CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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ALK (2p23) Rearrangement by FISH, Tissue

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Intended Use:
To detect rearrangement of the ALK (anaplastic large cell lymphoma) gene in patients with non-small cell lung cancers (NSCLC)[1]. Results are used to assess eligibility for Xalkori(crizotinib) therapy, which works by blocking certain kinases, including those produced by the abnormal ALK gene.

Testing Schedule:
Typically set up Tuesdays and Thursdays, with cases posted in SoloWeb on Wednesdays/Fridays. The testing schedule will be adjusted week to week to maximize workflow efficiencies. Expected Turn-Around-Time (TAT) is 2-4 days from receipt of testing order at CPAL.

Method:
Fluorescent In Situ Hybridization (FISH).
This test utilizes the IVD Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular, Inc.). The specific probes used are LSI 3'-ALK Spectrum Orange and LSI 5'-ALK Spectrum Green[1]. Lung tissue is fixed in formalin for >6 and <48 hours. Sections of this paraffin-embedded tissue are cut (4-6 microns) and mounted on positively charged glass slides. Using the pathologist-circled, specific tumor area of the H&E slide as a reference, the target area on the unstained slide is marked by diamond-tip etcher on the back of the slide. The probe set is hybridized to the target

area and 50 (or 100, if 10 to 50% of initial cells are positive) interphase nuclei are analyzed (BioView Duet Image Analysis System with manual scoring).

Specimen (IMPORTANT):

One H&E stained, cover slipped with ***invasive tumor area circled by a pathologist***. 3 unstained (no cover slip) serial sections of ***formalin-fixed, paraffin-embedded lung tissue*** on slides. **Blocks will not be accepted**. Specimens for which no desired sampling area (tumor) is indicated will be returned to the client so that the proper region of interest can be indicated and resubmitted to CPAL.

Specimens should be transported and stored at room temperature.

Reference Ranges/Interpretation:

The sample is considered positive for ALK gene rearrangement if >50% of the first 50 cells scored are positive. This suggests rearrangement of the ALK locus and a tumor that may be responsive to ALK inhibitor therapy.

The sample is considered negative for ALK gene rearrangement if <10% of the first 50 cells scored are positive. A negative result suggests no rearrangement of the *ALK* gene region at 2p23.

If 10-50% of the first 50 cells scored are positive, an additional 50 cells are evaluated by a second reviewer. The sample is then considered positive if > or = 15% of all 100 cells scored are positive . The sample would be considered negative for ALK gene rearrangement if <15% of all cells scored are positive.

Clinical Background:

Lung cancer is the leading cause of cancer death in the United States. Non-small cell lung carcinoma (NSCLC) accounts for 75% to 80% of all lung cancers with an overall 5-year survival rate of 10% to 15%[2]. Standard chemotherapy regimens have had marginal success in improving clinical outcomes. Targeted treatments may be used as novel molecular changes are identified.

Rearrangements of the *ALK* locus are found in a subset of lung carcinomas and their identification may guide important therapeutic decisions for the management of these tumors. The fusion of *EML4* (echinoderm microtubule-associated protein-like 4) gene with the *ALK* (anaplastic large cell lymphoma kinase) 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 in adenocarcinoma and younger male patients who were light or nonsmokers[3]. Recent studies have demonstrated that lung cancers harboring *ALK* rearrangements are resistant to epidermal growth factor receptor

tyrosine kinase inhibitors, but may be highly sensitive to ALK inhibitors, like Xalkori (crizotinib). Clinical studies have demonstrated that Xalkori treatment of patients with tumors exhibiting *ALK* rearrangements can halt tumor progression or result in tumor regression[4]. This FISH assay is a FDA-approved companion diagnostic test for Xalkori, which the FDA approved to treat certain patients with late-stage (locally advanced or metastatic), non-small cell lung cancers that harbor anaplastic lymphoma kinase (*ALK*) gene rearrangements[5]. It can be used to identify patients who will benefit from Xalkori therapy.

Limitations of Procedure:

This test is intended to be used for therapeutic purposes in pulmonary carcinoma.

This FISH assay does not rule out other chromosome abnormalities.

While results may indicate the likely response to ALK inhibitor therapy, selection of treatment remains a clinical decision.

FISH assay results may not be informative if the specimen quality and/or specimen slide preparation is inadequate.

References:

1. Vysis ALK Break Apart FISH Probe Kit package insert, June 2012, Abbott Molecular Inc., Des Plaines, IL

2. NCCN Clinical Practice Guidelines in Oncology™. Non-small cell lung cancer. v 2.2010. Available at: http://www.nccn.org/professionals/physician_gls/PDF/nscl.pdf . Accessed July 22, 2010

3. Shaw AT, Yeap BY, Mino-Kenudson M, et al. Clinical features and outcome of patients with non-small–cell lung cancer who harbor *EML4-ALK*. *J Clin Oncol*. 2009;27:4247-4253.

4. Shaw AT, Yeap BY, Solomon BJ, et al: Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring *ALK* gene rearrangement: a retrospective analysis. Lancet Oncol 2011;12:1004-1012

5. XALKORI® (crizotinib) [package insert]. New York, NY: Pfizer; 2012.