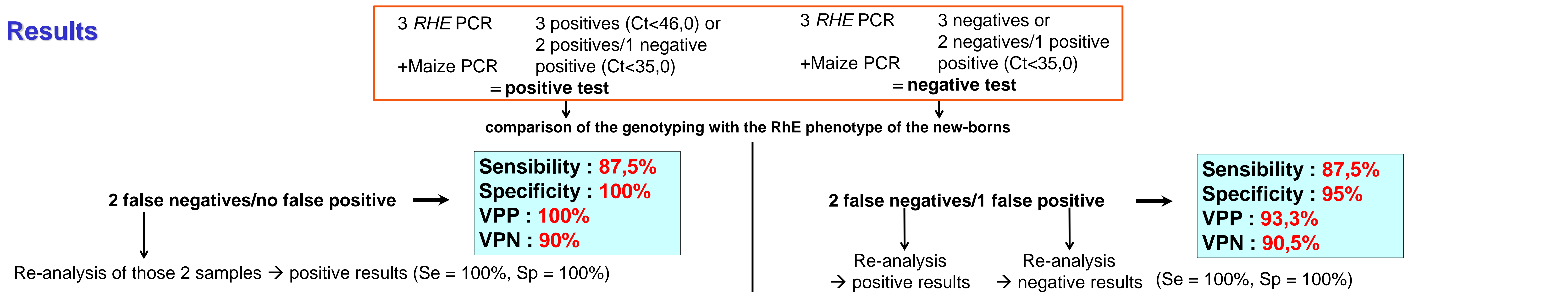

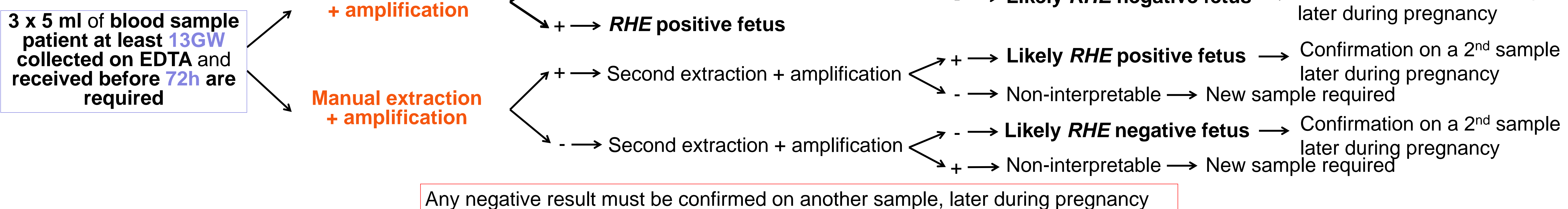### Non invasive fetal *RHE* genotyping

Methods

Automatic DNA extraction

Extraction — EasyMag Biomerieux
Amplification — ABI 7300

34 plasmas (-20°C) from RH:-3 pregnant women 12-38 GW

Plasma volume 800 µl
Elution volume 70 µl

5 µl DNA in 10 µl final volume
RHE PCR* in triplicate
+ 1 PCR Maize (Taqman)

Manual DNA extraction

Extraction — QIAamp MinElute®Virus Vaccum Kit
Amplification — ABI 7300

36 plasmas (-20°C) from 34 RH:-3 pregnant women 12-38 GW

Plasma volume 500 µl
Elution volume 40 µl

5 µl DNA in 10 µl final volume
RHE PCR *in triplicate
+1 PCR Maize (Taqman)

* adapted published method (Finning *et al.* Transfusion, 2007, 47: 2126-33)

Results

| 3 *RHE* PCR +Maize PCR | 3 positives (Ct<46,0) or 2 positives/1 negative positive (Ct<35,0) = **positive test** | 3 *RHE* PCR +Maize PCR | 3 negatives or 2 negatives/1 positive positive (Ct<35,0) = **negative test** |

comparison of the genotyping with the RhE phenotype of the new-borns

2 false negatives/no false positive →
Sensibility : 87,5%
Specificity : 100%
VPP : 100%
VPN : 90%

Re-analysis of those 2 samples → positive results (Se = 100%, Sp = 100%)

2 false negatives/1 false positive →
Sensibility : 87,5%
Specificity : 95%
VPP : 93,3%
VPN : 90,5%

Re-analysis → positive results   Re-analysis → negative results   (Se = 100%, Sp = 100%)

Interpretation

3 x 5 ml of **blood sample patient at least 13GW collected on EDTA and received before 72h are required**

Automatic extraction + amplification
- → Second extraction + amplification
  - + → Non-interpretable → New sample required
  - - → **Likely *RHE* negative fetus** → Confirmation on a 2nd sample later during pregnancy
+ → *RHE* positive fetus

Manual extraction + amplification
+ → Second extraction + amplification
  - + → **Likely *RHE* positive fetus** → Confirmation on a 2nd sample later during pregnancy
  - - → Non-interpretable → New sample required
- → Second extraction + amplification
  - - → **Likely *RHE* negative fetus** → Confirmation on a 2nd sample later during pregnancy
  - + → Non-interpretable → New sample required

Any negative result must be confirmed on another sample, later during pregnancy

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Monitoring of allo-immunized anti-RH3 women in France

positive antibody screen at first trimester of pregnancy

Antibody Identification : anti-RH3

Non invasive fetal *RHE* genotyping from maternal blood at first trimester of pregnancy → **Antibody Titration and/or Dosage**

If fetus negative → **No specific antenatal monitoring**

If fetus positive

Anti-RH3 < 700 UCHP (titre < 8)

Anti-RH3 ≥ 700 UCHP or ≥ 1/8

Specific antenatal monitoring
- Normal ultrasonography
- Antibody titration and/or dosage every 4 weeks from 18 GW (or only at the 7th and the 8th month of pregnancy if anti-RH3 < 40 UCHP or < 1)

Specific antenatal monitoring
- **Weekly ultrasonography and middle cerebral artery Doppler velocimetry**
- **Antibody titration and/or dosage every 2 weeks from 18 GW**

Specific postnatal monitoring of the newborn (blood counts, direct Coombs test, bilirubin dosages…)

Conclusion

Non invasive fetal *RHE* genotyping will be prescribed to RH3-alloimmunized pregnant women who would have required clinical and biological supervision.

The test will allow to diagnose the real RH3-incompatible pregnancies in order to either legitimize or avoid this heavy and costly monitoring.