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EGFR
- Change in Testing Procedure –

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Affected Tests:

Mnemonics:	EGFR	EGFRREALK
Test Name/ Lab Nexus ID:	EGFR	EGFR with reflex to ALK FISH
Cerner Test Number:	7100020	
Specimen:	1. Six unstained, paraffin embedded formalin fixed tissue sections on slides representing serial sections from the selected tissue should be submitted along with a serial section that is stained appropriately (H&E). 2. Tissue section thickness should be between 5 and 10 microns. 3. The stained slide must be examined by a pathologist and the tumor tissue on the slide must be indicated. The percentage of neoplastic cell content must also be indicated. 4. The unstained serial sections must be received without cover slips. 6. Slides containing tissue sections are stored at room temperature. Avoid temperature extremes.	

Effective Date: Testing will begin on January 27, 2015 with samples received on January 26, 2015.

CPT Codes: When ordering the test as a companion diagnostic, there may be a modifier needed for proper reimbursement. Review of coding for this test is recommended.

Performed: Monday through Friday

Procedural Change:
The current test will be replaced with the recently FDA-approved companion diagnostic.

Background:
EGFR or epidermal growth factor receptor is a protein found on the surface of cells to which epidermal growth factor (EGF) binds. When EGF attaches to EGFR, it activates the enzyme tyrosine kinase thus triggering reactions that cause cells to grow and multiply. The EGFR molecule has 3 regions, one projects outside the cell and contains the site for binding EGF, the second is embedded in the cell membrane and the third projects into the cytoplasm of the cell's interior. Receptor tyrosine kinases, like EGFR, have been shown not only to be key regulators of normal cellular processes but also have a critical role in the development and progression of many types of cancers.

EGFR dimerization stimulates its intrinsic intracellular protein, tyrosine kinase activity. As a result, autophosphorylation of several tyrosine residues in the C-terminal domain of EGFR occurs. This elicits downstream activation and signaling by several other proteins that associate with the phosphorylated tyrosines through their own phosphotyrosine-binding SH2 domains. These downstream signaling proteins initiate several signal transduction cascades, principally the MAPK, AKT and JNK pathways, leading to DNA synthesis and cell proliferation. Mutations that lead to EGFR overexpression have been associated with a number of cancers as these mutations could lead to constant activation of EGFR which could result in uncontrolled cell division.

The identification of EGFR as an oncogene has led to the development of anticancer therapeutics directed against EGFR including gefitinib and erlotinib for lung cancer and cetuximab for colon cancer. Cetuximab is an example of a monoclonal antibody inhibitor (IgG type). Monoclonal antibodies block the extracellular ligand binding domain, and with the binding site blocked, signal molecules can no longer attach there and activate tyrosine kinase.

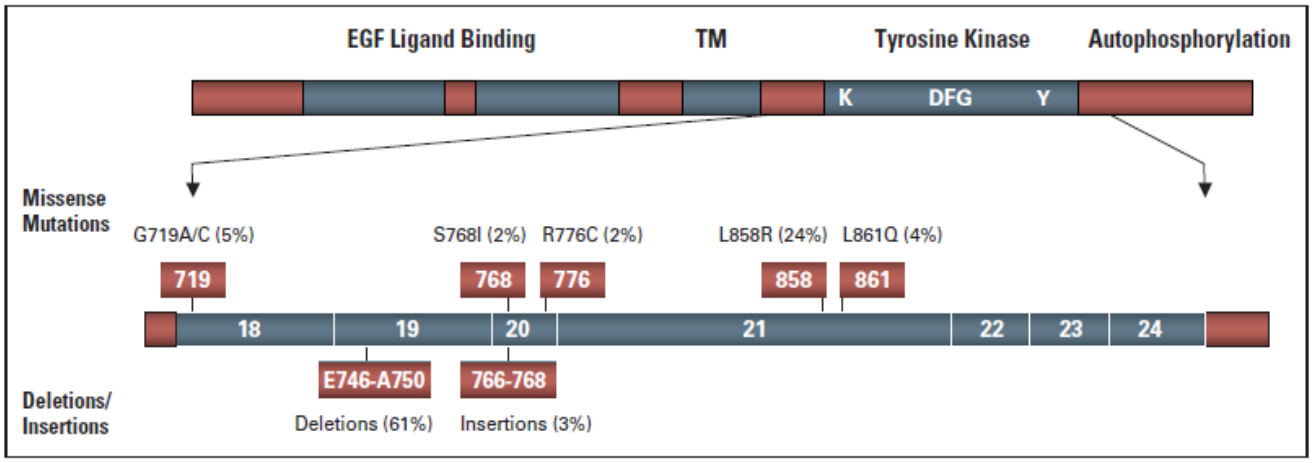

Fig 1. Summary of epidermal growth factor receptor (EGFR) mutations. The different domains of EGFR are shown. Also shown is an expanded view of the tyrosine kinase domain and the locations and frequencies of the different EGFR mutations. The shown mutations are those identified in patients treated with either gefitinib or erlotinib. The deletion and insertion mutations are combined. EGF, epidermal growth factor; TM, transmembrane domain.

