Test Definition: BLBLF

B-Cell Lymphoblastic Leukemia/Lymphoma, FISH, Tissue

Overview

Useful For

Detecting 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) using **client-specified** probe set(s) in paraffin-embedded specimens

Evaluating specimens in which chromosome studies are unsuccessful

This test **should not be used** to screen for residual B-ALL/LBL.

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 1 probe set (2 individual fluorescence in situ hybridization [FISH] probes). Additional charges will be incurred for all reflex and ancillary probe sets. Analysis charges are based on the number of cells analyzed per probe. Analysis charges do not apply if no cells are available for evaluation.

This test may be ordered in 2 distinct ways allowing different combinations of probes to be analyzed based on the clinical question.
1. Standard (**diagnostic**) B-lymphoblastic leukemia/lymphoma (BLBL) FISH panel
2. Individual BLBL FISH probes chosen, per **client request**, from probes listed below

If individual BLBL FISH probes are wanted, the specific probes requested must be noted on the request form or in the reason for referral. If no FISH probes are indicated, the standard (diagnostic) panel will be performed.

The standard (**diagnostic**) panel for patients aged **30 years or younger** includes testing for the following abnormalities, using the FISH probes listed:
t(1;19)(q23;p13) or *TCF3::PBX1* fusion, request probe PBX1/TCF3
Hyperdiploidy or +4,+10,+17, request probe D4Z1/D10Z1/D17Z1
t(8;14)(q24.21;q32) or *IGH::MYC* fusion, request probe MYC/IGH

t(8q24.21;var) or *MYC* rearrangement, request probe MYC break-apart
t(9;22)(q34;q11.2) or *BCR::ABL1* fusion, request probe ABL1/BCR
t(11q23;var) or *KMT2A* rearrangement, request probe KMT2A break-apart
t(12;21)(p13;q22), *ETV6::RUNX1* fusion and iAMP21, request probe ETV6/RUNX1
t(14q32;var) or *IGH* rearrangement, request probe IGH 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:
t(1q25;var) or *ABL2* rearrangement, request probe ABL2 break-apart
t(5q32;var) or *PDGFRB* rearrangement, request probe PDGFRB break-apart
t(9p24.1;var) or *JAK2* rearrangement, request probe JAK2 break-apart
t(9q34;var) or *ABL1* rearrangement, request probe 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 following probe sets will be performed as a secondary panel:
t(1;19)(q23;p13) or *TCF3::PBX1* fusion, request probe PBX1/TCF3
t(1q25;var) or *ABL2* rearrangement, request probe ABL2 break-apart
Hyperdiploidy or +4,+10,+17, request probe D4Z1/D10Z1/D17Z1
t(5q32;var) or *PDGFRB* rearrangement, request probe PDGFRB break-apart
t(8;14)(q24.21;q32) or *IGH::MYC* fusion, request probe MYC/IGH
t(8q24.21;var) or *MYC* rearrangement, request probe MYC break-apart
t(9p24.1;var) or *JAK2* rearrangement, request probe JAK2 break-apart
t(9q34;var) or *ABL1* rearrangement, request probe ABL1 break-apart
t(11q23;var) or *KMT2A* rearrangement, request probe KMT2A break-apart
t(12;21)(p13;q22), *ETV6::RUNX1* fusion and iAMP21, request probe ETV6/RUNX1
t(14q32;var) or *IGH* rearrangement, request probe IGH break-apart

In the following situations, additional (reflex) testing may be performed at the laboratory's discretion and may be influenced by available karyotype results or other FISH testing.

When a *KMT2A* rearrangement is identified, testing with 1 or more dual-fusion FISH probe sets may be performed in an attempt to identify the translocation partner for the following abnormalities:
t(4;11)(q21;q23) or *KMT2A::AFF1* fusion, request probe AFF1/KMT2A
t(6;11)(q27;q23) or *KMT2A::AFDN* fusion, request probe AFDN/KMT2A
t(9;11)(p22;q23) or *KMT2A::MLLT3* fusion, request probe MLLT3/KMT2A
t(10;11)(p12;q23) or *KMT2A::MLLT10* fusion, request probe MLLT10/KMT2A
t(11;19)(q23;p13.1) or *KMT2A::MLLT1* fusion, request probe KMT2A/ELL
t(11;19)(q23;p13.3) or *KMT2A::ELL* fusion, request probe KMT2A/MLLT1

In the absence of *BCR::ABL1* fusion, when an extra ABL1 signal is identified, testing using the ABL1 break-apart probe set may be performed to evaluate for the presence of a potential variant translocation involving *ABL1*, t(9;var)(q34;?).

