Development and validation of a One-Step RT-Real Time PCR kit for detection and quantification of BCR-ABL p210

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Chronic myeloid leukemia (CML) is a cancer of the white blood cells which is characterized by a BCR-ABL1 fusion gene, commonly known as the Philadelphia chromosome (Ph). This marker is found in more than 90% of CML cases and in 3% to 40% of patients with acute lymphoblastic leukemia (ALL) (Westbrook et al. 1992). It is caused by a reciprocal translocation between chromosome 9 and 22, t(9; 22)(q34; 11), (Bartram et al. 1983). Ph contains the chimeric oncogene Bcr-Abl, which produces an active Bcr-Abl tyrosine kinase that is involved in the pathogenesis of CML. The introduction of the first target-specific tyrosine kinase inhibitor (TKI) into clinical practice has been a significant progress in the treatment of CML. Detecting and identifying the translocation not only provides useful information for diagnosis and prognosis of those types of leukemia, but also provides the means for monitoring the Minimal Residual Disease (MRD). MRD describes the remainder of neoplastic cells that persists in the patient during the different phases of chemotherapy and which cannot be detected by methods of cytology (e.g. analysis of cell morphology).
Assays based on qPCR are considered the gold standard methods for MRD monitoring, as they allow detection and quantification of fusion transcripts with high sensitivity. Although many devices are on the market, almost all of them require a reverse transcription (RT) to be performed before the test. This is time-consuming and increases the number of steps, thus increasing the risk of with RNA degradation and contamination of the analysis run.
In order to improve this kind of analysis, AB ANALITICA s.r.l. designed and developed a one-step RT-Real-Time PCR assay, that start directly from extracted RNA. The assay was designed according to the EAC guidelines. The validation steps will include the evaluation of: analytical specificity, analytical sensitivity (detection limit and linearity range), Limit of Blank (LoB) and Limit of Detection (LoD) of clinical samples, reproducibility, diagnostic specificity, diagnostic sensitivity, and accuracy. Documents for IVD-CE mark will be prepared. First experiments demonstrated the test to have high analytical sensitivity (about 0,5 copies per reaction) and a good range of linearity. The test will be calibrated using WHO International Standards and is going to be validated by laboratories associated with LabNet.
Owed to the fact that many onco-hematological disorders can be caused by a range of mutations in the same gene as well as in multiple genes, Real-Time PCR is not the most suited technology to study and analyze these conditions. In this context the use of Next Generation Sequencing (NGS) is a great opportunity, to advance personalized medicine. AB ANALITICA, in collaboration with the Department of Experimental and Clinical Medicine at the University of Florence, Italy, started a feasibility study regarding the development of a diagnostic tool for myeloproliferative neoplasms (MPNs). Understanding underlying mechanisms and finding a way to overcome the obstacles in pre- and post-analysis will help to develop an innovative and valuable method to improving and advancing personalized therapy in hematological oncology.

Westbrook, Carol A., et al. "Clinical significance of the BCR-ABL fusion gene in adult acute lymphoblastic leukemia: a Cancer and Leukemia Group B Study (8762)."*Blood*80.12 (1992): 2983-2990.
Bartram, Claus R., et al. "Translocation of c-abl oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia." (1983): 277-280.