Principle of Test:

The *therascreen* EGFR RGQ PCR kit is a real-time PCR test for the qualitative detection of exon 19 deletions and exon 21 (L858R) substitution mutations of the epidermal growth factor receptor (EGFR) gene in DNA derived from formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tumor tissue. Using Scorpions and ARMS (Allele Refractory Mutation System) technologies, the *therascreen* EGFR RGQ PCR kit enables the detection of 21 mutations in exons 18, 19, 20 and 21 of the EGFR oncogene against a background of wild type genomic DNA.

The *therascreen* EGFR RGQ PCR kit comprises 8 separate PCR amplification reaction mixes: 7 mutation-specific reactions in exons 18, 19, 20, and 21 of the EGFR oncogene and a wild-type control in exon 2.

Allele- or mutation-specific amplification is achieved by ARMS (Amplification Refractory Mutation System) which exploits the ability of *taq* DNA polymerase to distinguish between a matched and a mismatched base at the 3' end of a PCR primer. When the primer is fully matched, the amplification proceeds with full efficiency. When the 3' base is mismatched, only low-level background amplification may occur. Therefore, a mutated sequence is selectively amplified even in samples where the majority of DNA does not carry the mutation.

Detection of amplification is performed using Scorpions. Scorpions are bifunctional molecules containing a PCR primer covalently linked to a probe. The probe incorporates the fluorophore carboxyfluorescein (FAM™) and a quencher. The latter quenches the fluorescence of the fluorophore. When the probe binds to the ARMS amplicon during PCR, the fluorophore and quencher become separated, leading to a detectable increase in fluorescence.

Results Interpretation:
The test is intended to be used to select patients with NSCLC for whom Gilotrif (afatinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated. Safety and efficacy of Gilotrif (afatinib) have not been established in the patients whose tumors have L861Q, G719X, S768I, exon 20 insertions, and T790M mutations, which are also detected by the *therascreen* EGFR RGQ PCR kit. Among the 21 EGFR mutations detected by the *therascreen* EGFR RGQ PCR kit, safety and efficacy of Gilotrif has been established for the mutations shown in Table 1, but has not been established for the 6 mutations listed in table 2 below.

Table 1. List of mutations – Safety and efficacy of GILOTRIF established

Mutation	Exon	Base change
Deletions	19	2238_2255del18
		2235_2249del15
		2236_2250del15
		2239_2253del15
		2239_2256del18
		2240_2254del15
		2240_2257del18
		2239_2248TTAAGAGAAG>C
		2239_2251>C
		2237_2255>T
		2239_2258>CA
		2238_2252>GCA
		2238_2248>GC
		2235_2252>AAT
L858R	21	2573T>G

Table 2. List of mutations - Safety and efficacy of GILORIF not established

Mutation	Exon	Base change
T790M	20	2369C>T
L861Q	21	2582T>A
G719A	18	2156G>C
S768I	20	2303G>T
Insertions	20	2319_2320insCAC
		2310_2311insGGT

The following interpretive comment will appear with each report:
Interpretive Information: The epidermal growth factor receptor is over expressed in 40-80% of patients with non-small cell lung cancer (NSCLC). In patients who are unresponsive to standard chemotherapy, a subset responds to treatment with small molecular tyrosine kinase inhibitors (TKIs) of the EGFR intracellular domain, such as gefitinib and erlotinib. Most EGFR mutations (80-90%) are in the tyrosine kinase coding domain in exons 18-21. Two of the most common sensitizing mutations are short in-frame deletions of exon 19 (45% of all mutations) and the L858R substitution in exon 21 (40-45% of all mutations). Another mutation, T790M in exon 20, has been shown to cause secondary resistance to gefitinib. In patients who develop resistance, approximately

43-50% acquire the T790M mutation. Altogether, EGFR mutations are seen in 10-20% of all NSCLC patients. Other exon 20 mutations may be associated with resistance to gefitinib treatment, but variability exists between different individuals.

This assay identifies 21 somatic mutations in the EGFR oncogene by real time PCR in which the safety and efficacy of Gilotrif (afatinib) has been established and 6 somatic mutations in which it has not. In some instances, additional characterization is performed by Sanger sequencing in order to fully characterize the specific mutations (deletions/insertions) identified. The lower limit of detection of this assay is 1-4%. Paraffin embedded, formalin fixed (PEFF) tumor tissue is subjected to manual macrodissection, isolation and purification of the genomic DNA. The presence of non-tumor cellular components in the submitted specimen may affect the sensitivity of this assay. A result of "NOT DETECTED" does not preclude the presence of an EGFR mutation since results depend on percent mutant sequences, adequate specimen integrity, absence of inhibitors and sufficient DNA to be detected. As with any laboratory assay, the results of this assay should be considered in the entire context of the clinical presentation.

This test is FDA approved as a companion diagnostic when Gilotrif® (afatinib) treatment is being considered. For use in other clinical situations, the performance characteristics were determined by The Central Pennsylvania Alliance Laboratory, LLC. It has not otherwise been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.

Validation:
All categories of mutations were validated using method comparison with either previously tested samples and/or reference material.

References:

1. therascreen® EGFR RGQ PCR Kit Instructions for Use (Handbook), QIAGEN Manchester Ltd, Skelton House, Lloyd Street North, Manchester, M15 6SH, UK, July 2013.
2. Journal of Clinical Oncology. Vol23 (14).2005 pp3227-3234.
3. NEJM 352;8 2005 pp786-792.
4. Clinical Cancer Res 2008:14, pp4877-4882.