

Comparison of the Performance of the Cepheid *Xpert HemosIL* Factor II and Factor V and the ViennaLab *FV-PTH-MTHFR* StripAssay Kits for Molecular Thrombophilia Profiling

Rouba Hoteit, Sylvana Hassanieh, and Rami A. Mahfouz

Aims: To compare the performance of two assays used for the detection of mutations/polymorphisms in the Factor V, Factor II, and methylenetetrahydrofolate reductase genes among patients referred for the management of a thrombotic event. **Materials and Methods:** We tested 40 different patient samples using two assays, the ViennaLab *FV-PTH-MTHFR* StripAssay and the Cepheid *Xpert HemosIL*. **Results:** The two assays were 100% concordant in their produced results with no samples failing the testing procedures in both. **Conclusion:** This is the first report to evaluate the performance of the ViennaLab *FV-PTH-MTHFR* StripAssay and the Cepheid *Xpert HemosIL*. Both assays can be introduced to the operation of molecular diagnostic laboratories to cover the referrals from different disciplines, especially in tertiary care centers with emergency departments.

Introduction

THE ASSOCIATION OF Factor II (G20210A), Factor V Leiden (FVL; G1691A), and methylenetetrahydrofolate reductase (MTHFR) gene mutations with an increased risk for venous thrombosis has been well documented. One manifestation of venous thrombosis is venous thromboembolic disorders (VTE). VTE are serious disorders accounting for high morbidity and mortality rates, with an annual incidence of 1/1000 (Jadaon, 2011). In case of a blood vessel injury, a cascade of chemical reactions responsible for blood coagulation or clotting is initiated. Several enzymes and proteins are involved in this cascade known as blood clotting factors. Thrombin is a clotting factor produced in the liver in an inactive form called prothrombin (Factor II). Upon injury, thrombin gets activated by Factor X and converts fibrinogen into fibrin, thus leading to the formation of the blood clot. Although the mutated molecule of prothrombin is structurally similar and its function is comparable to the normal molecule, prothrombin G20210A mutation leads to VTE by causing higher levels of the clotting factor prothrombin and thus hypercoagulability (Jadaon, 2011). Through molecular genetic testing, the G20210A mutation can be detected and the patient can be assessed for the heterozygosity or homozygosity of the gene, which is formed by the transition of guanine to adenine in the 3'-untranslated region of the prothrombin (Factor II) gene at position 20210 (Gessoni *et al.*, 2012).

Another clotting factor that causes hypercoagulation in this cascade is Factor V. The gene that codes Factor V is referred to as *F5*. *FVL* mutation is characterized by a single-nucleotide polymorphism (SNP) in exon 10, with a G to A substitution at position 1691, G1691A. It also exhibits the replacement of arginine by glutamine (R506Q) at one of three activated Protein C cleavage sites in Factor Va (Gessoni *et al.*, 2012). This mutation in Factor V makes it resistant to cleavage and induces its inactivation by activated protein C. This results in a higher amount of Factor V in the chain of reactions producing more thrombin and consequently leading to hypercoagulation (Shaheen *et al.*, 2012). Similar to Factor II, Factor V mutations can be determined through genetic testing and evaluated to be homozygous or heterozygous for the condition.

Another risk factor for venous thrombosis is hyperhomocysteinemia. This is a condition where homocysteine accumulates in plasma due to altered activity of MTHFR. The MTHFR C677T and A1298T mutations are the most frequent cause of reduction of the MTHFR enzymatic activity (Gil-Prieto *et al.*, 2009).

Patients carrying one of these mutations are at increased risk of developing venous thromboembolism (VTE). For instance, an individual who is heterozygous for *FVL* is 5–10 times more prone to hypercoagulation than an individual who carries no mutations. Furthermore, a homozygous state for the same gene increases the risk of VTE by 80-fold (Arslan *et al.*, 2011). As for prothrombin, G20210A increases the risk

of VTE by twofold (Poort *et al.*, 1996). Carrying multiple genetic defects is yet more severe and predisposes to higher hypercoagulation risk, with double heterozygosity for FVL and G20210A being the most common combination and may be the most critical due to the synergistic effect of these two mutations (Margaglione *et al.*, 1999).

