MAYO CLINIC LABORATORIES

Test Definition: EGFRW

EGFR Targeted Mutation Analysis with ALK Reflex, Tumor

Overview

Useful For

Identifying non-small cell lung cancers that may benefit from treatment with epidermal growth factor receptor -targeted therapies or anaplastic lymphoma kinase inhibitors

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
_PBCT	Probe, +2	No, (Bill Only)	No
_PADD	Probe, +1	No, (Bill Only)	No
_PB02	Probe, +2	No, (Bill Only)	No
_PB03	Probe, +3	No, (Bill Only)	No
_IL25	Interphases, <25	No, (Bill Only)	No
_I099	Interphases, 25-99	No, (Bill Only)	No
_I300	Interphases, >=100	No, (Bill Only)	No
LCAF	ALK (2p23), Lung Cancer, FISH, Ts	Yes	No

Additional Tests

Test Id	Reporting Name	Available Separately	Always Performed
SLIRV	Slide Review in MG	No, (Bill Only)	Yes

Testing Algorithm

When this test is ordered, the EGFR Gene, Mutation Analysis, 51 Mutation Panel, Tumor will always be performed. All specimens without an EGFR mutation will be automatically reflexed to testing for the ALK (2p23) rearrangement. Specimens with an identified EGFR mutation will result in cancellation of the LCAF test.

When this test is ordered, slide review will always be performed at an additional charge.

Method Name

Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type
Varies

Specimen Required
Pathology report must accompany specimen in order for testing to be performed.

Preferred:
Specimen Type: Tissue
Container/Tube: Tissue block
Collection Instructions: Submit a formalin-fixed, paraffin-embedded tissue block.

Acceptable:
Specimen Type: Tissue
Container/Tube: Slides
Specimen Volume: 12 unstained, positively charged, unbaked slides or 2 hematoxylin and eosin-stained slides (will not be returned) and 10 unstained, positively charged, unbaked slides
Collection Instructions: Submit 12 unstained, positively charged, unbaked slides cut at 5-microns or 2 hematoxylin and eosin-stained slides and 10 unstained, positively charged, unbaked slides with 5-micron thick sections of the tumor tissue.

Forms
If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
Molecular Genetics: Inherited Cancer Syndromes Patient Information (T519)
Oncology Test Request (T729)

Specimen Minimum Volume
See Specimen Required

Reject Due To

Specimens that have been decalcified (all methods) Low tumor percentage Insufficient amount of tumor Specimens that have not been formalin-fixed, paraffin-embedded	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)		
	Refrigerated		

Clinical & Interpretive

Clinical Information

Targeted cancer therapies are defined as antibody or small molecule drugs that block the growth and spread of cancer by interfering with specific cell molecules involved in tumor growth and progression. Multiple targeted therapies have been approved by the FDA for treatment of specific cancers. Molecular genetic profiling is often needed to identify targets amenable to targeted therapies and to minimize treatment costs and therapy-associated risks. Epidermal growth factor receptor (EGFR) protein is activated by the binding of specific ligands, resulting in activation of the RAS/MAPK pathway. Activation of this pathway induces a signaling cascade, ultimately leading to cell proliferation. Dysregulation of the RAS/MAPK pathway is a key factor in tumor progression for many solid tumors. Targeted therapies directed to tumors harboring activating mutations within the *EGFR* tyrosine kinase domain (exons 18-21) have demonstrated some success in treating a subset of patients with non-small cell lung cancer (NSCLC). As a result, the mutation status of *EGFR* can be a useful marker by which patients are selected for EGFR-targeted therapy.

