MAYO CLINIC LABORATORIES

Test Definition: BALAF

B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), FISH, Adult, Varies

Overview

Useful For

Detecting a neoplastic clone associated with the common chromosome abnormalities and classic rearrangements seen in adult patients with B-cell acute lymphoblastic leukemia/lymphoma (B-ALL/LBL)

An adjunct to conventional chromosome studies for patients with B-ALL/LBL

Evaluating specimens in which standard cytogenetic analysis is unsuccessful

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
BALAB	Probe, Each Additional (BALAF)	No, (Bill Only)	No
BAL3B	Probe, Tri-color (BAL)	No, (Bill Only)	No

Testing Algorithm

This test includes a charge for the probe application, analysis, and professional interpretation of results for 2 probe sets (4 individual fluorescence in situ hybridization [FISH] probes). Additional charges will be incurred for all reflex or additional probe sets performed.

The initial panel includes testing for the following abnormalities using the probes listed:
t(9;22), BCR/ABL1
t(X;14)(p22.33;q32)/ t(Y;14)(p11.32;q32), CRLF2/IGH

If results for the initial panel are negative, the following reflex probe sets will be performed as a secondary panel:
1q25 rearrangement, ABL2 break-apart
5q32 rearrangement, PDGFRB break-apart
9p24.1 rearrangement, JAK2 break-apart
9q34 rearrangement, ABL1 break-apart
t(Xp22.33;var) or t(Yp11.32;var), CRLF2 rearrangement
t(Xp22.33;var) or t(Yp11.32;var), P2RY8 rearrangement

Finally, if results for the secondary panel are negative, 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), ETV6/RUNX1 & iAMP21
14q32 rearrangement, IGH break-apart
11q23 rearrangement, MLL(KMT2A) break-apart
7p-, IKZF1/CEP7

When a KMT2A (MLL) rearrangement is identified, reflex testing will be performed to identify the translocation partner. Probes include identification of:
t(4;11)(q21;q23) AFF1/MLL
MLLT4(AFDN)/MLL
t(6;11)(q27;q23)
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

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

Special Instructions

- B-Lymphoblastic Leukemia/Lymphoma Algorithm
- Acute Leukemias of Ambiguous Lineage Testing Algorithm

Method Name

Fluorescence In Situ Hybridization (FISH)

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

This test is only performed on specimens from patients with B-cell acute lymphoblastic leukemia/lymphoma (B-ALL/LBL) who are 31 years of age or older.

This test is intended to be ordered when the entire B-ALL/LBL fluorescence in situ hybridization (FISH) panel is needed for an **adult** patient.
-If this test is ordered on a patient 30 years of age or younger, this test will be canceled and automatically reordered by the laboratory as BALPF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Pediatric, FISH, Varies.
-If this test is ordered and the laboratory is informed that the patient is on a Children's Oncology Group (COG) protocol, this test will be canceled and automatically reordered by the laboratory as COGBF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Children's Oncology Group Enrollment Testing, FISH, Varies

If PHLDF / Philadelphia Chromosome-like Acute Lymphoblastic Leukemia (Ph-like ALL), Diagnostic FISH, Varies, is ordered concurrently with this test, PHLDF testing will be canceled. The probes offered in PHLDF are included within this test, when appropriate.

If limited B-cell ALL FISH probes are preferred, order BALMF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Specified FISH, Varies.

At follow-up, conventional cytogenetic studies (CHRBM / Chromosome Analysis, Hematologic Disorders, Bone Marrow) and targeted B-ALL FISH probes can be evaluated based on the abnormalities identified in the diagnostic study. Order BALMF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Specified FISH, Varies and request specific probes or abnormalities.

If the patient clinically relapses, a conventional chromosome study may be useful to identify cytogenetic changes in the neoplastic clone or the possible emergence of a new therapy-related myeloid clone.

For patients with B-cell lymphoma, order BLPMF / B-Cell Lymphoma, Specified FISH, Varies.

For testing paraffin-embedded tissue samples from patients with B-ALL/LBL, order BLBLF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma, FISH, Tissue. If a paraffin-embedded tissue sample is submitted for this test, it will be canceled and BLBLF will be added and performed as the appropriate test.

Additional Testing Requirements

At diagnosis, conventional cytogenetic studies (CHRBM / Chromosome Analysis, Hematologic Disorders, Bone Marrow) and this panel should be performed per National Comprehensive Cancer Network guidelines. If there is limited specimen available, only this test will be performed.

Shipping Instructions

Advise Express Mail or equivalent if not on courier service.

Necessary Information

A reason for testing and a flow cytometry and/or bone marrow pathology report should be submitted with each specimen. The laboratory will not reject testing if this information is not provided; however, appropriate testing and interpretation may be compromised or delayed in some instances.If 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: Bone marrow
Container/Tube:
Preferred: Yellow top (ACD)
Acceptable: Green top (heparin) or lavender top (EDTA)
Specimen Volume: 2-3 mL
Collection Instructions:
1. It is preferable to send the first aspirate from the bone marrow collection.
2. Invert several times to mix bone marrow.
3. Send bone marrow specimen in original tube. **Do not aliquot.**

Acceptable
Specimen Type: Whole blood
Container/Tube:
Preferred: Yellow top (ACD)
Acceptable: Green top (heparin) or lavender top (EDTA)
Specimen Volume: 6 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**

Forms

If not ordering electronically, complete, print, and send a Hematopathology/Cytogenetics Test Request (T726) with the specimen.

