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AUTOMATION IN THROMBOGENETICS: IMPLEMENTATION OF ANYPLEX™ II THROMBOSIS SNP PANEL ASSAY (SEEGENE)

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Dicht bij het leven

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Background

Thrombosis is the formation of a blood clot inside a blood vessel, obstructing the blood flow of the cardiovascular system. Several thrombosis associated single nucleotide polymorphisms (SNPs) have been identified and reported to significantly increase the risk of venous thrombosis.

Pipetting and washing steps of our current reference method, Line Probe Assay (AID), for the detection of Factor V Leiden and Factor II mutation (G20210A) require a lot of hands on time. In contrast, Seegene offers a complete automated platform from extraction to result.

In addition to Factor V Leiden and Factor II mutation, the Anyplex™ Thrombosis SNP Panel assay (Seegene) detects 4 other thrombosis-associated SNPs in one PCR reaction: Factor V (H1299R, Y1702C), and MTHFR (C677T, A1298C).

Methods

- The test was performed following the manufacturer's instructions.
- Barcode labelled whole blood was directly placed on board of the MICROLAB STARlet platform. Extraction (STARMag96 Universal Cartridge Kit) and PCR set up were performed consecutively.
- The PCR reaction took place in the CFX96 Cycler (Bio-Rad). Interpretation of meltingcurves was done by the Seegene Viewer Software. The results were directly sent to the Laboratory Information System.
- 13 samples (9 patient and 4 INSTAND EQC) with 7 different genotype combinations were included in this study checking the accuracy.
- Five samples with different genotype combinations were tested in three runs on three days on two cyclers to verify the reproducibility.

Fig. 1: Automated workflow: extraction and PCR-set up on the STARlet, PCR on the CFX96, interpretation with the Seegene Viewer software.

Fig. 2: The Anyplex ™ II Thrombosis SNP Panel Assay contains Primer mix (TOCE Oligo Mix), Enzymes (Polymerase and UDG) and buffer, RNAse-free water and Positive control.

Results

The use of PCR plastics not recommended by the manufacturer can lead to false negative and invalid results because the software had problems to distinguish between real and background fluorescence signal. After using the correct PCR tubes (BIOplastics) all results were concordant with the reference method. None of the samples were inhibited. The turnaround time is about 3,5 hours from sample to result including less than 20 minutes hands on time.

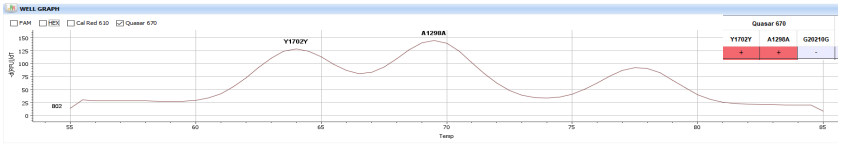

Fig. 3: The use of PCR plastics not recommended by the manufacturer can lead to false interpretation: the 3rd melting curve in the Quasar 670 channel is not recognized by the software

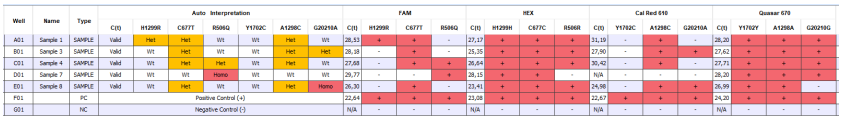

Fig. 4: The interpretation is done by the Seegene Viewer software and the results were directly sent to the Laboratory Information System.

	Factor V Leiden (R506Q)	Factor II (G20210A)
Sample 1	Wild Type	Wild Type
Sample 2	Wild Type	Wild Type
Sample 3	Wild Type	Heterozygote
Sample 4	Heterozygote	Wild Type
Sample 5	Heterozygote	Heterozygote
Sample 6	Homozygote	Heterozygote
Sample 7	Homozygote	Wild Type
Sample 8	Wild Type	Homozygote
Sample 9	Wild Type	Homozygote
Sample 10	Wild Type	Wild Type
Sample 11	Heterozygote	Wild Type
Sample 12	Wild Type	Heterozygote
Sample 13	Wild Type	Wild Type

Conclusion

The Anyplex™ Thrombosis SNP Panel Assay met all our validation criteria and was successfully implemented in our routine diagnostic laboratory. The whole workflow from extraction to release of the results is an automated process with few hands on time and full traceability of operator and used batchnumbers.

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