Development and validation of a kit for the detection of the *PML-RARA* bcr2 fusion transcript for monitoring of acute promyelocytic leukemia

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Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) characterized by M3 cytomorphology. Most APL patients express *PML-RARA* fusion transcripts, which result from the fusion of the *PML* (on chromosome 15) and *RARA* (retinoic acid receptor alpha, on chromosome 17) genes by a t(15;17)(q22;q21) translocation. The chimeric *PML-RARA* protein binds DNA with a higher affinity, blocking the differentiation of hematopoietic stem cells at the promyelocytic stage.
The breakpoints in the *RARA* gene are always found in intron 2. In contrast, the PML locus contains three breakpoint cluster regions (bcr), which are located in intron 6 (bcr1, 55% of translocations), exon 6 (bcr2, 5% of translocations) and intron 3 (bcr3, 40% of translocations). As a consequence, three isoforms of the *PML-RARA* fusion transcript exist; they are denoted as long (bcr1), variant (bcr2) and short (bcr3), respectively [1].
Since the expression of *PML-RARA* transcript and the occurrence of APL are closely correlated, PCR-based detection of the *PML-RARA* fusion transcript is used in diagnosis and monitoring of APL [2].
Most patients (up to 90%) that are treated with ATRA (all-trans retinoic acid) in combination with chemotherapy achieve complete remission [3]. However, relapse occurs in about 20% of cases indicating the need for a detection system that allows precise monitoring of the minimal residual disease (MRD) [3].
Currently, AB ANALITICA offers two assays for the detection of the most frequent *PML-RARA* transcript isoforms bcr1 and bcr3 (about 95% of APL cases).
In order to complete the panel for the monitoring of MRD we have developed a qPCR test for the detection of *PML-RARA* bcr2 transcripts that is based on dual-labeled probe technology.
The assay was designed according to the EAC guidelines and clinical samples were provided by a laboratory associated with the LabNet [4].
Validation was carried out on the Applied Biosystems StepOne Plus, 7300, and 7500 Fast Dx Real-Time PCR Systems. On each of the instruments**,** analytical sensitivity and specificity as well as reproducibility of the assay were assessed with dilutions of DNA fragments containing the target region. The diagnostic sensitivity and specificity were tested using cDNA from 6 positive and 9 negative samples.
A compatibility study was conducted on the Bio-Rad Dx Real-Time System.
The assay showed high analytical sensitivity on all instruments, with a detection limit of 5 transcript copies per reaction and a linear range between 5 and 10^6 transcript copies per reaction. Neither nonspecific amplification nor cross-reaction with the *PML?RARA* bcr3 transcript was observed.
In all tests the assay was highly reproducible with low inter- and intra-assay variability (<1.4%).
Diagnostic sensitivity and specificity as well as the accuracy of the assay were 100%.
This system for the detection of *PML-RARA* bcr2 transcripts completes the AB ANALITICA panel of diagnostic products for the management of APL patients. This panel allows timely detection of disease relapse and enables the clinician to offer the patient a proper treatment.

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