MAYO CLINIC LABORATORIES

Test Definition: BLBLF

B-Cell Lymphoblastic Leukemia/Lymphoma, FISH, Tissue

Overview

Useful For

Detecting a neoplastic clone in paraffin-embedded specimens associated with the common chromosome abnormalities seen in patients with B-cell lymphoblastic leukemia/lymphoma

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
_IL25	Interphases, <25	No, (Bill Only)	No
_I099	Interphases, 25-99	No, (Bill Only)	No
_I300	Interphases, >=100	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
_PBCT	Probe, +2	No, (Bill Only)	No

Testing Algorithm

This test includes a charge for application of the first probe set (2 fluorescence in situ hybridization [FISH] probes) and professional interpretation of results. Additional charges will be incurred for all reflex probes performed. Analysis charges will be incurred based on the number of cells analyzed per probe set. If no cells are available for analysis, no analysis charges will be incurred.

This FISH test allows different combinations of probes to be utilized based on the patient's age and clinical question, including the standard (diagnostic) B-cell lymphoblastic lymphoma (B-LBL) FISH panel and the individual B-LBL FISH probes (per client request).

The FISH panel for patients **30 years and younger** includes testing for the following abnormalities using the FISH probes listed:
+9/9p-, CDKN2A/D9Z1
t(9;22) BCR/ABL1
11q23 rearrangement, MLL (KMT2A) break-apart
-17/17p-, TP53/D17Z1
t(1;19)(q23;p13), PBX1/TCF3
Hyperdiploidy, +4,+10,+17: D4Z1/D10Z1/D17Z1
t(12;21)(p13;q22), ETV6/RUNX1 fusion and iAMP21
14q32 rearrangement, IGH break-apart
8q24.1 rearrangement, MYC break-apart

If the initial FISH panel demonstrates normal or nonclassical abnormalities, the Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-like ALL) panel will be performed.

The Ph-like ALL panel includes testing for the following kinase activating chromosome abnormalities, using the FISH probes listed below:
1q25 rearrangement, ABL2 break-apart
5q32 rearrangement, PDGFRB break-apart
9p24.1 rearrangement, JAK2 break-apart
9q34 rearrangement, ABL1 break-apart

The initial FISH panel for patients **older than 30 years of age** includes testing for the following abnormalities using the FISH probes listed:
t(9;22) BCR/ABL1

If BCR/ABL1 fusion is not observed, the Ph-like ALL panel will be performed. The Ph-like ALL panel includes testing for the following kinase activating chromosome abnormalities, using the FISH probes listed below:
1q25 rearrangement, ABL2 break-apart
5q32 rearrangement, PDGFRB break-apart
9p24.1 rearrangement, JAK2 break-apart
9q34 rearrangement, ABL1 break-apart

If the previous FISH probe sets demonstrate normal or nonclassical abnormalities, the following probe sets will be performed:
t(1;19)(q23;p13), PBX1/TCF3 fusion
Hyperdiploidy, +4,+10,+17: D4Z1/D10Z1/D17Z1
t(12;21)(p13;q22), ETV6/RUNX1 fusion and iAMP21
14q32 rearrangement, IGH break-apart
11q23 rearrangement, MLL (KMT2A) break-apart

When an MLL (KMT2A) rearrangement is identified, reflex testing will be performed to identify the translocation partner. Probes include identification of:
t(4;11)(q21;q23) AFF1/MLL
t(6;11)(q27;q23) MLLT4(AFDN)/MLL
t(9;11)(p22;q23) MLLT3/MLL
t(10;11)(p12;q23) MLLT10/MLL
t(11;19)(q23;p13.1) MLL/ELL
t(11;19)(q23;p13.3) MLL/MLLT1.

In the absence of BCR/ABL1 fusion, when an extra ABL1 signal is identified, reflex testing will be performed using the ABL1 break-apart probe set to evaluate for the presence or absence of an *ABL1* rearrangement.

In the absence of ETV6/RUNX1 fusion, when an extra ETV6 signal is identified, reflex testing will be performed using the ETV6 break-apart probe set to evaluate for the presence or absence of an *ETV6* rearrangement.

