Fluorescence *In Situ* Hybridization (FISH) for B-Cell Chronic Lymphocytic Leukemia

Background Information

B-cell chronic lymphocytic leukemia (B-CLL) is a neoplasm of small B lymphocytes that involves the peripheral blood, bone marrow, and, in some patients, the lymph nodes. Most patients with B-CLL are older than 50 years of age at diagnosis, and most cases will follow an indolent clinical course.

In recent years, researchers have described chromosomal abnormalities in B-CLL that are correlated with prognosis and resistance to fludarabine.[1-3] Deletions of chromosome 17p involving the *TP53* locus have been identified in approximately 10% of B-CLL cases and are associated with an adverse prognosis. Similarly, deletions of chromosome 11q involving the *ATM* gene are reported in approximately 20% of B-CLL cases and likewise are associated with poor outcomes. In contrast, cases of B-CLL with deletions of chromosome 13q as a sole abnormality, detected in approximately 40% of cases, are associated with a favorable prognosis. Trisomy of chromosome 12 has been identified in approximately 10% of B-CLL and is associated with atypical morphologic features and an intermediate prognosis.

Interphase fluorescent *in situ* hybridization (FISH) is superior to metaphase cytogenetic studies for detecting these chromosomal abnormalities due to the low proliferative rate of B-CLL cells in culture.[2,4]

Clinical Indications

Cleveland Clinic Laboratories offers FISH analysis for abnormalities of chromosomes 17p, 11q, 13q, and trisomy 12 to assist in the clinical evaluation of patients with B-CLL. Peripheral blood and bone marrow specimens involved by B-CLL are suitable for analysis.

Interpretation

Cases are classified as positive for del(11q), del(13q), or trisomy 12 when the corresponding abnormal signal pattern is observed in >10% of nuclei.

Cases are classified as positive for 17p deletion when loss of the 17p signal is detected in >15% of nuclei.

Limitations of the Assay

False negative results may occur if the neoplastic cells represent <15% (for 17p deletions) or <10% (for all other abnormalities) of the total cellularity in the sample analyzed.

This assay should be employed only in cases with an established diagnosis of B-CLL. The abnormalities identified by the test are not specific to B-CLL.

FISH studies do not exclude the presence of other chromosomal abnormalities that also may influence the prognosis of B-CLL.

Methodology

FISH analysis is performed on gravity preparations of peripheral blood or bone marrow samples. Cells are hybridized with a set of five probes described below (Vysis, Inc., Downer's Grove, Ill.). Probes are combined into two probe mixtures, each performed on a separate slide. 100 cells are evaluated for each probe.

Probe Set 1:

- Del(17p):
  The LSI p53 (17p13.1) probe is a ~145 kb sequence labeled in Spectrum Orange.
- Del(11q23):
  The LSI ATM probe is a ~500 kb probe that encompasses the entire ATM locus at 11q22.3 and is labeled in Spectrum Green.

Probe Set 2:

- Del(13q):
  Abnormalities of chromosome 13q are detected using two probes to this region: The LSI D13S319 probe is a ~130 kb sequence that hybridizes to chromosome 13q14.3 and is labeled in Spectrum Orange.

  The LSI 13q34 probe is a ~550 kb probe to the 13q34 region and is labeled in Spectrum Aqua.
- CEP12:
  The CEP12 probe hybridizes to the centromeric region of chromosome 12 and is labeled in Spectrum Green.

References

1. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Kröber A, Bullinger L, Döhner K, Bentz. M, Lichter, P. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*. 2000;343:1910-1916.
2. Shanafelt TD, Geyer SM, and Kay NE. Prognosis at diagnosis: integrating molecular biologic insights into clinical practice for patients with CLL. *Blood.* 2004;103:1202-1210.
3. Stilgenbauer S, Bullinger L, Lichter P, Dohner H., German CLL Study Group (GCLLSG). Chronic lymphocytic leukemia. Genetics of chronic lymphocytic leukemia: genomic aberrations and V(H) gene mutation status in pathogenesis and clinical course. *Leukemia*. 2002;16:993-1007.
4. Stilgenbauer S, Lichter P, Dohner H. Genetic features of B-cell chronic lymphocytic leukemia. *Rev Clin Exp Hematol.* 2000;4:48-72.

Test Overview

Test Name	FISH for B-CLL
Reference Range	Disomic for each probe
Specimen Requirements	8 mL of whole blood in two lavender (EDTA) 4 mL tubes OR bone marrow in EDTA
Test Ordering Information	CLLFSH
CPT Code	88368 (x5)

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