In Lebanon, the prevalence of these mutations is relatively high where a study by Hoteit *et al.* showed that of 2248 referred cases, 25 cases were found to be simultaneously positive for the three mutations at a prevalence rate of 1.1%. Compared with other populations, this prevalence rate is considered high (Hoteit *et al.*, 2012). Thus, thrombophilia is a serious problem, and a quick molecular testing for diagnosis is highly appreciated in the field.

Genetic testing for disease predisposition is perceived as one of the potential benefits of the Human Genome Project (Collins and McKusick, 2001). Molecular diagnostics has markedly improved the diagnosis and workup of different clinical conditions, including the hypercoagulable state or thrombophilia, where different genes are involved. At the American University of Beirut Medical Center (AUBMC), most of the referrals for inherited thrombophilia workup are from the departments of obstetrics/gynecology, vascular surgery, hematology/oncology, and neurology. The high rate of referrals among the obstetrics team is due to the increase in recurrent abortions and failures of *in vitro* fertilization. As for the other departments, the rate of referrals is associated with deep vein thrombosis in the field of cardiac surgery, thrombosis and coagulopathy in hematology/oncology, and stroke among the neurology department patients. The importance of genetic testing for thrombophilia in several clinical and surgical fields requires the introduction of this test to all molecular diagnostics laboratories (Hoteit *et al.*, 2012). In this study, we compared Cepheid's *Xpert HemosIL Factor II and Factor V Combo Assay* and ViennaLab's *FV-PTH-MTHFR StripAssay* on a sample of referred cases for workup.

The Cepheid's *Xpert HemosIL Factor II and Factor V Combo Assay* is a qualitative genotyping test that allows the detection of the most common mutations in *FII* and *FV*, G20210A and *FVL*, respectively. This test uses real-time polymerase chain reaction (RT-PCR) techniques to genotype the samples. It is a simple test, all the elements needed for the PCR are available in the provided cartridge and only whole blood needs to be added. Since the cartridges are self-contained, there is no risk of cross-contamination. As for the ViennaLab's *FV-PTH-MTHFR StripAssay*, it uses the reverse hybridization method for biotinylated PCR products. It is a three-step protocol: DNA isolation from whole blood, PCR amplification using biotinylated primers, and finally, hybridization of the products on a test strip containing immobilized allele-specific oligonucleotide probes. In addition to the most common mutations in *FII* and *FV*, the *FV-PTH-MTHFR StripAssay* covers an additional mutation in the *MTHFR* gene (C677T).

Materials and Methods

Study samples and DNA extraction

We assessed 40 cases referred for thrombophilia workup at the AUBMC. For the ViennaLab's *StripAssay*, DNA was extracted based on the manufacturer's recommendations, and the genomic material was stored at -20°C for later use.

PCR and reverse hybridization (ViennaLab assay)

To test the various genotypic profiles of the Factor V, prothrombin, and methylenetetrahydrofolate genes, the *FV-PTH-MTHFR StripAssay* (ViennaLab) was used according to the manufacturer's protocol recommendation. This assay screens for the G1691A, G20210A, and C677T mutations of the Factor V, prothrombin, and *MTHFR* genes, respectively, whereby *in vitro*, the different gene sequences are simultaneously amplified and biotin labeled in a single amplification reaction (Multiplexing). Briefly, 5 μL of DNA was added to 15 μL of already prepared PCR amplification mix in the presence of 5 μL of 0.2 U/ μL Taq polymerase enzyme (AmpliTaq; Perkin Elmer). The thermocycler (Px2; Thermo Hybaid) program consists of an initial step of 94°C for 2 min, followed by 30 cycles of 94°C for 15 s, 58°C for 30 s, 72°C for 30 s, and a final extension step of 72°C for 3 min. Finally, the amplification products are selectively hybridized to a test strip that contains allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.

RT-PCR (Cepheid assay)

The *Xpert HemosIL Factor II and Factor V Assay* is a qualitative, *in vitro* diagnostic genotyping test for the detection of Factor II and Factor V alleles from sodium citrate or EDTA-anticoagulated whole blood. The test was performed on the Cepheid GeneXpert Dx System. This test is intended to provide results for Factor II (G20210A) and *FVL* (G1691A) mutations. The GeneXpert cartridge consists of multiple chambers for the complete automation of the nucleic acid extraction from whole blood, PCR, and product analysis and quantification. The cartridges contain chambers for sample introduction, the lysis buffer, the purification and elution buffers, and all of the RT-PCR reagents and enzymes. All of the sample-processing wastes are retained within the cartridge (Gessoni *et al.*, 2012). Detection of *FVL* and prothrombin 20210A was made through a single step of introducing 50 μL of sodium citrate or EDTA-anticoagulated whole blood into the cartridge. The cartridge was then introduced into the GeneXpert Dx instrument, and the results were read in 33 min.