If a MYC rearrangement is identified, both the BCL2 and BCL6 probe sets will be performed.

For more information, see B-Lymphoblastic Leukemia/Lymphoma Algorithm.

Special Instructions

- B-Lymphoblastic Leukemia/Lymphoma Algorithm

Method Name

Fluorescence In Situ Hybridization (FISH)

NY State Available

Yes

Specimen

Specimen Type

Tissue

Ordering Guidance

This test does not include a pathology consult. If a pathology consultation is requested, PATHC / Pathology Consultation should be ordered and the appropriate fluorescence in situ hybridization (FISH) test will be ordered and performed at an additional charge.

For testing non-paraffin bone marrow or blood samples from patients with B-cell acute lymphoblastic leukemia/lymphoma, order either BALPF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), FISH, Pediatric, Varies or BALAF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), FISH, Adult, Varies, depending on the patient's age. If non-paraffin bone marrow or blood sample is received for this test, it will be canceled, and either BALPF or BALAF, depending on patient's age, will be added and performed as the appropriate test.

For patients with B-cell lymphoma, order BLYM / B-Cell Lymphoma, FISH, Tissue.

Shipping Instructions

Advise Express Mail or equivalent if not on courier service.

Necessary Information

A reason for testing and pathology report are required for testing to be performed. Send information with specimen. Acceptable pathology reports include working drafts, preliminary pathology, or surgical pathology reports. The laboratory will not reject testing if this information is not provided; however. appropriate testing and interpretation may be compromised or delayed. If not provided, an appropriate indication for testing may be entered by Mayo Clinic Laboratories.

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Tissue
Preferred: Tissue block
Collection Instructions: Submit a formalin-fixed, paraffin-embedded tumor tissue block. Blocks prepared with alternative fixation methods may be acceptable; provide fixation method used.
Additional Information:
1. Paraffin-embedded specimens can be from any anatomic location (skin, soft tissue, lymph node, etc).
2. Bone specimens that have been decalcified will be attempted for testing, but the success rate is approximately 50%.

Acceptable: Tissue slides
Collection Instructions: 20 Consecutive, unstained, 5-micron thick sections placed on positively charged slides and 1 hematoxylin and eosin-stained slide.

Forms
If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-Hematopathology/Cytogenetics Test Request (T726)
-Children's Oncology Group Test Request (T829)

Specimen Minimum Volume
Fifteen consecutive, unstained, 5-micron thick sections placed on positively charged slides, and 1 hematoxylin and eosin-stained slide.

Reject Due To
All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information
In the United States, the incidence of B-lymphoblastic leukemia/lymphoma (B-ALL/LBL) is roughly 6000 new cases per year or approximately 1 in 50,000 individuals. B-ALL/LBL accounts for approximately 70% of all childhood leukemia cases (ages 0 to 19 years), making it the most common type of childhood cancer. It has a peak incidence at 2 to 5 years of age. This incidence decreases with age before increasing again at around 50 years of age. B-ALL/LBL is slightly more common in male patients than female patients. There is also an increased incidence of B-ALL/LBL in individuals with genetic conditions such as Down syndrome, Fanconi anemia, Bloom syndrome, ataxia telangiectasia, Li-Fraumeni syndrome, X-linked agammaglobulinemia, and severe combined immunodeficiency. The overall cure rate for B-ALL/LBL in children is approximately 90%, and about 45% to 60% of adults have long-term disease-free survival. Of note, CRLF2/IGH rearrangements are more commonly observed in patients with Down syndrome or of Hispanic descent.

Specific cytogenetic abnormalities are identified in the majority of cases of B-ALL/LBL, either by conventional

chromosome studies or fluorescence in situ hybridization studies. Each of the genetic subgroups is important to detect and can be critical prognostic markers. For example, a decision for early transplantation may be made if t(9;22)(q34;q11.2), KMT2A rearrangement, iAMP21, or a hypodiploid clone is identified. In contrast, if the ETV6/RUNX1 fusion or hyperdiploidy is identified, the patient has a more favorable prognosis and transplantation is rarely initially considered.

