GeneXpert in the diagnosis of risk factors for thrombophilia: evaluation of its use in a small laboratory

Gianluca Gessoni[1,2], Sara Valverde Sara[2], Rosa Canistro[3], Fabio Manoni[4]

[1]Transfusional Service, Chioggia, Venice; [2]Service of Laboratory Medicine, Chioggia, Venice; [3]Service of Oncology and Haematology, Chioggia, Venice; [4]Service of Laboratory Medicine, Monselice, Padua, Italy

Dear Sir,

In this letter we report our experience with the implementation of the GeneXpert analyzer for combined detection of factor V Leiden (FVL) and prothrombin G20210A (PRO-G) variant in our laboratory.

This study was performed in Chioggia, in a small clinical laboratory (about 1,500,000 test/year) situated in a non-teaching, 300-bed general hospital. For this study we evaluated results obtained using the HemosIL GeneXpert test for the combined detection of FVL and PRO-G in a series of 142 consecutive patients observed between March and May 2011. After approval from the local Ethics Committees, this study was carried out according to the Principles of the Declaration of Helsinki; informed consent was obtained from all subjects.

The GeneXpert system performs hands-off sample processing and real-time, multiplex polymerase chain reaction (PCR) for the detection of DNA or RNA. In this platform, sample preparation, amplification, and real-time detection are all fully automated and completely integrated. The system consists of an instrument, a personal computer and disposable fluid cartridges that have been designed to complete sample preparation and real time-PCR (RT-PCR) to detect both FVL and PRO-G in about 33 minutes in a single analytical run. Our instrument contains two randomly accessible modules that are each capable of performing separate sample preparation and RT-PCR tests. Each module contains a syringe drive for dispensing fluids, an ultrasound source for lysing cells or spores if necessary, and an ICORE thermocycler for performing transcription, real-time PCR, and amplicon detection[1].

The patented single-use cartridges contain chambers for holding samples, reagents or other materials, a valve body composed of a plunger and syringe barrel, a rotary valve system for controlling the movement of fluids between chambers, a capture matrix for binding the DNA, a sample preparation control in the form of a dry bead, dry reagents for real-time PCR and an integrated PCR tube that can be automatically filled by the instrument. To eliminate test-to-test contamination, all fluids including amplicons are contained within the disposable cartridge. The instrument never comes into contact with any fluids within the cartridge. Up to four different targets or groups of targets can be simultaneously detected in each sample by employing multiplex PCR techniques and real-time fluorescence technologies such as TaqMan, molecular beacon and scorpion probes[2].

All the samples considered in this study were obtained from patients of Italian ancestry aged from 14 to 69 years (mean 49 years); 93 (65%) were females and 49 (35%) were males. Among the 142 subjects considered, 33 were heterozygous for FVL, 2 were FVL homozygotes, 9 were heterozygous for PRO-G, 4 were combined FVL and PRO-G heterozygotes and 94 subjects were normal; these data are reported in Table I.

Table I - Results obtained by using the GeneXpert HemosIL Factor II and Factor V assay.

Subjects' Classification	Number
Normal Subjects	94 (66.2%)
FVL homozygous	2 (1.4%)
FVL heterozygous	33 (23.2%)
PRO-G heterozygous	9 (6.3%)
PRO-G + FVL double heterozygous	4 (2.8%)
Total	142

In the 3 months of use, we observed 11 invalid sample results (7.7%); the prevalence of invalid samples decreased from the first to the third month of analytical activity as the operators' gained experience. All these samples were retested after 1/5 sample

dilution and give valid results. Thus, in order to obtain 142 valid results we performed 149 tests, with a ratio of 1.04 tests/results.

FVL mutation is the most important genetic risk factor for venous thromboembolism, and the mutation in the 3' untranslated region of the prothrombin gene seems to be another mild risk factor for thrombotic events. It has been clearly shown that factor V mutation Arg 506 is frequently co-inherited with the prothrombin 20210 variant[3]. The association of the two prothrombotic alleles indicates that the prothrombin variant is an additional risk factor for venous thromboembolism and might contribute to thrombotic manifestations[3]. As previously reported, we observed a high prevalence of the two described risk factors for venous thrombosis in the population of Chioggia in patients studied to detect thrombosis risk factors and in the general population[4]. Therefore, a rapid, simple and cheap test for the simultaneous detection of the point mutations of FVL and the prothrombin variation is required in laboratory practice. Various different methods for the simultaneous detection have been described in the literature. All these methods require DNA extraction followed by an amplification reaction and, often, further analysis of the amplification products. e.g. enzyme digestion. We report here our field experience with the use of a fast, simple test for simultaneous detection of FVL and the prothrombin G20210A variant, using allele-specific primers which specifically recognize the mutated genes and a real-time PCR reaction involving a fluorescent reaction[5].

The first advantage of the simultaneous FVL and prothrombin G20210A variant screening is the 50% reduction in costs and labour, because patients with a mutated allele of factor V and/or the prothrombin variant are identified in a single step. In our experience, with a ratio of 1.04 tests per result, the overall cost was €112/patient for the combined detection of FVL and PRO-G. Moreover the test is cost-effective with regards to the reimbursement rate established by the National Health Service (€122.50 for each test, code 91.29.4).

In our opinion the GeneXpert system is an important new development in the field of molecular diagnostics. It automates all of the steps of a nucleic acid amplification test in a disposable, microfluid cartridge. Although it is a moderately complex test, it is simple enough to be performed reliably by individuals without a background in nucleic acid diagnostics. The user needs only to add sample preparation reagents and the sample to the cartridge. It has independently controlled and operated analysis modules that facilitate testing of individual samples in a random-access mode. The test incorporates an internal control that ensures that the entire test system is functioning properly, and a probe check control step is performed before PCR to verify reagent rehydration, probe integrity, and PCR tube filling in the cartridge. In summary, the GeneXpert HemosIL Factor II and Factor V assay enables genetic tests to be performed in complete automation – from the addition of whole blood and reagents to a cartridge to final results, without the need for a further analytical phase i.e. DNA extraction or a devoted room for molecular biology assays.

The Authors declare no conflicts of interest.

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Correspondence: Gianluca Gessoni
Transfusional Service
Ospedale Civile
Via Madonna Marina 500
30015 Chioggia, Italy
e-mail: ggessoni@asl14chioggia.veneto.it