



Validation of a DNA Microarray Test System for the
Detection of Two Single Nucleotide Polymorphisms
(SNP'S), C282Y (845 G TO A) and
H63D (187 C to G) of the HFE Gene

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Hereditary Haemochromatosis (HH)

- HH is an inherited disorder concerning iron metabolism (Fix and Kowdley , 2008).
- The most common mutations implicated in HH are those of the HFE gene (Santos et al, 2012).
- HFE genotyping should be considered in individuals with unexplained elevated transferrin saturation and or both serum ferritin measurements (Santos et al, 2012).

Aims

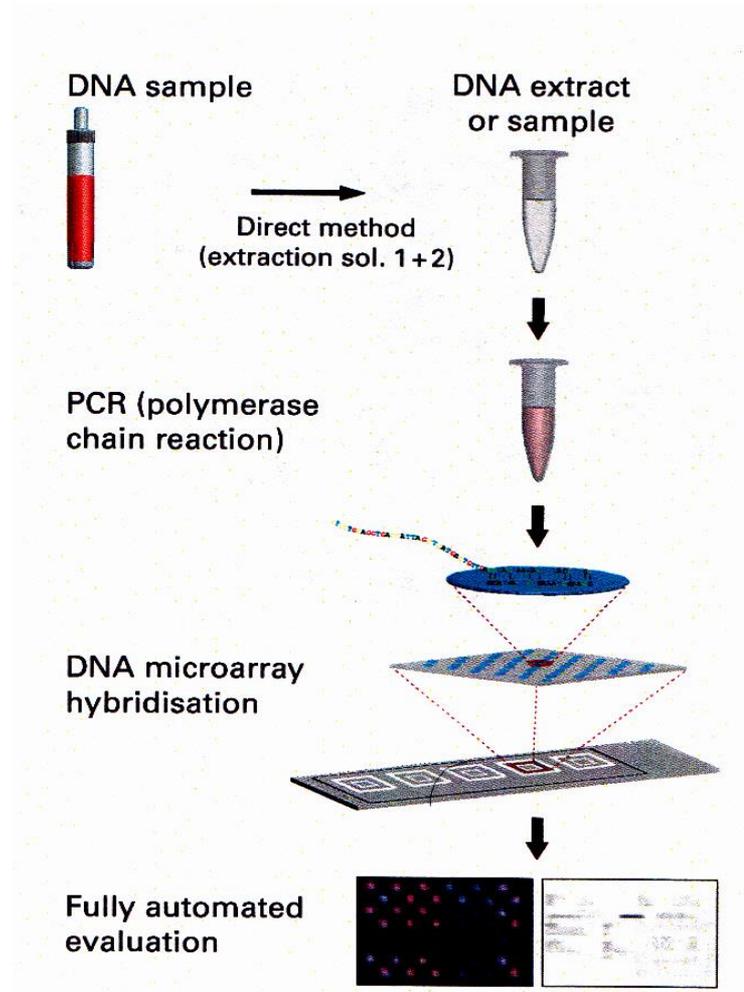
- To determine the sensitivity, specificity and accuracy of the EUROIMMUN EUROArray Haemochromatosis (2 SNP) Direct test kit.
- To validate the EUROIMMUN EUROArray Haemochromatosis (2 SNP) Direct test kit.
- To perform a cost comparison.

DNA Microarrays

- A DNA microarray consists of DNA molecules or probes which are applied to a solid carrier material (Seung Min, et al 2009).
- The probes are located at defined positions and differ from each other by their DNA sequence (EUROIMMUN, 2015).
- When patient DNA contains segments that match the probes of the microarray, the complementary DNA regions bind (EUROIMMUN, 2015).

