Evaluation study of the Genvinset® Factor II G20210A kit for the detection of the G20210A mutation associated with thrombophilia in a genetic analysis center

Introduction and objective

Thrombophilia consists in the predisposition to form blood clots, caused by an underlying hypercoagulation state attributable to inherited or acquired disorders of blood coagulation or fibrinolysis. Prothrombin is one of the proteins involved in the coagulation cascade, and the mutation G20210A on it has been associated with an increased risk of thrombosis.
The objective is to evaluate the performance of the IVD kit Genvinset® Factor II G20210A in a clinical laboratory, where clinical diagnosis is routinely performed. The laboratory has a genetic diagnosis area that provides direct service to both medical professionals and patients.

Materials and methods

- **Samples:**

A total of 25 DNA clinical samples were analysed. DNA was extracted from whole blood with the EXM3000 automatic extraction system (Zybio, CE-IVD).
Samples were previously analysed during the diagnostic routine of the laboratory, as part of their Thrombogen service, using predesigned TaqMan SNP Genotyping Assay (Applied Biosystems) by real-time PCR.

- **Reagents:**

Genvinset® Factor II G20210A, lot 230112. Controls provided with the kit were included in the PCR.

- **Equipment:**

7500 Real-Time PCR System (Applied Biosystems), with 7500 Software v2.3 for the data analysis.

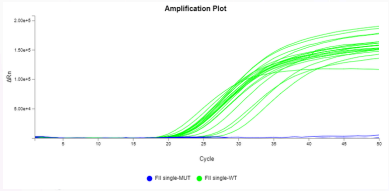
- **Data analysis:**

Conducted based on the information regarding interpretation of results shown in the instructions for use of the product. Genotypes were obtained by analyzing amplification curves and scatter plot.

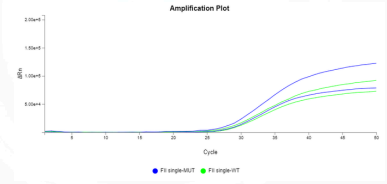
- **Acceptance criteria:**

1. C1 (wt/wt) must show exponential signal in HEX channel, and no signal in FAM.
2. C2 (mut/mut) must show exponential signal in FAM, and no signal in HEX.
3. Contamination control (RB) must show absence of amplification in both channels
4. DNA samples with amplification curves with Ct>35 must be considered doubtful and should be retested performing a new DNA extraction.

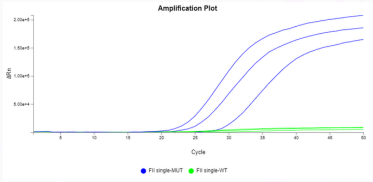
Results



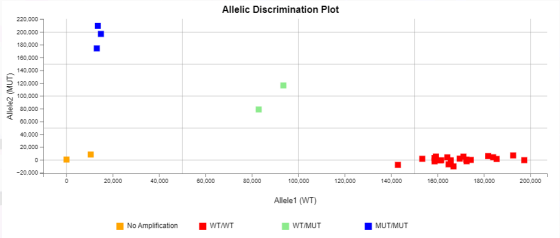
Homozygous wild-type samples



Heterozygous samples



Homozygous mutant samples



Sigmoidal curves with Ct<35 were obtained in the kit controls. No curves were obtained in any of the channels in the contamination control wells. Adequate amplification curves (exponential with sigmoid shape) were obtained in the samples under study. The behaviour of the samples agrees with their theoretical genotype, no non-specific signal is observed in the FAM or the HEX channel.

The genotypes obtained are distributed as follows:

	Genvinset® Factor II G20210A		
Previous method	Homozygous wild-type (WT)	Heterozygous	Homozygous mutant (MUT)
Homozygous wild-type (WT)	21	0	0
Heterozygous	0	2	0
Homozygous mutant (MUT)	0	0	2

Conclusions

The amplification curves obtained are clear and distinguishable from the background signal. The assignment of genotypes is unambiguous, either based on amplification curves or scatter plots.
A 100% concordance was obtained in the genotypes with Genvinset® with respect to the previous method.
The fact that the equipment is validated for a large number of real-time equipment is an advantage over the laboratory's routine system.
The information included in the instructions for use is useful and easy to understand and follow.
This kit could be used in the laboratory's diagnostic routine for the determination of the G20210A variant in Factor II.

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