



PRE-TEST INFORMATION

Affinitymap description and purpose of the test

Affinitymap is a test for KIR and HLA-C genotyping to assess the compatibility between KIR receptors on the surface of maternal NK (Natural Killer) lymphocytes within the uterine cavity, and HLA-C immuno-histocompatibility system of the foetus.

Each individual inherits half of their genetic asset from the father and half from the mother; therefore each embryo has a share of genetic material of paternal origin that is recognized as *non-self* from the maternal uterine environment.

During pregnancy the maternal immune system develops a "tolerance" toward the embryo first and then the foetus, despite this being recognized as partially *non-self*. The pregnancy success is therefore due to the ability of the maternal immune system, through KIR receptors, to tolerate the embryo and not attack it as an external element.

In case of optimal compatibility, the maternal-foetal tolerance process will develop properly leading to a pregnancy; conversely, an incompatibility between maternal KIR/NK genes and foetal HLA-C will result in maternal NK population activation, resulting in an immune response that could compromise the success of pregnancy or lead to the onset of preterm birth.

Immuno-histocompatibility complex HLA –C

HLAs, or human leukocyte antigens, are proteins encoded by over 200 genes located on chromosome 6 that are part of the major histocompatibility complex (MHC). The histocompatibility complex has the function of presenting on its surface, to the cells of the immune system (T lymphocytes), the antigens of infectious agents or considered as *non-self*. The presentation of the antigen by the foetal HLA-C to the maternal immune system (T-NK lymphocytes) allows the identification of the same as *self* or *non-self*, inducing a specific immune response.

Two main groups of HLA molecules can be distinguished depending on their tissue distribution and function: class I molecules, consisting of HLA-A, HLA-B and HLA-C antigens, and class II molecules, which include HLA-DR, HLA-DQ and HLA-DP antigens.

The majority of HLA-C alleles can be divided into two main groups (HLA-C 1 and 2) which differs for the 80th amino acid (aspartic or lysine) of the HLA-molecule C. It is through this region that HLA-C binding with maternal KIR receptors occurs. The specific NK immune response is strongly influenced by the foetal genotype C1 and C2.

Table 1: HLAC Alleles C 1 and 2

HLA-C Group	HLA-C Alleles
C1	HLA-C*01, HLA-C*03, HLA-C*07, HLA-C*08, HLA-C*12, HLA-C*14 and HLA-C*16
C2	HLA-C*02, HLA-C*04, HLA-C*05, HLA-C*06, HLA-C*15, HLA-C*17 and HLA-C*18

KIR Genes and NK Lymphocytes

Natural Killer Cells (NK) are a lymphocyte subpopulation involved in the immune response. NK have membrane receptors called Killer-cell Immunoglobulin-like Receptors (KIR) that recognize proteins encoded by the HLA-C allele from the embryo's trophoblast. KIR receptors are encoded by a family of 16 genes, all located on chromosome 19 (19q13.4), characterized by high homology but also by wide variability. KIR receptors belong to the IgSF immunoglobulin superfamily include 2 or 3 Ig domains, a transmembrane region and a cytoplasmic tail that can be short (with activating effect) or long (with inhibitory effect). The activation state of NK cells is determined by the combination of these 16 KIR genes, as they express both surface receptors with activating effect and receptors with inhibitory effect.

KIR genes are organized into two main haplotypes: A and B

- Haplotype A causes to mother-foetus incompatibility as it determines an activation of the NK population
- Haplotype B causes to mother-foetus tolerance as it determines an inhibitory effect of NK.

Table 2: KIR gene alleles and haplotypes

KIR haplotype	KIR Genes * and haplotypes
A	KIR2DL3, KIR2DP1, KIR2DL1, KIR3DP1, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3, KIR2DS4
B	KIR2DL2, KIR2DL4, KIR2DL5, KIR3DL2, KIR3DL3, KIR3DS1, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, KIR2DP1 KIR3DP1

* KIR's genes nomenclature from International Comittee (<http://www.ebi.ac.uk/ipd/kir/>) (1)

Affinitymap indications

Affinitymap is indicated in all cases where maternal-foetal immunological compatibility for KIR/HLA-C genotype should be investigated for an adequate take-over in the PMA pathway or in the preconception phase.

It is particularly suitable for:

- ✓ Couples with recurrent miscarriage history and/or repeated implantation failures
- ✓ Couples with history or risk of pre-eclampsia
- ✓ Selection of sperm donors in male assisted fertilization

- ✓ Selection of egg donors in heterologous female assisted fertilization
- ✓ Selection of donors for embryo donation/double donation of gametes

Affinitymap testing selection

- ☐ Affinitymap KIR: KIR genotype study
- ☐ Affinitymap HLA-C: HLA-C genotype study
- ☐ Affinitymap HLA-C/KIR: KIR genotype and HLA-C genotype study

How to propose Affinitymap

Affinitymap can be performed in case of homologous or heterologous conceptions.

