

What is an HLA?

HLA stands for the Human Leukocyte Antigen locus otherwise known as the Major Histocompatibility complex, or MHC. This is large collection of genes on human chromosome 6 that governs the response of the immune system to infection by viruses and bacteria and directs antibody production against foreign substances or antigens. Leukocytes are the white blood cells found in the circulation. Different classes of these cells produce antibodies (lymphocytes) and others ingest bacteria and debris in tissues (macrophages and neutrophils). The HLA system comprises two classes of proteins which are expressed at cell surface and are also involved in recognition of 'self'. Like blood groups, a particular pattern of HLAs is an important consideration in rejection of transplants and grafts as well as their role in protection against and transmission of infections.

What is B*5701?

There are at least 30M possible genotypes in the HLA system. HLA B is a sub class of the HLA system and, based on DNA sequence variations, over 650 different versions of the gene have been identified in the human population, which code for the antigen. Ordinarily, we can only carry two of these genes; one inherited from each parent. The HLA-B*57 family consists of at least 16 closely related genes and the products of the *5701 gene family members have been associated with abacavir hypersensitivity. Around 5-8% of the Caucasian population express *57-like antigens, so this represents a common HLA genotype. This genotype is much more rare in other ethnic groups; it is found in less than 2% of people of African origin.

What assay is used?

Firstly, DNA is extracted from your blood and this DNA sample is tested for the presence or absence of the *5701 gene family members. As a control, the DNA is also tested, at the same time, for gene sequences that are present in everyone so that a negative result for *57-like sequences is always accompanied by a positive result for other genes. We carry out at least two different tests that detect different parts of the genes. At this stage, negative results will be reported as such. Any suspect positives for the *57 family are then retested using DNA sequencing techniques to exclude genes that are not associated with hypersensitivity (e.g. HLA-B*5702 or *5703).

What is the technology?

The test is performed using a technique called the Polymerase Chain Reaction, or PCR. In a series of steps, a specific target DNA sequence within the *57 gene family is amplified using a heat stable enzyme called Taq Polymerase. Successive heating and cooling steps, using a complex mixture of components, produce millions of copies of the target fragment of the gene. This fragment can then be detected using gel electrophoresis and a fluorescent dye that shows the presence of DNA under UV light. No fragment, or DNA band, is seen in people who do not carry the *5701 gene. If a band is present, present, this is DNA sequenced to determine the presence of *5701 genes.

How has the test been validated?

Many years of scientific research have allowed the identification of the gene, and its specific DNA sequence is well known. We have adapted tests from this literature and have run control assays on hundreds of DNA samples to show that the test can show both the presence and absence of the *5701 gene. Sequence data from our experiments and from external laboratories clearly show that the test produces only the PCR fragments we expect from the known sequence of the gene.

What is the specificity of the test?

As we know, there are 11 members of the *5701 gene family and our tests predict the presence of *5701, *5706, *5708 and *5710. The last three family members are very rare and there are, as yet, no data as to whether these two antigens are not included in hypersensitivity.

Do you get false negatives?

Because the way the test is carried out, false negatives are unlikely. As we employ at least two distinct tests and an internal DNA control we have confidence in our predictions.

What is a SNP (pronounced snip)snip?

A SNP, or a Single Nucleotide Polymorphism, is a term for the variation in the DNA sequences at any base pair (nucleotide pair) position. This SNP may be a normal variation or a mutation in gene sequences. Each base pair position can be filled by one of the 4 different nucleotides, A C G or T, which make up all your DNA sequences. The Human genome contains billions of these nucleotides and it is predicted that at least 10 million of them vary and are termed SNPs.

Are there other SNPs that can be tested? Do they improve the predictive quality of the test?

Yes, and we are constantly upgrading our information on these genes and refining our tests so that specific members of the gene family can be identified in simple assays. The predictive quality of the test is very high, but clinical information on the relationship between the presence of the different members of the *57 family of antigens and side-effects of therapy is still scarce.

Can the test fail? If so, why?

It is possible for the test to 'fail'. Any complete failure would be rare as we have many controls built into the system. A test failure is nearly always due to the quality of the DNA with which we carry out the test. If your blood sample has been incorrectly stored or 'mistreated' before it gets to us, then the extraction of DNA of an acceptable quality may be impossible.

How does the laboratory accreditation relate to the test?

The British Standards Institute (BSI) accredits our laboratory, under UKAS guidelines, to the internationally recognised BS EN ISO 9001: 2000 quality standard. This controls everything we do and demands that all our processes and assays are rigorously tested and verified to the highest scientific and medical standards. Essentially our services have to do exactly what we say they do.

Where can I obtain information on the clinical utility of the test?

Please download our clinical FAQs at:
www.delphicdiagnostics.com/pdf/EN_PGx_5701_EU_CLIN_FAQ.pdf.

How can I obtain this test?

Delphic has operated a routine clinical service for HLA-B*5701 testing since 2005. Our laboratory is certified to ISO 9001:2000. Our turnaround time is 7 days from receipt of sample as our UK laboratory. The cost is €90 per sample. Whole blood samples can be stored and shipped at ambient temperature enabling weekly shipments. For details on how to set up an account and for full Sample-handling Instructions go to www.delphicdiagnostics.com/products/pharmacogenetics/5701eu/index.php. Alternatively, please contact us (below) and we will be able to set-up an account and receive samples immediately.

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