Methods

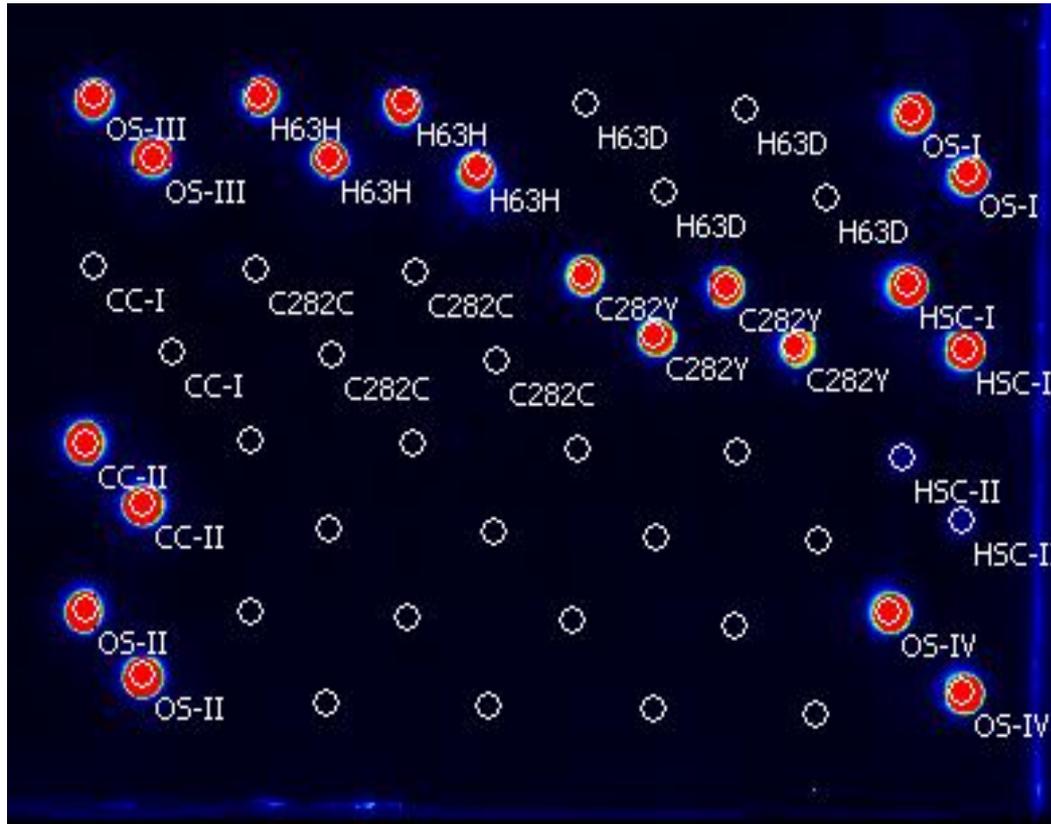
- The sample material used in this study was whole blood EDTA.



- DNA Extraction
- PCR Amplification
- Hybridisation
- Microarray Scan and Evaluation

Figure 1. Principles of DNA Microarray (EUROIMMUN, 2015)

Results



- Results are evaluated by the scan software.
- Patient DNA bound to the DNA probes of the microarray is interpreted as fluorescence.

Figure 2. Microarray result depicting homozygous C282Y mutation and wild type H63D test sample

Results

Sensitivity	Specificity	PPV	NPV
100%	100%	100%	100%

- Of the 137 samples analysed 4 yielded not valid results.
- Cost Comparison

Method	Institute	Cost per Test
DNA Microarray	Tallaght Hospital	€ 26.25
Real Time PCR	External Laboratory	€101.96

Discussion

- Results determined by the DNA microarray test system and the reference method were in agreement.
- As part of this study National Institute of Biological Standards and Control (NIBSC) samples were analysed.
- The inclusion of NIBSC samples provided comparison of the DNA microarray test system with the 'gold standard technique' in genetic testing, DNA sequencing.

Discussion

- Of the 137 samples analysed 4 yielded not valid results. These were compound heterozygous C282Y/H63D test results.
- The fluorescence signals from the microarray test fields failed to meet the fluorescence threshold for result interpretation. In an attempt to overcome this, amendments to the washing procedure of the test slides was proposed.
- Further testing would be required to determine if this amendment will resolve this limitation.
- Cost analysis proved a significant financial advantage was gained by performing the test in house

Conclusion

- To conclude the DNA microarray test system was found to be an accurate, easy to use high throughput test system for diagnostic use.
- However ,further investigation will be required to implement the DNA microarray test system. The suggested washing procedure amendments require testing to determine whether they will overcome the limitation regarding the invalid test results.



Acknowledgements

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- Seung Min, Y., Hyun Choi, J., Yup Lee, S. and Choon Yoo, N. (2009). Applications of DNA Microarray in Disease Diagnostics. *J. Microbiol. Biotechnol.*, 19(7), pp.635-646.