GENDX
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New Robust HLA-B*27 Detection Assay Based On Real-Time PCR

Introduction

Recently, requests for accurate HLA typing have been significantly raised in the area of personalized health care. Evidence has been presented by several groups worldwide that HLA genes, and in particular some HLA alleles, are associated with diseases or alternatively with adverse effects of certain drugs.

The HLA-B*27 allele is present in 5-10% of the Caucasian population and is associated with various autoimmune diseases, the most common of which is ankylosing spondylitis (AS). Furthermore, it has also been shown that certain alleles within the groups of HLA-B*27 (and HLA-B*57) slow down the development and progress of AIDS in HIV positive patients, as compared to patients without this allele.

A test, specific for these kinds of HLA typing a) requires in most cases a presence / absence scoring and b) is performed by non-HLA specialized laboratories, which requires a different design, compared to the tests used for tissue typing.

Here, we present a HLA-B*27 detection assay, which is based on real-time PCR, and will show that this is a quick, robust, and reliable assay which can be easily applied in laboratories routinely performing molecular biology assays.

Materials and Methods

Real-time PCR assays were performed on the RotorGene Q (QIAGEN)
The assay consists of:

- a generic HLA-B gene amplification
- a generic detection (In this manner, all samples will result in a detectable signal, confirming successful amplification.)
- specific probe 1 for detection of HLA-B*27 (The probe cannot discriminate between HLA-B*27 and HLA-B*73 alleles.)
- specific probe 2 for exclusion of HLA-B*73

Using this strategy, 0.18205% of samples is expected result in a positive signal with both probes, based on the allele frequencies of Table 1. In these samples, the presence or absence of HLA-B*27 cannot be confirmed. If needed, the presence of HLA-B*73 alleles can be excluded by Pyrosequencing.

Results

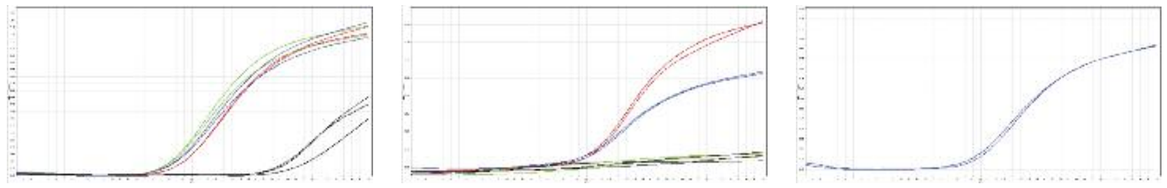
Figure 1 shows the amplification of 3 cell line samples, the results are as expected:

- The amplification control of all samples is positive
- With specific probe 1, samples with HLA-B*27 and/or HLA-B*73 alleles are positive
- With specific probe 2, only samples with HLA-B*73 alleles can be detected

Samples with a positive signal with specific probe 1, can be positive for HLA-B*27 and/or HLA-B*73. Both alleles can clearly be discriminated using Pyrosequencing. Starting from dispensation 14, a clearly different pattern is obtained in samples containing the HLA-B*27 or HLA-B*73 allele (Figure 2).

Figure 1: real-time detection of HLA-B*27 positive and negative samples

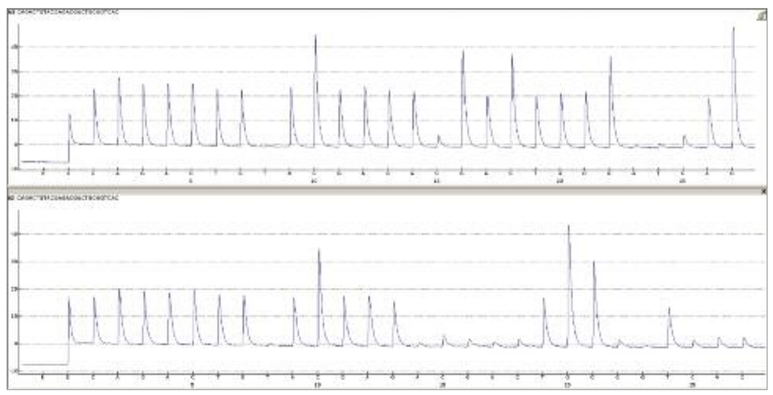
Three amplifications were performed in duplicate. Panel A: generic amplification detection, Panel B: HLA-B*27 probe, Panel C: HLA-B*73 probe. Green line: IHWG9253 (HLA-B*15:12, 46:01), red line: IHWG9067 (HLA B*27:05,-), blue line: IHWG9267 (HLA-B*51:01:01,73:01), black line: NTC.



Panel A | Panel B | Panel C

Figure 2: Pyrosequencing analysis of HLA-B*27 and HLA-B*73 positive

Upper panel: HLA-B*27 positive sample.
Lower panel: HLA-B*73 positive sample.



Conclusion

- A robust, easy to perform assay has been developed for detection of HLA-B*27.
- The assay, including data analysis can be performed in 2 hours.
- Since probe 1 cannot discriminate between HLA-B*27 or HLA-B*73, two alternatives strategies can be applied to exclude the presence of HLA-B*73.
- Pyrosequencing is a powerful tool to discriminate between HLA-B*27 and HLA-B*73.

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