EGFR HOTSPOT PCR ANALYSIS ASSAY RESULT

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

Value:

Cleveland Clinic EGFR Hotspot PCR Analysis

Laboratory Accession Number: 00000000000
Case #: 000-000000
Part ID: xxxxxx
Sample Type: FFPET

% Tumor: 20

RESULT:
NOT DETECTED

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

EGFR encodes for epidermal growth factor receptor, a tyrosine kinase receptor that activates canonical MAPK and P13K/AKT/mTOR pathways to promote cell proliferation and growth. EGFR gain-of-function due to activating mutations, amplification and fusions are detected in many tumor types. Some activating mutations confer EGFR-tyrosine kinase inhibitor resistance.

METHODS:
The EGFR Hotspot PCR analysis is a rapid, automated, cartridge-based system to detect 51 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 EGFR mutation assay on the Idylla system (Biocartis US, Itasca, IL) to qualitatively test for 51 biomarkers of the EGFR gene. EGFR 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 of the transmembrane region of the EGFR 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 51 EGFR variants detected by this assay are clinically relevant and actionable, most with therapeutic drug treatments (see EGFR Detected Variants below).

EGFR NM_005228.3 Detected Variants:
S768I (p.Ser768Ile, c.2303G>T),

T790M (p.Thr790Met, c.2369C>T)
L858R (p.Leu858Arg, c.2573T>G, c.2573_2574delinsGT and c.2573_2574delinsGA),
L861Q (p.Leu861Gln, c,2582T>A)
G719-mutant (G719A, p.Gly719Ala, c.2156G>C; G719S, p.Gly719Ser; c.2155G>A; G719C, p.Gly719Cys, c.2155G>T and c.2154_2155delinsTT)
Exon 19 deletion mutations: 9bp deletions (c.2238_2248delinsGC, c.2239_2248delinsC, c.2240_2248del, c.2239_2247del), 12 bp deletions (c.2239_2251delinsC, c.2240_2251del), 15 bp deletions (c.2235_2249del, c.2236_2250del, c.2239_2253del, c.2240_2254del, c,2238_2252del, c.2237_2251del, c.2235_2252delinsAAT, c.2237_2252delinsT, c.2234_2248del, c.2236_2253delinsCTA, c.2237_2253delinsTA, c.2235_2251delinsAG, c.2236_2253delinsCAA, c.2230_2249delinsGTCAA), 18 bp deletions (c.2240_2257del, c.2237_2255delinsT, c.2239_2256del, c.2236_2253del, c.2239_2258delinsCA, c.2237_2254del, c.2238_2255del, c.2237_2257delinsTCT, c.2236_2255delinsAT, c.2236_2256elinsATC, c.2237_2256delinsTT, c.2237_2256delinsTC, c.2235_2255delinsGGT), 21 bp deletions (c.2238_2258del, c.2236_2256del), 24 bp deletion (c.2253_2276del)
Exon 20 insertion mutations: D770_N771insG (p.Asp770_Asn771insGly, c.2310_2311insGGT),
A767_V769dup (p.Ala767_Val769dup, c.2300_2308dup and c.2309_2310delinsCCAGCGTGGAT), S768_D770dup (p.Ser768_Asp770dup, c.2303_2311dup), H773dup (p.His773dup, c.2317_2319dup)

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 51 EGFR 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. 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