Specimen Minimum Volume

Blood: 2 mL
Bone Marrow: 1 mL

Reject Due To

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

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

In the United States, the incidence of acute lymphoblastic leukemia (ALL) is roughly 6000 new cases per year (as of 2019). ALL accounts for approximately 70% of all childhood leukemia cases (ages 0-19 years), making it the most common type of childhood cancer. Approximately 85% of pediatric cases of ALL are of B-cell lineage (B-ALL) and 15% are of T-cell lineage. It has a peak incidence at 2 to 5 years of age. The incidence decreases with increasing age, before increasing again at around 50 years of age. ALL is slightly more common in male patients than female patients. There is an increased incidence of ALL in individuals with Down syndrome, Fanconi anemia, Bloom syndrome, ataxia telangiectasia, X-linked agammaglobulinemia, and severe combined immunodeficiency. The overall cure rate for ALL in children is about 90%, and about 45% to 60% of adults have long-term disease-free survival. *CRLF2/IGH* rearrangements are more commonly observed in patients with Down syndrome or of Hispanic descent.

Specific genetic abnormalities are identified in the majority of cases of B-ALL, either by conventional chromosome studies or fluorescence in situ hybridization (FISH) studies. Each of the B-ALL genetic subgroups is important to detect and can be critical prognostic markers. The decision for early transplantation may be made if t(9;22)(q34;q11.2), *MLL*

(*KMT2A*) translocations, *RUNX1* duplication/amplification (iAMP21) or a hypodiploid clone is identified. In contrast, if the *ETV6/RUNX1* fusion is detected by FISH or hyperdiploidy is identified by chromosome studies, the patient has a favorable prognosis and transplantation is rarely considered.

A newly recognized World Health Organization entity *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.* Deletion of *IKZF1* often accompanies this entity. Some patients who have failed conventional therapies have demonstrated favorable responses to targeted therapies in clinical trials 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/lymphoblastic lymphoma (LBL). Additional cytogenetic techniques such as chromosomal microarray (CMAH / Chromosomal Microarray, Hematologic Disorders, Varies) may be helpful to resolve questions related to ploidy (hyperdiploid clone vs doubled hypodiploid clone) or to resolve 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. 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*	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
	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*	All ages	Unfavorable
	t(Xp22.33;var) or t(Yp11.32;var), *P2RY8*	All ages	Unfavorable
	-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 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.

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

Fluorescence in situ hybridization (FISH) is not a substitute for conventional chromosome studies because the latter detects chromosome abnormalities associated with other hematological disorders that would be missed by this FISH panel test.

Bone marrow is the preferred specimen type for this FISH test. If bone marrow is not available, a blood specimen may be used if there are malignant cells in the blood specimen (as verified by a hematopathologist).

Supportive Data

Each probe was independently tested and verified on unstimulated peripheral blood and bone marrow specimens.

Normal cutoffs were calculated based on the results of 25 normal specimens. Each probe set was evaluated to confirm the probe set detected the abnormality it was designed to detect.

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. doi: 10.1182/blood-2006-10-051912
2. Moorman AV: The clinical relevance of chromosomal and genetic abnormalities in B-cell precursor acute lymphoblastic leukemia. Blood Rev. 2012;26:123-135. doi: 10.1016/j.blre.2012.01.001
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;371(11):1005-1015. i: 10.1056/NEJMoa1403088
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. doi: 10.1182/asheducation-2014.1.174
5. 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, deletion of *IKZF1* on chromosome 7, and gain of chromosomes 4, 10, and 17 are detected using enumeration strategy probes. Rearrangements involving *ABL2, PDGFRB, MYC, JAK2*, *ABL1, MLL, IGH*, *CRLF2,* and *P2RY8* are detected using a dual-color break-apart (BAP) strategy probe. Dual-color, dual-fusion fluorescence in situ hybridization (D-FISH) strategy probe sets are used to detect t(X/Y;14), t(9;22), t(12;21), t(1;19), and in reflex testing when rearrangements of the *MLL* and *IGH* genes are detected. Amplification of *RUNX1* (21q22) is detected using a D-FISH probe strategy. 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)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

7 to 10 days

Specimen Retention Time

4 weeks

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 x4,88275 x2, 88291 - FISH Probe, Analysis, Interpretation; 2 probe sets
88271 x2, 88275 - FISH Probe, Analysis; each additional probe set (if appropriate)
88271 - FISH Probe (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
BALAF	Adult ALL (B-cell), FISH	In Process

Result ID	Test Result Name	Result LOINC® Value
609538	Result Summary	50397-9
609539	Interpretation	69965-2
609540	Result Table	93356-4
609541	Result	62356-1
GC065	Reason for Referral	42349-1
GC066	Specimen	31208-2
609542	Source	31208-2
609543	Method	85069-3
609544	Additional Information	48767-8
609545	Disclaimer	62364-5
609546	Released By	18771-6