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TAG-SNP VERSUS SSP GENOTYPING FOR DETERMINING THE HLA-B27 STATUS

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Dicht bij het leven

Background

The LAMP (Loop-mediated isothermal amplification) human HLA-B27 tag detection KIT (LaCAR) detects the Single Nucleotide Polymorphism rs4349859, which tags for the European most ankylosing spondylitis (AS) associated subtypes B*27:02 and B*27:05. AS is a common, highly heritable inflammatory arthritis affecting primarily the spine and pelvis and affects approximatively 0,5% of the population. >90% of AS patients are HLA-B27 positive. In LAMP the target sequence is amplified at a constant temperature around 65°C using together three sets of primers and a polymerase.

Methods

- The assay was performed following the manufacturer's instructions.
- 1 µl EDTA blood was added to 200 µl Lysis Buffer, after an incubation of 1-10', 5 µl of the lysed specimen is transferred to 20 µl Reaction buffer.
- After the LAMP amplification (65°C for 30') 2 hold steps were added: 2'' at 90°C, 7' at 35°C. Thereafter, melting curve analysis was performed, starting from 35°C and proceeding until 80°C, at a linear rate of 1°C every 15''.
- 101 positive and 74 negative samples were included in this study checking the accuracy.
- The used reference method was AllSet+™ Gold SSP B27 Low Resolution Kit (One Lambda).
- 4 samples with different genotypes were tested in three runs on three days to verify the reproducibility.

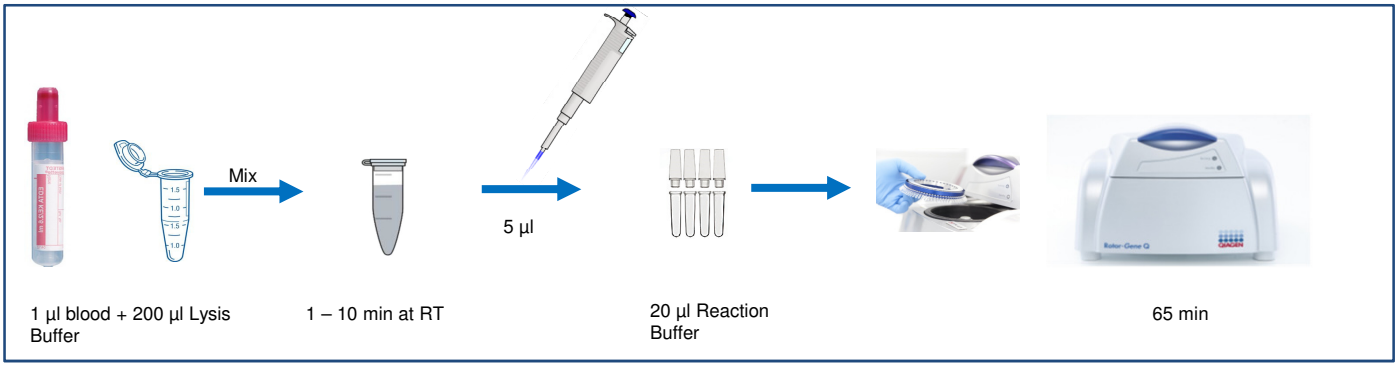

Fig. 1: Workflow

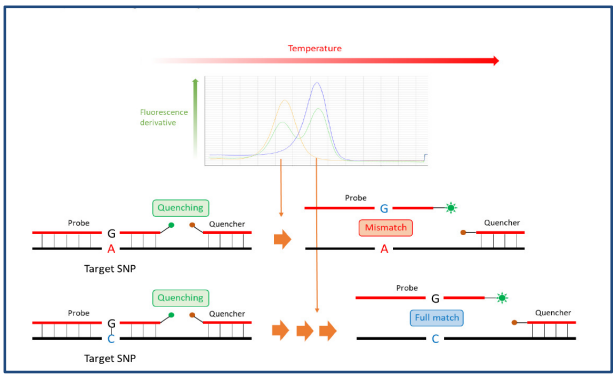

Fig. 2: Principle of melting curve analysis: the probe has been designed to hybridize the amplified mutated DNA fragment of rs4349859.

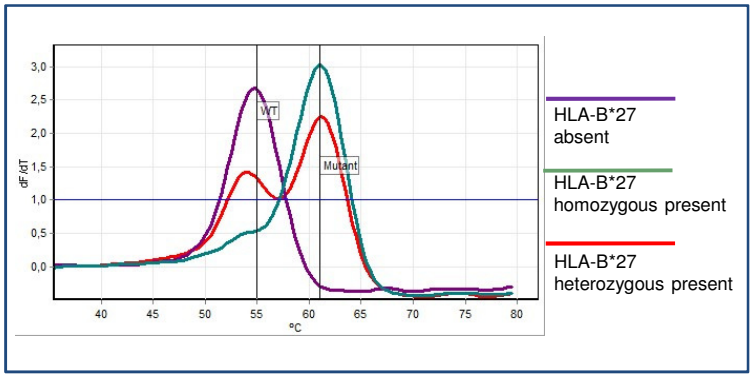

Fig. 3: Melting curve of 3 different samples on the Rotor Gene Q.

Results

- **Accuracy**

No sample showed inhibition.
172 out of 175 results were concordant with the reference method. All three false negative specimens were positive for HLA-B*27:05. The analytical sensitivity and specificity was 97% respectively 100% which is in line with the literature (Lehr *et al*. J Reuhmatol, 2017).

	TAG-SNP +	TAG-SNP -	Total
SSP +	98	3	101
SSP -	0	74	74
Total	98	77	175

- **Reproducibility**

All 4 samples (2 HLA-B27 absent and 2 HLA-B27 present) gave the same result on all three days (12/12).

Conclusion

HLA-B27 tag detection kit is an easy, accurate, rapid and cost-effective method and saves time compared to end-point PCR followed by gel electrophoresis. A big advantage is that no preliminary DNA extraction is required.

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