Results

As shown in Table 1, the 40 samples that were run simultaneously using the two assays were 100% concordant. No sample failed the procedure using any of the two techniques.

Discussion

Analysis of the mutations and polymorphisms in genes implicated in thrombosis is gaining more importance in the clinical and laboratory management of patients with a history of a thromboembolic event. The Factor V, Factor II, and *MTHFR* genes are among the most important and fundamental to be first tested in this clinical setting. Different platforms are used by diagnostic laboratories to test for the corresponding gene mutations/SNPs, and their availability largely depends on several parameters mainly related to cost of technology, test turnaround time, and reimbursement issues. Two very important assays available are the ViennaLab *FV-PTH-MTHFR StripAssay* and the Cepheid *Xpert HemosIL*.

TABLE 1. COMPARISON OF FACTOR II AND FACTOR V GENE MUTATION RESULTS USING GENEXPERT AND VIENNALAB ASSAYS

Sample ID	Specimen	Results using GeneXpert assay	Results using ViennaLab assay	Concordance
1	PB	FII normal; FV heterozygous	FII normal; FV heterozygous	Yes
2	PB	FII normal; FV normal	FII normal; FV normal	Yes
3	PB	FII normal; FV heterozygous	FII normal; FV heterozygous	Yes
4	PB	FII normal; FV normal	FII normal; FV normal	Yes
5	PB	FII heterozygous; FV heterozygous	FII heterozygous; FV heterozygous	Yes
6	PB	FII normal; FV homozygous	FII normal; FV homozygous	Yes
7	PB	FII normal; FV homozygous	FII normal; FV homozygous	Yes
8	PB	FII normal; FV homozygous	FII normal; FV homozygous	Yes
9	PB	FII heterozygous; FV normal	FII heterozygous; FV normal	Yes
10	PB	FII normal; FV normal	FII normal; FV normal	Yes
11	PB	FII normal; FV heterozygous	FII normal; FV heterozygous	Yes
12	PB	FII normal; FV heterozygous	FII normal; FV heterozygous	Yes
13	PB	FII normal; FV normal	FII normal; FV normal	Yes
14	PB	FII normal; FV homozygous	FII normal; FV homozygous	Yes
15	PB	FII heterozygous; FV normal	FII heterozygous; FV normal	Yes
16	PB	FII heterozygous; FV normal	FII heterozygous; FV normal	Yes
17	PB	FII normal; FV heterozygous	FII normal; FV heterozygous	Yes
18	PB	FII heterozygous; FV normal	FII heterozygous; FV normal	Yes
19	PB	FII normal; FV normal	FII normal; FV normal	Yes
20	PB	FII normal; FV normal	FII normal; FV normal	Yes
21	PB	FII heterozygous; FV normal	FII heterozygous; FV normal	Yes
22	PB	FII normal; FV normal	FII normal; FV normal	Yes
23	PB	FII normal; FV normal	FII normal; FV normal	Yes
24	PB	FII normal; FV normal	FII normal; FV normal	Yes
25	PB	FII normal; FV normal	FII normal; FV normal	Yes
26	PB	FII normal; FV normal	FII normal; FV normal	Yes
27	PB	FII heterozygous; FV heterozygous	FII heterozygous; FV heterozygous	Yes
28	PB	FII normal; FV normal	FII normal; FV normal	Yes
29	PB	FII normal; FV normal	FII normal; FV normal	Yes
30	PB	FII normal; FV normal	FII normal; FV normal	Yes
31	PB	FII normal; FV normal	FII normal; FV normal	Yes
32	PB	FII normal; FV normal	FII normal; FV normal	Yes
33	PB	FII normal; FV heterozygous	FII normal; FV heterozygous	Yes
34	PB	FII normal; FV normal	FII normal; FV normal	Yes
35	PB	FII normal; FV normal	FII normal; FV normal	Yes
36	PB	FII normal; FV homozygous	FII normal; FV homozygous	Yes
37	PB	FII normal; FV normal	FII normal; FV normal	Yes
38	PB	FII heterozygous; FV normal	FII heterozygous; FV normal	Yes
39	PB	FII normal; FV normal	FII normal; FV normal	Yes
40	PB	FII normal; FV normal	FII normal; FV normal	Yes

PB, peripheral blood; FII, Factor II; FV, Factor V.

