Detection of IDH1/IDH2 Mutations Using Multiplex PCR/Single-base Extension in Formalin-Fixed, Paraffin-Embedded Giomi Tissues

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Abstract
Acute myeloid leukemia (AML) is a heterogeneous disease with a variable prognosis. The detection of IDH1/IDH2 mutations is critical for prognosis, as these mutations are associated with a higher remission rate and longer survival when compared to non-mutated AML. The detection of these mutations is important in the diagnosis and treatment of AML.

We performed a blinded study of 30 paraffin-embedded glioma specimens previously screened for IDH1 or IDH2 mutations by a PCR-based method. The results of our study showed that the sensitivity and specificity of the PCR-based method was high, with a sensitivity of 96% and specificity of 95%.

Results
We established a Shaposh® assay for the detection of IDH1 and IDH2 mutations as described in Methods. Figure 1 shows the capillary electropherograms of our control (Panel D) which is a typical example of a wild-type sample. Some example cases demonstrating wild-type (IDH1 R132H or IDH2 R1408T) and mutated samples are shown in Figure 2. As the Shaposh® assay should theoretically provide a better signal to noise ratio than the reference assay, we evaluated the specificity of the Shaposh® assay in the detection of a mutant allele in the wild-type background. Figure 3 demonstrates a titration and shows that the ability to detect a mutant allele in the Shaposh® assay is still present (Fig. C and D) while the mutant allele of the sequencing assay disappears into background. Further evaluation (Figure 3) shows that between 20 and 50% mutant allele can be detected with the Shaposh® assay.

Discussion
We have developed a simple, one tube assay to detect the major IDH1 and IDH2 mutations commonly found in human cancer. Data analysis was much faster and easier to perform than the traditional sequencing method. Results could be obtained in 1 day from DNA extraction to analysis. Equivalent results were obtained when comparing DNA extracted from formalin-fixed, paraffin-embedded tissue to those extracted from fresh-frozen tissue samples. The simplicity of our test allows potential adaptation of this assay to full automation.

Table 1. Primer PCR and Shaposh® Primer Sequences

Table 2. Shaposh® and Sequence Comparison Data

References