Table 3: How to propose Affinitymap in different fertilizations:

	Female Partner (Pregnant)	Male Partner	Oocytes Donor	Semen Donor
Natural Conception	KIR + HLA-C	HLA-C	n.a.	n.a.
Homologous ART	KIR + HLA-C	HLA-C	n.a.	n.a.
Female heterologous ART	KIR	HLA-C	HLA-C	n.a.
Male heterologous ART	KIR + HLA-C	n.a.	n.a.	HLA-C
ART with double gametes donation or embryo donation	KIR	n.a.	HLA-C	HLA-C

**n.a.: not applicable*

Results:

Recent studies have shown that specific combinations between the maternal KIR genotype and the paternal HLA-C genotype, are more common in women with recurrent miscarriages (2). In particular, maternal KIR AA genotype has been associated with a higher risk of recurrent interruptions of pregnancy, pre-eclampsia or intrauterine growth delay than heterozygous AB genotype or homozygotes BB genotype (3). This increased risk is related to embryonic expression of the HLAC type 2 genotype in both heterozygous and homozygous.

Otherwise, if the maternal genotype is KIR AB or KIR BB there will be no increased risk for the above-exposed complications regardless of the HLA-C genotype of the foetus.

Table 4: Risk level for implantation during pregnancy based on maternal KIR genotype and foetal HLA-C group derived from maternal and paternal genotypes

Maternal KIR Genotype	Foetal HLA-C		
	C1/C1	C1/C2	C2/C2
AA	neutral	risk*	risk
AB	neutral	neutral	neutral
BB	neutral	neutral	neutral

* The risk increases when the HLA-C allele belonging to the HLA-C2 group is of paternal origin.

To deduce the HLA-C genotype of the foetus it is necessary to distinguish between homologous or heterologous conception.

Homologous Fertilization

In homologous, natural or medically assisted conception, both genotypes of the two partners within the couple should be considered.

The presence of a **genotype KIR AB or BB in the female partner**, regardless of the genotype HLA-C of the two partners, does not reduce implantation probability nor increases the risk of pregnancy complications.

If the female partner has **the KIR AA genotype**, the genotype HLA-C of the two partners must be evaluated.

- If both partners show HLA-C C1/C1 genotype there is no reduction of implantation probability nor increases the risk of pregnancy complications.
- If one or both partners have C1/C2 or C2/C2 HLA-C genotype, there is a possibility that the foetus inherits a C1/C2 or C2/C2 genotype and this reduces the possibilities of implantation and increases the risk of complications in pregnancy.

Heterologous Fertilization

In heterologous conception, the genotype of the pregnant woman and other genotypes should be considered in relation to the specific type of heterologous fertilization that will be performed.

The presence of a **KIR AB or BB genotype of the pregnant woman**, regardless of the HLA-C genotype of the pregnant woman and/or partner and/or donors, does not reduce the probability of implantation nor increases the risk of complications of pregnancy.

If the pregnant woman has **the KIR AA genotype**, the HLA-C genotype in the pregnant woman and/or partner and/or in the donors shall be evaluated:

- Female heterologous fertilization:
 - If the male partner and the donor have HLA-C C1/C1 genotype there is no reduction of implantation chances nor increased risk of pregnancy complications.
 - If the male partner and the donor show HLA-C C1/C2 or C2/C2 genotype there is a chance that the fetus shows a C1/C2 or C2/C2 genotype and therefore there could be a reduction in the implantation rate and an higher risk of pregnancy complications.
- Male heterologous fertilization:
 - If the sperm donor has HLA-C C1/C1 genotype there is no reduction of chance of implantation nor increases the risk of pregnancy complications.
 - If the sperm donor has HLA-C C1/C2 or C2/C2 genotype there is a possibility that the fetus shows a genotype C1/C2 or C2/C2 and therefore there could be a reduction in the implantation rate and an higher risk of pregnancy complications.
- Heterologous fertilization with double donation or embryo donation:
 - If donors have HLA-C C1/C1 genotype there is no reduction of chance of implantation nor increases the risk of pregnancy complications.
 - If the donors have HLA-C C1/C2 or C2/C2 genotype there is a possibility that the foetus shows a C1/C2 or C2/C2 genotype and therefore there could be a reduction in the implantation rate and an higher risk of pregnancy complications.

Technical Note

Analysis method

- **HLA-C Genotyping**

Affinitymap allows the detection and the genotyping of the following HLA-C- gene alleles: HLA-C: HLA-C*01, HLA-C*02, HLA-C*03, HLA-C*04, HLA-C*05, HLA-C*06, HLA-C*07, HLA-C*08, HLA-C*12, A-C*14, HLA-C*15, HLA-C*16, HLA-C*17 and HLA*18. The test is performed on DNA extracted from peripheral blood followed by PCR amplification of HLA-C genetic markers and subsequent typing by specific SSO (Sequence Specific Oligonucleotide) probes linked to Luminex microspheres (Lifecodes, Inmucor).

