MAYO CLINIC LABORATORIES

Test Definition: BLBLF

B-Cell Lymphoblastic Leukemia/Lymphoma, FISH, Tissue

Overview

Useful For

Detecting, at diagnosis, recurrent common chromosome abnormalities associated with B-cell acute lymphoblastic leukemia/lymphoma (B-ALL/LBL) and Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-like ALL) in paraffin-embedded specimens

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 the probe application, analysis, and professional interpretation of results for one probe set (2 individual fluorescence in situ hybridization [FISH] probes). Additional charges will be incurred for all reflex or additional probe sets performed. No analysis charges will be incurred if an insufficient number of representative cells are available for analysis.

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 initial (diagnostic) panel for patients aged **30 years or younger** includes testing for the following abnormalities using the FISH probes listed:
+9/9p-, CDKN2A/D9Z1
t(9;22)(q34;q11.2), 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
8q24.2 rearrangement, MYC break-apart

If results for the initial panel are negative or demonstrate nonclassical abnormalities, the Philadelphia chromosome-like

acute lymphoblastic leukemia (Ph-like ALL) panel will be performed as a secondary panel. The Ph-like ALL panel includes testing for the following kinase activating chromosome abnormalities, using the FISH probes listed below, as well as *IKZF1* deletion, which often accompanies Ph-like ALL.
1q25 rearrangement, ABL2 break-apart
5q32 rearrangement, PDGFRB break-apart
9p24.1 rearrangement, JAK2 break-apart
9q34 rearrangement, ABL1 break-apart

The FISH initial (diagnostic) panel for patients aged **31 years or older** includes testing with the following FISH probe:
t(9;22)(q34;q11.2), BCR/ABL1

If *BCR::ABL1* fusion is not observed, the Ph-like ALL panel will be performed as a secondary panel. 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

Finally, if results for the Ph-like panel are negative or demonstrate nonclassical abnormalities, the following probe sets will be performed as a tertiary panel:
t(1;19)(q23;p13), PBX1/TCF3 fusion
Hyperdiploidy, +4,+10,+17, D4Z1/D10Z1/D17Z1
t(12;21)(p13;q22) or iAMP21, ETV6/RUNX1
14q32 rearrangement, IGH break-apart
11q23 rearrangement, MLL (KMT2A) break-apart

Appropriate ancillary probes may be performed at consultant discretion to render comprehensive assessment. Any additional probes will have the results included within the final report and will be performed at an additional charge.

When an *MLL*(*KMT2A*) rearrangement is identified, appropriate reflex testing will be performed to identify the translocation partner. Probes include identification of t(4;11)(q21;q23) *AFF1*::*MLL(KMT2A)*, t(6;11)(q27;q23) *MLLT4(AFDN)*::*MLL(KMT2A)*, t(9;11)(p22;q23) *MLLT3*::*MLL(KM2TA)*, t(10;11)(p12;q23) *MLLT10*::*MLL(KMT2A)*, t(11;19)(q23;p13.3) *MLL(KMT2A)*::*MLLT1*, or t(11;19)(q23;p13.1) *MLL(KMT2A)*::*ELL.* In the event an 11q23 translocation is (or has been) identified by chromosome or FISH analysis, only the targeted *MLL(KMT2A)* reflex probe will be performed if applicable.

In the absence of *BCR::ABL1* fusion, when an extra ABL1 signal is identified, reflex testing may be performed at the laboratory's discretion using the ABL1 break-apart probe set to evaluate for the presence of a potential variant translocation involving *ABL1*, t(9;var)(q34;?). Laboratory discretion may be influenced by available karyotype or other FISH results.

In the absence of *ETV6::RUNX1* fusion, when an extra ETV6 signal is identified, reflex testing may be performed at the laboratory's discretion using the ETV6 break-apart probe set to evaluate for the presence or absence of a potential variant translocation involving *ETV6*, t(12;var)(p13;?). Laboratory discretion may be influenced by available karyotype or

other FISH results.

If a *MYC* rearrangement is identified, both the BCL2 and BCL6 break-apart 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 consultation. If a pathology consultation is requested, order PATHC / Pathology Consultation, and appropriate testing will be added at the discretion of the pathologist and performed at an additional charge.