Rearrangements of the anaplastic lymphoma kinase (*ALK*) locus are found in a subset of lung carcinomas (generally *EGFR* wildtype tumors) and their identification by fluorescence in situ hybridization (FISH) may guide important therapeutic decisions for the management of these tumors. The fusion of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene with the *ALK* gene results from an inversion of chromosome band 2p23. The *ALK-EML4* rearrangement has been identified in 3% to 5% of NSCLC with the majority occurring in adenocarcinoma and younger male patients who were light or nonsmokers. Recent studies have demonstrated that lung cancers harboring *ALK* rearrangements are resistant to EGFR tyrosine kinase inhibitors but may be highly sensitive to ALK inhibitors, like crizotinib (Xalkori). The drug crizotinib works by blocking certain kinases, including those produced by the abnormal *ALK* gene. Clinical studies have demonstrated that crizotinib treatment of patients with tumors exhibiting *ALK* rearrangements can halt tumor progression or result in tumor regression. The *ALK/EML4* FISH assay is an FDA-approved companion diagnostic test for crizotinib, which was recently approved by the FDA to treat certain patients with late-stage (locally advanced or metastatic), non-small cell lung cancers that harbor *ALK* gene rearrangements. It is useful for the identification of patients with lung cancer who will benefit from crizotinib therapy.

Reference Values

An interpretive report will be provided.

Interpretation

An interpretive report will be provided.

Cautions

A negative (wildtype) EGFR result does not rule out the presence of a mutation that may be present but is below the limits of detection for this assay (approximately 10%).

A negative (wildtype) EGFR result does not rule out the presence of other activating mutations in the EGFR gene.

Rare polymorphisms exist that could lead to false-negative or false-positive EGFR results.

The ALK fluorescence in situ hybridization (FISH) test is intended to be used for therapeutic purposes in pulmonary carcinoma. This FISH assay does not rule out other chromosome abnormalities.

While results of these tests may indicate the likely response to EGFR-targeted therapies or anaplastic lymphoma kinase (ALK)-inhibitor therapies, selection of treatment remains a clinical decision.

Clinical Reference

1. Sharma SV, Bell DW, Settleman J, Haber DA: Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer. 2007 Mar;7(3):169-181. doi: 10.1038/nrc2088
2. Gao G, Ren S, Li A, et al: Epidermal growth factor receptor-tyrosine kinase inhibitor therapy is effective as first- line treatment of advanced non-small-cell lung cancer with mutated EGFR: a meta-analysis from six phase III randomized controlled trials. Int J Cancer. 2012 Sep 1;131(5):E822-829. doi: 10.1002/ijc.27396
3. Mok TS: Personalized medicine in lung cancer: what we need to know. Nat Rev Clin Oncol. 2011 Aug 23;8(11):661-668. doi: 10.1038/nrclinonc.2011.126
4. Cheng L, Alexander RE, Maclennan GT, et al: Molecular pathology of lung cancer: key to personalized medicine. Mod Path 2012 Mar;25(3):346-369. doi: 10.1038/modpathol.2011.215

Performance

Method Description

All ordered specimens will undergo *EGFR* testing. The *EGFR* test is a qualitative polymerase chain reaction (PCR)-based assay employing fluorescently labeled probes that are used to detect exon 18 (G719A/C/S), exon 21 (L858R, L861Q), exon 20 (T790M, S768I) mutations, exon 19 deletions and exon 20 insertions of the *EGFR* gene.

Exon	Mutation	Protein change	Nucleotide change	Genotyp
18	G719A	p.Gly719Ala	c.2156G>C	G719A/C/S
	G719C	p.Gly719Cys	c.2155G>T	
	G719C	p.Gly719Cys(2)	c.2154_2155delinsTT	
	G719S	p.Gly719Ser	c.2155G>A	
19	Deletion 9	p.Leu747_Ala750delinsPro	c.2238_2248delinsGC	Exon 19 deletion
			c.2239_2248delinsC	
		p.Leu747_Ala750delinsSer	c.2240_2248del	
		p.Leu747_Glu749del	c.2239_2247del	
	Deletion 12	p.Leu747_Thr751delinsPro	c.2239_2251delinsC	
		p.Leu747_Thr751delinsSer	c.2240_2251del	
	Deletion	p.Glu746_Ala750del	c.2235_2249del	

