



October 17, 2011

## LUNG CARCINOMA MOLECULAR ANALYSIS

### EGFR Mutation and ALK Rearrangement analysis for Lung Carcinoma

Dahl-Chase Diagnostic Services is now offering a lung carcinoma molecular analysis that includes: Epidermal Growth Factor Receptor (EGFR) Mutation Analysis and FISH for ALK Rearrangements.

#### Background Information

Non small-cell lung carcinoma (NSCLC) accounts for approximately 85% of lung cancers. Targeted therapies based on genetic alterations in the tumor are appropriate for selected cases, currently principally in non-squamous NSCLC. Identifying of mutations in oncogenes associated with NSCLC, physicians can distinguish patients who are more likely to benefit from certain therapies, such as tyrosine kinase inhibitors (TKI) erlotinib and gefitinib in case of EGFR mutation or crizotinib in case of ALK rearrangement.

#### EGFR Mutation Analysis

This test is used to detect specific mutations of the EGFR gene (exons 18-19, 20 and 21), including point mutations, deletions and insertions reported to occur in characteristic locations throughout the above exons. This testing allows identifying patients who are most likely to respond to targeted lung cancer therapy, including tyrosine kinase inhibitors erlotinib and gefitinib. These mutations occur in between 10% up to 50% of patients (lower in Caucasians, higher in Asian patients).

#### ALK Rearrangement

ALK rearrangements can be found in 2-7% of NSCLC, more commonly in adenocarcinomas of never or light smokers, whose tumors lack EGFR and KRAS mutations. Patients with ALK rearrangements, characteristically due to ALK-EML4 fusion, rendering tumors sensitive to ALK inhibitors such as crizotinib (while resistant to EGFR tyrosine kinase inhibitor therapies).

#### Interpretive Information

Activating EGFR mutations predict response or resistance to erlotinib or gefitinib therapy (with the exception of the T790M mutation, which is associated with resistance to this therapy). ALK rearrangement predicts response to crizotinib therapy.

### Clinical Indications

The American Society of Clinical Oncologists (ASCO) and National Comprehensive Cancer Network (NCCN) guidelines recommend lung cancer molecular analysis for patients with NSCLC who are being considered for EGFR TKI or crizotinib therapies.

### Methodology

**EGFR:** DNA was extracted by the MagneSil DNA extraction method. PCR amplification, PCR product clean-up, and primer extension were conducted according to the recommended protocol for the TrimGen Mutector™ II Assay for EGFR mutation detection using STA Core Reagents A and C and the EGFR Primer Sets GP07-01 and GP07-02. Capillary electrophoresis was performed using an ABI 3130 and fragment analysis was performed using the GeneMapper v4.0 software. The EGFR mutational status was determined by either the presence of a point mutation and/or insertion or deletion by an additional peak when compared to a wildtype and mutational controls.

**ALK:** The Vysis ALK Break Apart FISH Probe Kit (Vysis Inc., Des Plaines, IL) is an FDA approved In Vitro Diagnostic (IVD) assay designed to detect rearrangements involving the ALK gene via fluorescence *in situ* hybridization (FISH) in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue specimen to aid in identifying patients eligible for treatment with crizotinib. Detection of the ALK probe hybridization to the 2p23 ALK region is performed using a fluorescence microscope. The pattern of orange and green signals are analyzed in a minimum of 50 nuclei and interpreted for the presence or absence of ALK gene rearrangement. A test is considered present if one orange and one green signal are present in the same nucleus and separated by at least two signal diameters in  $\geq 15\%$  of analyzed nuclei. Alternatively, a single orange signal (deletion of green signal) in addition to a fused or broken apart signal may be seen.

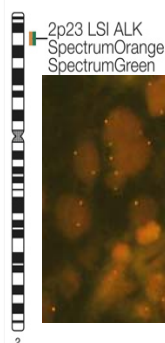


Figure A. An illustrative image of fluorescence in situ hybridization (FISH) demonstrating an ALK gene rearrangement (arrow). Adjacent orange and green signals are indicative of a fused signal (normal).

