AB WARFARIN TYPE:
Development of a pharmacogenetic test for personalized dosing of Warfarin (Coumadin®)

Elisabetta Bugin1-2, Katia Bortolozzo1, Dino Paladini1, Michela Pelloso1, Stefania Moz3, Dania Bozzato4, Paola Fogar5, Daniela Basso6, Carlo-Federico Zambon1-4, Mario Plebani1-4.

1 AB ANALITICA s.r.l., Padua, Italy
2 Department of Pharmacological Studies, University of Padua, Italy
3 Department of Laboratory Medicine, University Hospital of Padua, Italy
4 Medical Department (DIMED), University of Padua, Italy

INTRODUCTION
Warfarin (Coumadin®) is an anticoagulant, which is broadly used for prevention and treatment of thromboembolic events and disorders. Warfarin is an effective agent, but has a narrow therapeutic range and very pronounced interindividual differences concerning effective dosage as well as therapeutic response [1]. These characteristics necessitate a personalized treatment with adjustments of dosage and administration schedule on the basis of a regular INR (International Normalized Ratio) monitoring [2]. Recently, various genome-wide association studies have demonstrated the crucial role some variants of the genes CYP2C9, CYP4F2 and VKORC1 play in determining the effective pharmacological dose for Caucasian patients [3]. In 2011, the FDA (Food and Drug Administration) has revised the product insert of Coumadin® recommending the genotyping of VKORC1 and CYP4F2 before administration of the drug. This is seen as a necessary step towards a personalized treatment based on individual dose adjustments [4]. The aim of this study was to develop an innovative genotyping system for the genetic polymorphisms mainly determining the clinical response to Warfarin.

RESULTS AND CONCLUSIONS
All tested samples were accurately genotyped. This underlines the reliability of the assay. The mPCR/RLB assay is a convenient way to genotype polymorphisms in the genes CYP2C9, CYP4F2 and VKORC1 of patients under treatment with coumarin-type anticoagulants. Fig. 2 shows an example result of the RLB assay. The presence of a band shows the corresponding probe has bound the target DNA sequence. In order to facilitate interpretation of the strips and analyze the band pattern on a strip and assign the corresponding patient genotype. In order to evaluate the performance of the method, 150 patients under Warfarin therapy (target INR=2.5, average weekly dose = 31.25 mg) were analyzed. The device AB WARFARIN TYPE was capable of correctly assigning the genotype of all tested samples (the genotype had been determined by Real-Time PCR beforehand).

MATERIALS AND METHODS
Four polymorphisms were investigated in this study: rs9923231 in the VKORC1 gene, rs1799853 and rs1057910 in the CYP2C9 gene and rs2108622 in the CYP4F2 gene. Genotyping was performed by multiplex PCR combined with a reverse line blot (mPCR/RLB). The target sequences were amplified by PCR-SSP (Sequence-Specific Primer PCR), using biotinylated primers and the PCR products analyzed by direct sequencing to evaluate the specificity and efficacy of the PCR. For each assayed SNP two allele-specific oligonucleotide (ASO) probes recognizing a different allele were designed. An interpretation software was developed that is able to analyze the band pattern on a strip and assign the corresponding patient genotype. In order to evaluate the performance of the method, 150 patients under Warfarin therapy (target INR=2.5, average weekly dose = 31.25 mg) were analyzed. The device AB WARFARIN TYPE was capable of correctly assigning the genotype of all tested samples (the genotype had been determined by Real-Time PCR beforehand).

REFERENCES