This report showed the high concordance between these two assays. Although the turnaround time for the GeneXpert assay is 37 min, whereas that of the ViennaLab assay is 6 h, the latter has the advantage of providing the results for the MTHFR gene mutations/polymorphisms, which are very important markers to be considered in the workup of the indicated patients. For example, a study by Puri *et al.* (2013) in recurrent pregnancy losses found that low vitamin B12 increases homocysteine, specifically among T allele-carrying mothers, suggesting the T allele of MTHFR C677T is detrimental with B12 deficiency. Another study by Fekih-Mrissa *et al.* (2013) evaluated the role of the MTHFR C677T gene polymorphisms in ischemic stroke patients and concluded that, together with hyperhomocysteinemia, they are important risk factors to be considered in this category of patients. The rapid turnaround time for the Cepheid Xpert HemoSIL assay makes it an addition to the emergency units having

molecular testing on their algorithms for the clinical management of patients presenting with a thrombotic event.

Our experience with both assays is very encouraging, and both of them are available in our diagnostic test menu serving different disciplines and specialties in our tertiary care center.

Author Disclosure Statement

No competing financial interests exist.

References

- Arslan S, Manduz S, Epozturk K, *et al.* (2011) Association of deep venous thrombosis with prothrombotic gene polymorphism identified in lung cancer cases. *Mol Biol Rep* 38: 2395–3400.
- Collins FS, McKusick VA (2001) Implications of the human genome project for medical science. *JAMA* 285:540–544.

- Fekih-Mrissa N, Mrad M, Klai S, *et al.* (2013) Methylenetetrahydrofolate reductase (C677T and A1298C) polymorphisms, hyperhomocysteinemia, and ischemic stroke in Tunisian patients. *J Stroke Cerebrovasc Dis* 22:465–469.
- Gessoni G, Valverde S, Manoni F (2012) Evaluation of the GeneXpert assay in the detection of Factor V Leiden and prothrombin 20210 in stored, previously classified samples. *Clin Chim Acta* 413:814–816.
- Gil-Prieto R, Hernández V, Cano B, *et al.* (2009) Plasma homocysteine in adolescents depends on the interaction between methylenetetrahydrofolate reductase genotype, lipids and folate: a seroepidemiological study. *Nutr Metab (London)* 6:39.
- Hoteit R, Halas H, Hassanieh S, Mahfouz RA (2012) Laboratory referral rates of genetic tests for thrombophilia workup in a major referral center. *Genet Test Mol Biomarkers* 16:459–462.
- Hoteit R, Taher A, Nassar R, *et al.* (2012) Frequency of triple mutations involving factor V, prothrombin, and methylenetetrahydrofolate reductase genes among patients referred for molecular thrombophilia workup in a tertiary care center in Lebanon. *Genet Test Mol Biomarkers* 16:223–225.
- Jadaon MM (2011) Epidemiology of prothrombin G20210A mutation in the Mediterranean region. *Mediterr J Hematol Infect Dis* 3:e2011054.
- Margaglione M, D'Andrea G, Colaizzo D, *et al.* (1999) Coexistence of factor V Leiden and factor II A20210 mutations and recurrent venous thromboembolism. *Thromb Haemost* 82:1583–1587.
- Poort SR, Rosendaal FR, Reitsma PH, Bertina RM (1996) A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 88:3698–3703.
- Puri M, Kaur L, Walia GK, *et al.* (2013) MTHFR C677T polymorphism, folate, vitamin B12 and homocysteine in recurrent pregnancy losses: a case control study among north Indian women. *J Perinat Med* 16:1–6.
- Shaheen K, Alraies MC, Alraiyes AH, Christie R (2012) Factor V Leiden: how great is the risk of venous thromboembolism? *Cleve Clin J Med* 79:265–272.

Address correspondence to:
Rami Mahfouz, MD, MPH, IFCAP
Department of Pathology and Laboratory Medicine
American University of Beirut Medical Center (AUBMC)
P.O. Box 11-0236
Riad El Solh, Beirut 1107 2020
Lebanon

E-mail: rm11@aub.edu.lb