In the absence of *ETV6::RUNX1* fusion, when an extra ETV6 signal is identified, testing using the ETV6 break-apart probe set may be performed to evaluate for the presence or absence of a potential variant translocation involving *ETV6*, t(12;var)(p13;?).

When a *MYC* rearrangement is identified, both the BCL2 and BCL6 break-apart probe sets will be performed.

If an unbalanced *MYC* FISH result is identified (specifically 3' deletion with 5' retention), testing using IGK/MYC and MYC/IGL probes will be performed to assess for t(2;8)(p11;q24.21) *IGK*::*MYC* fusion or t(8;22)(q24.21;q11.2) *IGL*::*MYC* fusion.

If an unbalanced rearrangement of *BCL2* is identified, testing using the IGH/BCL2 probe set may be performed to identify a potential t(14;18)(q32;q21) or *IGH::BCL2* fusion.

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

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.

Mayo Clinic Hematopathology consultants are involved in the pre-analytic phase (tissue adequacy and probe selection, when applicable).

This test is **not appropriate** for testing blood and bone marrow from patients with B-lymphoblastic leukemia/lymphoma. If a non-paraffin embedded bone marrow or blood sample is received for this test, the test will be canceled and automatically reordered by the laboratory as BALAF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), FISH, Adult, Varies or BALFP / Pediatric B-Lymphoblastic Leukemia/Lymphoma Panel, FISH, Varies depending on the age of the patient.

Shipping Instructions

Advise Express Mail or equivalent if not on courier service.

Necessary Information

1. A list of probes requested for analysis is required. Probes available for this test are listed in the Testing Algorithm section.

2. 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.

3. The following information must be included in the report provided:

-Patient name

-Block number - **must be on all blocks, slides, and paperwork**

-Date of collection

-Tissue source

4. 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 (fresh tissue is **not acceptable**)

Collection Instructions:

1. Submit a formalin-fixed, paraffin-embedded tumor tissue block.
2. Blocks prepared with alternative fixation methods (eg, Prefer, Bouin's) will be attempted but are less favorable for successful results. Provide fixation method used.

Additional Information:

1. Paraffin-embedded specimens can be from any anatomic location (skin, soft tissue, lymph node, etc).
2. Decalcified paraffin-embedded specimens will have testing attempted; however, the success rate is approximately 50%. T**esting may be canceled** if sufficient tumor tissue is not presen**t**.
3. **Submitted fresh tissue specimens will be canceled upon receipt**. If only fresh tissue is available, embed in paraffin prior to sending.

Acceptable:

Specimen Type: Tissue slides

Slides: 1 Hematoxylin and eosin-stained and 2 unstained for each probe set

Collection Instructions:

1. If individual probe sets are chosen: Submit 1 slide stained with hematoxylin and eosin and, for each probe set ordered, submit 2 consecutive unstained, positively charged, unbaked slides with 5 micron-thick sections of the tumor tissue.
2. If a complete B-lymphoblastic leukemia/lymphoma (BLBL) panel is ordered: 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.

Additional Information: Broken or cracked unstained slides will be rejected. Stained slides that were broken and

repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

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 2 unstained per probe set requested

Reject Due To

Broken or cracked unstained slides	Reject
Broken or cracked stained slides that cannot be repaired	Reject

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. This incidence decreases with age before increasing again at around 50 years.