For testing non-paraffin bone marrow or blood specimens from patients with B-cell acute lymphoblastic leukemia/lymphoma, order either BALPF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Pediatric, FISH, Varies or BALAF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), FISH, Adult, Varies, depending on the patient's age. If a non-paraffin embedded bone marrow or blood specimen is received for this test, this test 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
1. A pathology report is required for testing to be performed. If not provided, appropriate testing and/or interpretation may be compromised or delayed. Acceptable pathology reports include working drafts, preliminary pathology, or surgical pathology reports.
2. The following information must be included in the report provided.??
1. Patient name?
2. Block number - must be on all blocks, slides, and paperwork??

3. Date of collection?
4. Tissue source?

3. A reason for testing must be provided. If this information is not provided, an appropriate indication for testing may be entered by Mayo Clinic Laboratories.

Specimen Required

Submit only 1 of the following specimens:

Preferred

Specimen Type: 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

Specimen Type: Tissue slides

Slides: 1 Hematoxylin and eosin stained and 20 unstained

Collection Instructions: Submit 1 slide stained with hematoxylin and eosin and 20 consecutive 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:

-Hematopathology/Cytogenetics Test Request (T726)

-Children's Oncology Group Test Request (T829)

Specimen Minimum Volume

Slides: 1 Hematoxylin and eosin stained and 15 unstained

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. 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.

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/lymphoblastic lymphoma (LBL). Additional cytogenetic techniques, such as chromosomal microarray (CMAH / Chromosomal Microarray, Hematologic Disorders, Varies), may be helpful in resolving either questions related to ploidy (hyperdiploid clone vs doubled hypodiploid clone) or certain clonal structural rearrangements, such as the presence or absence of intra-chromosomal amplification of chromosome 21 (iAMP21). A summary of the characteristic chromosome abnormalities identified in B-ALL is listed in the following table.

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

Leukemia type	**Cytogenetic change**	**Typical demographic**	**Risk category**
B-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* rearrangement	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* rearrangement	All ages	Variable
	t(X;14)(p22;q32)/t(Y;14)(p11;q32), *CRLF2::IGH*	Adolescent/ young adult	Unfavorable
	t(Xp22.33;var) or t(Yp11.32;var), *CRLF2* rearrangement	All ages	Unfavorable
	t(Xp22.33;var) or t(Yp11.32;var), *P2RY8* rearrangement	All ages	Unfavorable
	-17/17p-, *TP53*	All ages	Unfavorable
	t(8q24.2;var), *MYC* rearrangement *representing Burkitt or other mature B-cell lymphoma	Pediatric/ adolescent/ young adult	
	Complex karyotype (> or =4 abnormalities)	Adult	Unfavorable
	Low hypodiploidy/near triploidy	Adult	Unfavorable
	Near-haploid/hypodiploid	All ages	Unfavorable

	del(7p) *IKZF1*	All ages	Unfavorable in absence of *ERG* deletion
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*		
	t(Xp22.33;var) or t(Yp11.32;var), *CRLF2*		
	t(Xp22.33;var) or t(Yp11.32;var), *P2RY8*		

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 set.

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 an acute B-cell lymphoblastic leukemia/lymphoma or another neoplastic disorder.

Cautions

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

Fixatives other than formalin (eg, Prefer, Bouin's) may not be successful for fluorescence in situ hybridization (FISH) assays. Non-formalin fixed specimens will not be rejected.

Paraffin-embedded tissues that have been decalcified may not be successful for FISH analysis. The success rate of FISH studies on decalcified tissue is approximately 50%.

FISH studies will be attempted if sufficient tumor is present for analysis. If insufficient tissue/tumor is available for testing, 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. WHO Classification of Tumours. Vol 2.

Performance

Method Description

This test is performed using commercially available and laboratory-developed probes. Deletion of the *CDKN2A* locus on chromosome 9, the *TP53* locus 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 are detected. If separation of the *MYC* gene is identified, break-apart *BCL2* and *BCL6* will be evaluated using a dual-color BAP strategy probe.

Paraffin-embedded tissue samples 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 engraving tool on the back of the unstained slide to be assayed. The probe set is hybridized to the appropriate target areas, and 2 technologists each independently analyze 50 interphase nuclei (100 total) with the results expressed as the percent of 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 with regulatory requirements. Client provided paraffin blocks and extra unstained slides 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. It 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	102100-5

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