A newly recognized World Health Organization entity called BCR-ABL1-like ALL, also known as Philadelphia chromosome-like acute lymphoblastic leukemia, is increasing in importance due to the poor prognosis seen in pediatric, adolescent, and young adult ALL. Common features of this entity involve rearrangements with tyrosine kinase genes involving the following genes: *ABL2*, *PDGFRB*, *JAK2*, *ABL1*, *CRLF2*, and *P2RY8*, as well as deletions involving *IKZF1*. Patients who have failed conventional therapies have demonstrated favorable responses to targeted therapies when rearrangements involving these specific gene regions have been identified.

Evaluation of the *MYC* gene region is included in all diagnostic B-ALL panels to evaluate for Burkitt lymphoma. If a positive result is obtained, additional testing for the BCL2 and BCL6 gene regions will be performed.

Per National Comprehensive Cancer Network guidelines, a combination of cytogenetic and FISH testing is currently recommended in all pediatric and adult patients with B-ALL/LBL.

Table. Common Chromosome Abnormalities in B-cell Acute Lymphoblastic Leukemia/Lymphoma

Leukemia type	Cytogenetic change	Typical demographic	Risk category
B-cell acute lymphoblastic leukemia	t(12;21)(p13;q22), *ETV6/RUNX1*	Pediatric	Favorable
	Hyperdiploidy	Pediatric	Favorable
	t(1;19)(q23;p13.3), *PBX1/TCF3*	Pediatric	Intermediate to favorable
	t(9;22)(q34;q11.2), *BCR/ABL1*	All ages	Unfavorable
	iAMP21, *RUNX1*	Pediatric	Unfavorable
	del(9p), *CDKN2A*	All ages	Unknown
	t(11q23;var), *MLL*	All ages	Unfavorable
	t(4;11)(q21;q23), *AFF1/MLL*	All ages	Unfavorable
	t(6;11)(q27;q23), MLLT4(AFDN)/MLL	All ages	Unfavorable
	t(9;11)(p22;q23), *MLLT3/MLL*	All ages	Unfavorable
	t(10;11)(p12;q23), *MLLT10/MLL*	All ages	Unfavorable
	t(11;19)(q23;p13.1), *MLL/ELL*	All ages	Unfavorable
	t(11;19)(q23;p13.3), *MLL/MLLT1*	All ages	Unfavorable
	t(14q32;var), *IGH*	All ages	Variable
	-17/17p-, *TP53*	All ages	Unfavorable
	t(8q24.1;var), *MYC* **representing Burkitt or other mature B-cell lymphoma*	Pediatric/ adolescent/ young adult	
	Complex karyotype (> or =4	Adult	Unfavorable

	abnormalities)		
	Low hypodiploidy/near triploidy	Adult	Unfavorable
	Near-haploid/hypodiploid	All ages	Unfavorable
Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-like ALL)	t(1q25;var), *ABL2*	Pediatric/ adolescent/ young adult	Unfavorable
	t(5q32;var), *PDGFRB*		
	t(9p24.1;var), *JAK2*		
	t(9q34;var), *ABL1*		

Reference Values

An interpretive report will be provided.

Interpretation

A neoplastic clone is detected when the percent of cells with an abnormality exceeds the normal reference range for any given probe.

A positive result is not diagnostic for B-cell lymphoblastic lymphoma but may provide relevant prognostic information.

The absence of an abnormal clone does not rule out the presence of a neoplastic disorder.

Cautions

This test is not approved by the US Food and Drug Administration, and it is best used as an adjunct to clinical and pathologic information.

Fixatives other than formalin (eg, Prefer, Bouin's) may not be successful for fluorescence in situ hybridization (FISH) assays. Although FISH testing will not be rejected due to non-formalin fixation, results may be compromised.

Paraffin-embedded tissues that have been decalcified may be unsuccessful for FISH analysis.

FISH studies will be attempted if sufficient tumor is present for analysis. The pathologist reviewing the hematoxylin and eosin-stained slide may find it necessary to cancel testing. If no FISH signals are observed post-hybridization, the case will be released indicating a lack of FISH results.

