**SUMMARY**

- The Signature® KRAS/BRAF Mutations (RUO) assay is a multiplex assay with a streamlined workflow optimized for the molecular laboratory.
- The assay simultaneously detects and differentiates 12 KRAS mutations in codons 12 or 13, BRAF V600E, and an endogenous control (EC).
- The assay was evaluated using 143 DNA samples isolated from mCRC FFPE specimens and showed greater than 99% agreement with a laboratory-developed test (LDT) based on SNAPshot® technology.
- The Signature® KRAS/BRAF Mutations assay is an attractive, rapid and easy to use method compatible with routine clinical testing.

**INTRODUCTION**

K/RAS and BRAF are oncogenes involved in the epidermal growth factor receptor (EGFR) signaling pathway that control cell proliferation, differentiation, and migration. Mutations in these genes are associated with various clinical outcomes and may be predictive markers, or have a prognostic value, for anti-EGFR therapies based on monoclonal antibodies (cetuximab, panitumumab). Therefore, molecular screening of K/RAS/BRAF mutations using standardized, rapid and reproducible methods is important to identify patients who may or may not respond to anti-EGFR therapies. About 3,500 clinical specimens have been evaluated to date at the Rouen University Hospital using a laboratory-developed KRAS/SNaPshot® LDT. The goal of the present study was to determine the concordance between a novel research use assay, the Signature® KRAS/BRAF Mutations assay (Asuragen Inc.), and this clinically validated test.

**MATERIALS & METHODS**

Genomic DNA from FFPE blocks was isolated using a laboratory-validated method based on the RecoverAll® Total Nucleic Acid Isolation Kit for Tissue (Ambion). Signature® KRAS/BRAF Mutations (RUO) assays were performed according to the kit instructions for use (Asuragen Inc.). The procedure summarized in Figure 1A. Briefly, DNA samples (1 to 4 µg) were multiplex PCR in 25 µl reactions using 2.5 µl of AmpliTaq Gold® (Applied Biosystems) and biotin-modified primers targeting the KRAS codon 13 region, BRAF codon 600, and a conserved distant genomic region to serve as an endogenous control (EC). The PCR products (5 µl) were then spotted on a liquid bead array containing oligonucleotide capture probes for the various regions under investigation. The bead array was designed to capture all target sequences and negative controls are included to assess the validity of the amplification, hybridization and detection steps in each run. Amplification and hybridization steps were performed in 96 well plates on an ABI 384 Well thermal cyclers (Applied Biosystems). The sample had previously been tested with a PCR/SnaPshot® LDT as previously described.

**RESULTS**

- Figure 2: Study design and sample set. (A) Archival microarray genomic DNA samples extracted from FFPE specimens and previously tested with a SNaPshot® LDT were tested with the Signature assay. Out of the 343 archived DNA, 177 samples could not be analyzed because of absence of DNA. (B) Description of the 343 residual samples in the sample comparison analysis. The name and sequence of each mutation represented in each sample are shown. The mutation status of each sample was determined by the Signature assay and were therefore considered for negative for K/RAS and BRAF for the calculation of performance in Figure 2.

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**Figure 3:  Representative examples of Signature assay results.**

**Figure 4:  Example of discrepancy analysis.**

**Figure 5:**  **K/RAS/BRAF Mutations 7 (top) and Signature KRAS/BRAF Mutations 7 (bottom)** samples (11.25 ng per PCR) are shown for the Signature assay and for the Signature assay (RUO). Positive results were confirmed by re-testing (overall agreement: 100%).

**Figure 6:  Summary of performance.**

- Table 1 shows the overall agreement between methods after discrepancy resolution was greater than 99% for both KRAS and BRAF mutation status.
- Table 2 shows 5 samples may be true positive representative of the differences between the 2 assays in terms of analytical sensitivity or ability to co-detect multiple mutations in the same sample (2 of these potential true positive samples were double positive for G12V and Signature with Signature). Yellow = false negative. Red = false positive.

**Figure 7:  Comparison of results obtained with a SNaPshot® assay and the Signature® KRAS/BRAF Mutations assay.**

- The Signature® KRAS/BRAF Mutations assay (RUO) is compatible with genomic DNA extracted from FFPE mCRC specimens and generates results in very close agreement with an existing, clinically-validated method. The ability to detect 12 different K/RAS mutations and BRAF V600E in a single assay with a streamlined workflow makes it an attractive method for routine clinical testing of mCRC patients. Further, the flexible multiplex assay format and the potential to increase the number of mutations detected suggest that the assay may be developed and validated for the rapid assessment of various relevant mutations in solid tumor specimens.

**CONCLUSION**

The Signature® KRAS/BRAF Mutations assay (RUO) is compatible with genomic DNA extracted from FFPE mCRC specimens and generates results in very close agreement with an existing, clinically-validated method. The ability to detect 12 different K/RAS mutations and BRAF V600E in a single assay with a streamlined workflow makes it an attractive method for routine clinical testing of mCRC patients. Further, the flexible multiplex assay format and the potential to increase the number of mutations detected suggest that the assay may be developed and validated for the rapid assessment of various relevant mutations in solid tumor specimens.

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