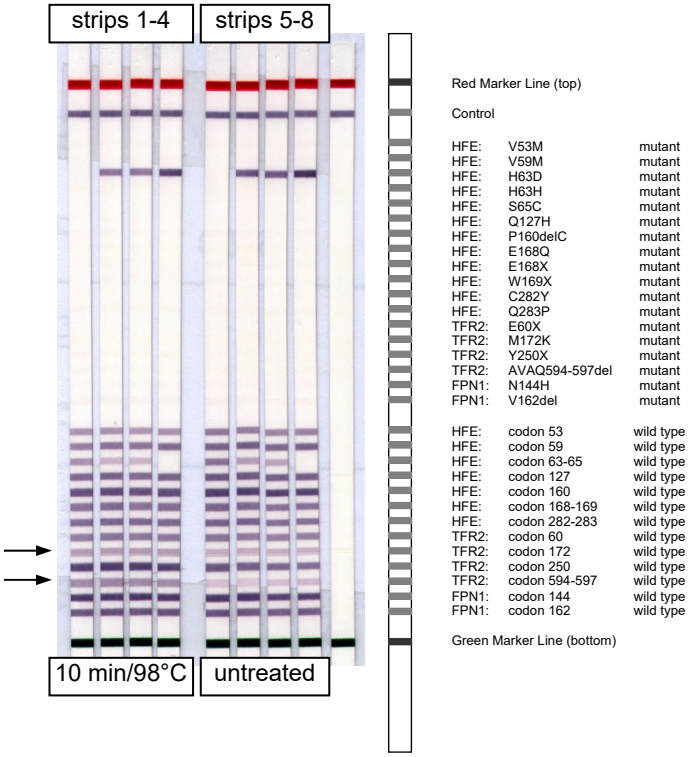
Potential effect of pre-heating DNA samples on the TFR-2 signal strength

Four DNAs were obtained during an external quality assessment trial. The samples came lyophilized and, according to the trial organizers, had been prepared by an in-house procedure based on chloroform extraction and isopropanol-ethanol precipitation. At ViennaLab the samples were re-dissolved in distilled water and analyzed following the standard protocol for Haemochromatosis StripAssay® A [REF 4-220] (teststrips 5-8). In a second run, DNA samples were first pre-heated to 98°C for 10 min. and rapidly cooled down on ice before setting up the PCR (teststrips 1-4).



It was observed that the signals for wild-type TFR-2 codon 594-597 and, to a smaller extent, codon 172 became more intense when DNA samples were pre-heated to 98°C before PCR.

Conclusion:

Since the ViennaLab GenXtract™ protocol already includes 98°C pre-heating, there is no need to perform an extra step when using the kit extraction system. However, if DNAs prepared by alternative methods lead to weak signals at some TFR-2 positions, results are likely to improve by pre-heating samples to 98°C for 10 min. immediately followed by cooling down on ice or in a cold block before setting up the PCR.