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. 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 intrachromosomal 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/lymphoma	t(12;21)(p13;q22), *ETV6::RUNX1*	Pediatric	Favorable
	Hyperdiploidy	Pediatric	Favorable
	t(1;19)(q23;p13.3), *TCF3::PBX1*	Pediatric	Intermediate to favorable
	t(9;22)(q34;q11.2), *BCR::ABL1*	All ages	Unfavorable
	iAMP21, *RUNX1*	Pediatric	Unfavorable
	t(11q23;var), *KMT2A* rearrangement	All ages	Unfavorable
	t(4;11)(q21;q23), *KMT2A::AFF1*	All ages	Unfavorable
	t(6;11)(q27;q23), *KMT2A::AFDN*	All ages	Unfavorable
	t(9;11)(p21.3;q23), *KMT2A::MLLT3*	All ages	Unfavorable
	t(10;11)(p12;q23), *KMT2A::MLLT10*	All ages	Unfavorable
	t(11;19)(q23;p13.3), *KMT2A::MLLT1*	All ages	Unfavorable
	t(11;19)(q23;p13.1), *KMT2A::ELL*	All ages	Unfavorable
	t(14q32;var), *IGH* rearrangement	All ages	Variable
	t(X;14)(p22;q32)/t(Y;14)(p11;q32), *IGH::CRLF2*	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
	t(8q24.21;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* deletion	All ages	Unfavorable in absence of *ERG* deletion
BCR::ABL1-like acute lymphoblastic leukemia/lymphoma	t(1q25;var), *ABL2* rearrangement	Pediatric/ adolescent/ young adult	Unfavorable
	t(5q32;var), *PDGFRB* rearrangement		
	t(9p24.1;var), *JAK2* rearrangement		
	t(9q34;var), *ABL1* rearrangement		
	t(Xp22.33;var) or t(Yp11.32;var), *CRLF2* rearrangement		

	t(Xp22.33;var) or t(Yp11.32;var), *P2RY8* rearrangement		

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.

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 existing clinical and pathologic information.

This fluorescence in situ hybridization (FISH) assay does not rule out other chromosome abnormalities.

Fixatives other than formalin (eg, Prefer, Bouin's) may not be successful for 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%, but FISH will be attempted if sufficient tumor is present for 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 insufficient tissue/tumor is available for testing.

If no FISH signals are observed post hybridization, the case will be released indicating a lack of FISH results.

Broken or cracked unstained slides are not compatible with the instrumentation used in our performing laboratories and will be rejected. Additional material may be requested.

Attempts will be made to repair broken or cracked stained slides; however, if they are unable to be repaired (ie, shattered in transit), they will be rejected and discarded due to safety concerns.

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;109(8):3189-3197
2. Moorman AV. The clinical relevance of chromosomal and genomic abnormalities in B-cell precursor acute lymphoblastic leukaemia. Blood Rev. 2012;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;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;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;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 fluorescence in situ hybridization (FISH) probes. Deletion of *IKZF1* on chromosome 7 and gain or losses of chromosomes 4, 10, and 17 are detected using enumeration strategy probes. Rearrangements involving *CRLF2, ABL2, BCL6, PDGFRB, MYC, JAK2*, *ABL1, KMT2A, IGH,* and *BCL2* are detected using dual-color break-apart (BAP) strategy probes. Dual-color, dual-fusion fluorescence in situ hybridization (D-FISH) strategy probe sets are used to detect t(X/Y;14), t(1;19), t(2;8), t(8;14), t(8;22), t(9;22), t(12;21), t(14;18) and in reflex testing when a rearrangement of the *KMT2A* gene is detected. Amplification of the *RUNX1* gene region is detected using a D-FISH probe to enumerate copies of the RUNX1 probe. For enumeration and BAP strategy probe sets, 100 interphase nuclei are scored; 200 interphase nuclei are scored when D-FISH probes are used. Results are expressed as the percent abnormal nuclei.(Unpublished Mayo method)

Formalin-fixed, paraffin-embedded tissues are cut at 5 microns and mounted on positively charged glass slides. The selection of tissue and the target areas on the hematoxylin and eosin (H and E)-stained slide is 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. The probe set is hybridized to the appropriate target areas and 2 technologists each 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 used for analysis are retained by the laboratory in accordance with regulatory requirements. Client provided paraffin blocks and extra unstained slides (if provided) will be returned after testing is complete.

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

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