## Mutation Sample – L858R (T2573G) most frequent mutation

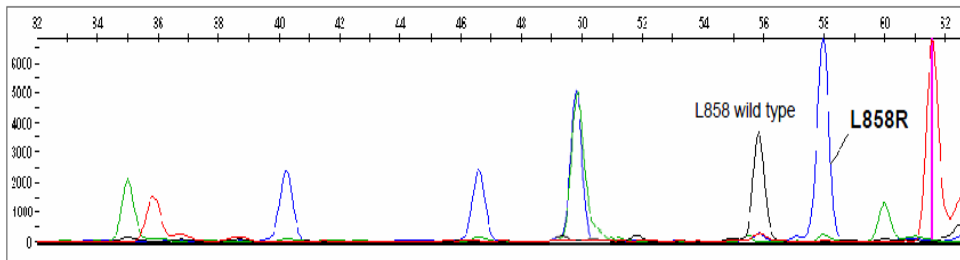


Figure B. A positive EGFR mutation result as depicted in a primer extension electropherogram. The presence of a blue peak adjacent to the wildtype L858 at position 56 base pairs confirms the presence of an L858R (T2573G) mutation.

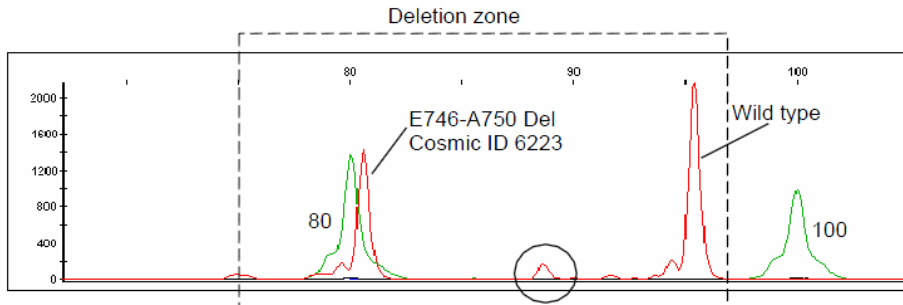


Figure C. A positive EGFR mutation result as depicted in a primer extension electropherogram. The presence of a red peak at 81 base pairs downstream of the exon 19 wildtype peak at 96 base pairs confirms the presence of an EGFR exon 19 deletion mutation.

## Test Overview

Test Name	EGFR Mutation Analysis	ALK p23 Rearrangements
<b>Individuals Suitable for Testing</b>	Patients with NSCLC who are being considered for treatment with EGFR TKI	Patients with NSCLC who are being considered for treatment with crizotinib
<b>Limitations</b>	Mutations of unknown clinical significance will not be detected by this assay. Mutations in low tumor cell populations may not be detected.	The probe set for this assay is only designed to detect breaks in the ALK gene region of chromosome 2 located at 2p23 and not to detect rearrangement partners, such as EML4.
<b>Reference Range</b>	EGFR mutation present (positive result) EGFR mutation not detected (negative result)	ALK gene rearrangement present (positive result) ALK gene rearrangement not detected (negative result)
<b>Specimen Requirements</b>	Formalin fixed tissue containing a sufficient amount of tumor (generally at least several mm of tumor tissue submitted in the tissue block)	Formalin fixed tissue containing a sufficient amount of tumor (generally at least several mm of tumor tissue submitted in the tissue block)
<b>Turn-around time</b>	7 - 10 Working days	7 - 10 Working days
<b>CPT Codes</b>	83891, 83892X2, 83900X2, 83901X9, 83907, 83909X2, 83912, 83912-TC, 83912-PC, 83917X8, 88380	88368X2

## References

1. NCCN Clinical Practice Guidelines in Oncology. Non-small cell lung cancer v.3.2011.

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## Our current molecular diagnostics test menu also includes:

- UroVysion bladder cancer FISH assay
- *HER2* gene amplification FISH assay
- KRAS Mutation Analysis of Colon Cancer
- BRAF V600E Mutation Analysis
- MSI Screening for Lynch Syndrome

**Dahl-Chase Diagnostic Services'** state-of-the-art laboratory is located in Bangor, Maine, but serves physicians, hospitals, laboratories, and clinics throughout northern New England, with specialty testing that draws from across the United States. Dahl-Chase Diagnostic Services is fully accredited by the College of American Pathologists (CAP), with CLIA certification. We offer cytopathology, including human papilloma virus (HPV) screening, surgical pathology, hematopathology, molecular diagnostics, and extensive flow cytometry testing. Dahl-Chase is a national leader in flow cytometric testing for paroxysmal nocturnal hemoglobinuria (PNH), a rare but serious blood disorder. Dahl-Chase Diagnostic Services is a subsidiary of **Dahl-Chase Pathology Associates, P.A.**, northern New England's leading provider of pathology services. Our 13 board-certified pathologists cover the breadth of subspecialty pathology.