KRAS HOTSPOT PCR ANALYSIS

Collected:	MM/DD/YY 0000
Result status:	Final
Resulting lab:	ILLUMINA CLARITY LIMS

Value:

Cleveland Clinic KRAS Hotspot PCR Analysis

Laboratory Accession Number: 000000000
Case #: P00-000000
Part ID: xxxxx
Sample Type: FFPET

% Tumor: 90

RESULT:
NOT DETECTED

INTERPRETATION:
A KRAS hotspot mutation was not detected in this specimen. Please see test limitations below.

KRAS proto-oncogene encodes for a small GTPase family protein, K-RAS, which plays a key role in cell proliferation and regulation. Activating KRAS hot spot mutations and amplifications are common in colorectal, non-small cell lung and pancreatic cancers.

METHODS:
The KRAS Hotspot PCR analysis is a rapid, automated, cartridge-based system to detect 21 DNA biomarkers using real-time PCR from formalin-fixed paraffin embedded tissues (FFPET), cytology cell blocks (CB) and alcohol fixed specimens. Samples with less than 10 percent tumor purity undergo tumor macrodissection prior to analysis. This assay utilizes the Idylla KRAS mutation assay on the Idylla system (Biocartis US, Itasca, IL) to qualitatively test for 21 biomarkers of the KRAS gene. KRAS assay cartridges contain dried reagents (enzymes, primers, and probes) and 1.8 mL liquefaction buffer to enable tissue to undergo liquefaction, cell lysis, extraction, real-time PCR amplification and detection. The assay was performed according to manufacturer's instructions. A conserved fragment in the intron 4/exon 5 junction of the KRAS gene is simultaneously amplified to act as a sample processing control (SPC) of the entire process from sample addition to result. Detection of the specific targets is performed using fluorescently labeled probes which are translated into genetic calls. The 21 KRAS variants detected by this assay are known to be oncogenic and clinically relevant (see KRAS Detected Variants below).

KRAS NM_033360.2 Detected Variants:
Exon 2:
G12C (p.Gly12Cys, c.34G>T)
G12R (p.Gly12Arg, c.34G>C)
G12S (p.Gly12Ser, c.34G>A)
G12A (p.Gly12Ala, c.35G>C)
G12D (p.Gly12Asp, c.35G>A)

G12V (p.Gly12Val, c.35G>T)
G13D (p.Gly13Asp, c.38G>A)

Exon 3:
A59-mutant (A59E, p.Ala59Glu, c.176C>A; A59G, p.Ala59Gly, c.176C>G; A59T, p.Ala59Thr, c.175G>A)
Q61K (p.Gln61Lys, c.181C>A and c.180_181delinsAA)
Q61H (p.Gln61His, c.183A>C and c.183A>T)
Q61-mutant (Q61L, p.Gln61Leu, c.182A>T; Q61R, p.Gln61Arg, c.182A>G)

LIMITATIONS:
During clinical validation, the assay limit of detection (LOD) was determined to be 5 percent variant allele fraction. Specimens must contain at least 10 percent tumor cells; if less than 10 percent tumor is utilized, a negative result is of indeterminate significance. Only the 21 KRAS variants described above can be detected by this technology. A negative result does not preclude the possibility of an alternative hotspot variant. Tumor heterogeneity, tumor burden, specimen degradation or other limitations of the technology may affect the sensitivity and LOD. Interfering substances, specifically formalin, decalcification agents, fixation agents containing heavy metals or preservation of buffy coats using Hank's Balanced Salt Solution (HBSS) can potentially affect assay performance. Cross-reactivity can occur in this assay. Where detected, a p.Gly13Asp can also result in a false positive p.Gly12Val mutation at low levels. A rare mutation, p.Gly12Trp (c.34_36delinsTGG), can be detected as p.Gly12Cys. Two mutations at codon 13, p.Gly13Asn (c.37_38delinsAA) and p.Gly13Glu (c.38_39delinsAA or c.38_39delinsAG) can be detected as p.Gly13Asp. This test cannot distinguish between somatic and germline variants.

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DISCLAIMER:
This test was developed and its performance characteristics determined by Cleveland Clinic's Robert J. Tomsich Pathology and Laboratory Medicine Institute (RT-PLMI). It has not been cleared or approved by the FDA. RT-PLMI is regulated under CLIA as certified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research.

Testing and interpretation performed at Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195. CLIA Number: 36D0656094

As reviewed by Elizabeth Azzato, MD, PhD