	15		c.2236_2250del	
		p.Leu747_Thr751del	c.2239_2253del	
			c.2240_2254del	
			c.2238_2252del	
		p.Glu746_Thr751delinsAla	c.2237_2251del	
		p.Glu746_Thr751delinsIle	c.2235_2252delinsAAT	
		p.Glu746_Thr751delinsVal	c.2237_2252delinsT	
		p.Lys745_Ala750delinsThr	c.2234_2248del	
		p.Glu746_Thr751delinsLeu	c.2236_2253delinsCTA	
		p.Glu746_Thr751delinsVal	c.2237_2253delinsTA	
		p.Glu746_Thr751delinsAla	c.2235_2251delinsAG	
		p.Glu746_Thr751delinsGln	c.2236_2253delinsCAA	
		p.Ile744_Ala750delinsValLys	c.2230_2249delinsGTCAA	
	Deletion 18	p.Leu747_Pro753delinsSer	c.2240_2257del	
		p.Glu746_Ser752delinsVal	c.2237_2255delinsT	
		p.Leu747_Ser752del	c.2239_2256del	
		p.Glu746_Thr751del	c.2236_2253del	
		p.Leu747_Pro753delinsGln	c.2239_2258delinsCA	
		p.Glu746_Ser752delinsAla	c.2237_2254del	
		p.Glu746_Ser752delinsAsp	c.2238_2255del	
		p.Glu746_Pro753delinsValSer	c.2237_2257delinsTCT	
		p.Glu746_Ser752delinsIle	c.2236_2255delinsAT	
			c.2236_2256delinsATC	
		p.Glu746_Ser752delinsVal	c.2237_2256delinsTT	
			c.2237_2256delinsTC	
			c.2235_2255delinsGGT	
		p.Leu747_Pro753del	c.2238_2258del	
		p.Glu746_Ser752del	c.2236_2256del	
		p.Ser752_Ile759del	c.2253_2276del	
		p.Thr790Met	c.2369C>T	
		p.Ser768Ile	c.2303G>T	
		p.Asp770_Asn771insGly	c.2310_2311insGGT	
		p.Val769_Asp770insAlaSerVal	c.2307_2308insGCCAGCGTG	
		p.Val769_Asp770insAlaSerVal	c.2309_2310delinsCCAGCGTGGAT	
		p.Asp770_Asn771insSerValAsp	c.2311_2312insGCGTGGACA	
		p.His773_Val774insHis	c.2319_2320insCAC	
		p.Leu858Arg	c.2573T>G	
			c.2573_2574delinsGT	
			c.2573_2574delinsGA	
		p.Leu861Gln	c.2582T>A	

A pathology review and macrodissection to enrich for tumor cells is performed prior to slide scraping.

The *ALK* fluorescence in situ hybridization (FISH) test uses an FDA-approved *ALK* dual-color, break-apart rearrangement probe kitset. The *ALK* probe consists of 2 probes that flank the *ALK* gene region at 2p23 (Abbott Molecular). Five-micron sections of formalin-fixed, paraffin-embedded tissue specimens are cut and mounted on positively-charged glass slides. The selection of tissue and the identification of target areas on the hematoxylin and eosin-stained slide are performed by a pathologist. The probe set is hybridized to the appropriate target areas and 2 technologists analyze 25 interphase nuclei each (50 total). Results are reported based on the guidelines include with the probe kit and package insert with the results expressed as the percent abnormal nuclei.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
5 to 14 days

Specimen Retention Time
Unused portions of blocks will be returned. Unused slides are stored indefinitely.

Performing Laboratory Location
Rochester

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
81235-*EGFR* (epidermal growth factor receptor) (eg, non-small cell lung cancer) gene analysis, common variants(eg, exon 19 deletions, L858R, T790M, G719S, L861Q)
88381-Microdissection, manual
88271 x 2-DNA Probe (if appropriate)
88274-Interphase in situ hybridization (if appropriate)
88291-Interpretation and report (if appropriate)

Test Definition: EGFRW

EGFR Targeted Mutation Analysis with ALK Reflex, Tumor

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
EGFRW	EGFR with ALK Reflex, Tumor	21665-5

Result ID	Test Result Name	Result LOINC® Value
616130	Result Summary	50397-9
616131	Result	21665-5
616132	Interpretation	69047-9
616133	Specimen	31208-2
616134	Source	31208-2
616135	Tissue ID	80398-1
616136	Released By	18771-6