Information sheet RhSafe®

What is RhSafe®

RhSafe® is a non-invasive prenatal test that determines the **fetal Rh(D) status** through the analysis of **circulating fetal DNA** (cell-free DNA, cfDNA) in maternal blood. The test is intended for pregnancies in which the **pregnant woman is Rh(D) negative** and the **partner is Rh(D) positive**, and it is useful in the management of the risk of maternal–fetal Rh incompatibility.

The Rh(D) factor is a protein located on the surface of red blood cells. If an Rh(D)-negative woman is carrying an Rh(D)-positive fetus, she may develop antibodies against fetal red blood cells. This phenomenon, known as **Rh alloimmunization**, may lead to **hemolytic disease of the fetus and newborn (HDFN)**. Determining fetal Rh(D) status during pregnancy allows for more appropriate clinical monitoring and targeted administration of anti-D immunoglobulin prophylaxis.

In recent decades, the use of anti-D immunoglobulin has significantly reduced this risk; however, not all Rh(D)-negative women require it, as approximately 40% carry an Rh(D)-negative fetus.

How is RhD status routinely assessed?

After birth, RhD status is usually determined through serological testing on blood samples. This method is rapid and effective, but in some cases it may fail to detect specific variants. Molecular techniques, on the other hand, allow direct analysis of the RHD gene and can be applied during pregnancy to assess fetal RhD status through a simple maternal blood sample.

Who is RhSafe® intended for and when to perform it

RhSafe® can be performed from the **10th week of pregnancy** onwards through a simple maternal blood draw. It can be performed in:

- singleton pregnancies;
- monochorionic twin pregnancies;
- pregnancies achieved through assisted reproduction, including donor conception.

To perform the test, it is necessary that:

- the pregnant woman is **documented as Rh(D) negative**;
- the biological father or donor is **documented as Rh(D) positive**.

Possible results of RhSafe®

RhSafe® may provide one of the following results:

- **RHD detected** → high probability that the fetus is Rh(D) positive;

- **RHD not detected** → high probability that the fetus is Rh(D) negative;
- **Inconclusive** → the result cannot be determined with sufficient reliability, for example due to low fetal DNA fraction or specific technical or biological conditions.

Methodology and performance of RhSafe®

RhSafe® is performed on circulating cfDNA extracted from maternal blood and analyzed using molecular biology techniques (Real-Time PCR).

A recent international review (Clausen & van der Schoot, 2025) has confirmed the high reliability of this approach: in routine screening programs involving over 70,000 pregnancies across several European countries, overall sensitivity exceeded 99.9% and specificity was close to 99%. In some cases, fetal genotyping demonstrated greater accuracy than neonatal serology, identifying discrepancies not detected postnatally.

cfDNA analysis is now considered a clinical standard in transfusion medicine and obstetrics, both in immunized pregnancies and in screening of non-immunized RhD-negative women, to guide targeted anti-D prophylaxis and avoid unnecessary administration.

Main limitations of RhSafe®

RhSafe® is a **screening test** and **does not replace postnatal testing** performed on neonatal blood samples. Test reliability also depends on the amount of fetal DNA present in the sample, which may be reduced in early gestational stages or under specific conditions.

In rare cases, the presence of RHD gene variants or particular biological conditions may complicate interpretation and lead to **false-positive, false-negative, or inconclusive results.**

The test does not distinguish whether the fetus carries **one or two copies of the RHD gene** and does not assess other red blood cell incompatibilities beyond the Rh(D) system.

The test exclusively evaluates RhD status: other blood group incompatibilities (e.g., Kell, Duffy, Kidd) are not assessed.

Turnaround time

Results are generally available within approximately **10 working days**. Turnaround time may be extended in cases requiring repeat testing, suboptimal results, or additional interpretative analyses.

Informed consent

To proceed with RhSafe® testing, the patient (or the legal guardian, in the case of a minor) must sign the appropriate informed consent form provided together with this information leaflet.