- **KIR Genotyping**

Affinitymap allows the detection and the genotyping of 16 KIR genes: KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR2DP1 and KIR3DP1. The test is performed on DNA extracted from peripheral blood followed by PCR amplification of genetic markers associated with KIR genes and subsequent typing by SSO (Sequence Specific Oligonucleotide) probes linked to Luminex microspheres (Lifecodes, Inmucor).

Sensitivity

The sensitivity is 98%.

Limits

Affinitymap does not guarantee the implantation of the embryo nor the success of the pregnancy. The test results and the planning of specific therapy and follow-up should be defined and explained by the reference doctor who requested Affinitymap.

Affinitymap is performed on DNA extracted from peripheral blood collected in a tube with EDTA anticoagulant. The use of test tubes containing other anticoagulants is not suitable for Affinitymap. In the case of blood transfusions or transplantation it is not possible to perform the test as it cannot be ensured that the DNA extracted from blood collection corresponds to the patient germinal DNA.

For Affinitymap analysis it is mandatory that the Test Request Form and informed consent are duly filled and signed. In case of missing information, the laboratory will contact the doctor to fill the missing parts. Such communication could modify the test and/or the reporting turn-around time.

References:

- (1) Marsh SGE et al. *Killer-cell Immunoglobulin-like Receptors (KIR) Nomenclature Report, 2002*; Human Immunology, 2003 64:648-654.
- (2) Hong Y et al. *Killer immunoglobulin-like receptor repertoire on uterine natural killer cell subsets in women with recurrent spontaneous abortions*. Eur J Obstet Gynecol Reprod Biol. 2008 Oct;140(2):218-23.
- (3) Hiby SE et al. *Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success*, J Exp Med 2004; 200: 957-65.
- (4) Hiby SE et al. *Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage*, Hum Repro, 2008; 23: 972-6.

AFFINITYMAP INFORMED CONSENT

I, the undersigned _____

Date of Birth _____ Place of Birth _____

Address _____ n. _____ ZIP CODE _____

Town _____ Country _____

Phone _____ E-mail _____

I DECLARE:

of having received, during the meeting with Doctor _____ on the date _____, detailed information about the genetic analysis I am about to perform, of having understood and considered all the aspects of the exam and of having understood the benefit and the purpose of the genetic test and its possible limits. I had the chance to ask all the questions I considered worthwhile and I received answers I consider complete. In particular:

- It has been explained to me the test purpose;
- It has been explained to me the test limits;
- I have discussed the possible risks, benefits and limits connected to the test;
- I have understood that the result of the genetic test may have medical and psychological consequences for my family and I;
- I have understood the meaning of possible test results (even unexpected);
- I've been informed about the people who will have access to the biological sample;
- I've been informed about the people who will have access to the test result;
- To have the possibility to revoke the consent at any time, by signing the relevant revocation act.

Therefore,

I AUTHORIZE:

the execution of/and/the following analysis: _____
on biological material taken from me.

INDICATION TO THE ANALYSIS: _____

FURTHERMORE, I DECLARE:

☐ I agree

☐ I DO NOT agree

To be informed about analysis results;

☐ I agree

☐ I DO NOT agree

to share the results with Dr. _____

☐ I agree	☐ I DO NOT agree	that biological material can be used in the future, in compliance with the current legislation on the protection of personal data, for further investigations for diagnostic purposes for the examined pathology at the centre that performs the analysis;
☐ I agree	☐ I DO NOT agree	that biological material can be used in the future, in compliance with the current legislation on the protection of personal data, for further investigations for diagnostic purposes for the examined pathology in other centres, even outside European Union;
☐ I agree	☐ I DO NOT agree	to be informed about results of further investigations for diagnostic purposes for the examined pathology;
☐ I agree	☐ I DO NOT agree	to be informed about analysis results even in relation to unexpected news, which may have a benefit in terms of therapy, prevention or awareness about reproductive choices;
☐ I agree	☐ I DO NOT agree	that biological material may be used in the future, in compliance with the current legislation on the protection of personal data, for research purposes and/or for studies aimed to the collectivity in medical, biomedical and the epidemiological field and for quality verification programs for the laboratory performance
☐ I agree	☐ I DO NOT agree	to be informed about the results of the research.

The undersigned declares that the above is true and undertakes to promptly communicate any change of opinion on the matter.

Date ____/____/____

Patient's signature:

The Specialist who collected the consent (name and surname): _____

Phone _____ E-Mail _____

Signature and stamp of the Specialist: _____