Supportive Data

For each probe set, blinded fluorescence in situ hybridization analysis was performed on 20 to 25 normal paraffin-embedded, formalin-fixed tissue controls and between 2 and 20 paraffin-embedded, formalin-fixed tissue samples from patients diagnosed with B-cell lymphoblastic leukemia or lymphoma. Results from the 25 controls were used to generate the normal cutoff values.

Clinical Reference

1. Moorman AV, Harrison CJ, Buck GA, et al: Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. Blood 2007. Apr 15;109(8):3189-3197
2. Moorman AV: The clinical relevance of chromosomal and genetic abnormalities in B-cell precursor acute lymphoblastic leukemia. Blood Rev. 2012 May;26(3):123-135

3. Roberts KG, Li Y, Payne-Turner D, et al: Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014 Sept 11;371(11):1005-1015
4. Mullighan CG: The genomic landscape of acute lymphoblastic leukemia in children and young adults. Hematology Am Soc Hematol Educ Program. 2014 Dec 5;2014(1):174-180
5. Arber DA, Orazi A, Hasserjian R, et al: The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016 May 19;127(20):2391-2405
6. Swerdlow SH, Campo E, Harris NL, et al, eds: WHO Classification of Tumours. Vol 2.WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. IARC Press; 2017

Performance

Method Description

This test is performed using commercially available and laboratory-developed probes. Deletion of the *CDKN2A* locus on chromosome 9, *TP53* on chromosome 17, and gain of chromosomes 4, 10, and 17 are detected using enumeration strategy probes. Rearrangements involving *ABL2, PDGFRB, MYC, JAK2, ABL1, MLL, ETV6,* and *IGH* are detected using a dual-color break-apart (BAP) strategy probe. Dual-color, dual-fusion fluorescence in situ hybridization strategy probe sets are used to detect t(9;22), t(12;21), t(1;19), and in reflex testing when rearrangements of the *MLL* gene is detected. If a *MYC* gene region separation is identified, break-apart *BCL2* and *BCL6* will be evaluated using a dual-color BAP strategy probe.

Paraffin-embedded tissues are cut at 5 microns and mounted on positively charged glass slides. The selection of tissue and the identification of target areas on the hematoxylin and eosin (H and E) stained slide are performed by a pathologist. Using the H and E-stained slide as a reference, target areas are etched with a diamond-tipped etcher on the back of the unstained slide to be assayed. For each probe set, the probes are hybridized to the appropriate target areas and 2 technologists each analyze 50 interphase nuclei (100 total) per probe set with the results expressed as the percent abnormal nuclei.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

7 to 10 days

Specimen Retention Time

Slides and H and E used for analysis are retained by the laboratory in accordance to CAP and NYS requirements. Client provided paraffin blocks and extra unstained slides (if provided) will be returned after testing is complete.

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

88271 x 2, 88291-DNA probe, each (first probe set), interpretation and report
88271 x 2-DNA probe, each; each additional probe set (if appropriate)
88271-DNA probe, each; coverage for sets containing 3 probes (if appropriate)
88271 x 2-DNA probe, each; coverage for sets containing 4 probes (if appropriate)
88271 x 3-DNA probe, each; coverage for sets containing 5 probes (if appropriate)
88274 w/modifier 52-Interphase in situ hybridization, <25 cells, each probe set (if appropriate)
88274-Interphase in situ hybridization, 25 to 99 cells, each probe set (if appropriate)
88275-Interphase in situ hybridization, 100 to 300 cells, each probe set (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
BLBLF	B-Lymphoblastic Leuk/Lymph, FISH,Ts	In Process

Result ID	Test Result Name	Result LOINC® Value
609452	Result Summary	50397-9
609453	Interpretation	69965-2
609454	Result Table	93356-4
609455	Result	62356-1
GC057	Reason for Referral	42349-1
609456	Specimen	31208-2
609457	Source	31208-2
609458	Tissue ID	80398-1
609459	Method	85069-3
609460	Additional Information	48767-8
609461	Disclaimer	62364-5
609462	Released By	18771-6