# Test Definition: BALAF

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

## 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 adult patients

As an adjunct to conventional chromosome studies in patients with B-ALL/LBL

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 |
|---|---|---|---|
| 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 (diagnostic) adult B-cell acute lymphoblastic leukemia (B-ALL) FISH panel includes testing for the following abnormalities using the FISH probes listed:
t(9;22)(q34;q11.2), BCR/ABL1
t(X;14)(p22.33;q32)/ t(Y;14)(p11.32;q32), CRLF2/IGH

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
t(Xp22.33;var) or t(Yp11.32;var) rearrangement, CRLF2 break-apart
t(Xp22.33;var) or t(Yp11.32;var) rearrangement, P2RY8 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
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
7p-, IKZF1/CEP7

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 analysis, only the targeted *MLL(KMT2A)* reflex probe will be performed if applicable.

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.

For more information see B-Lymphoblastic Leukemia/Lymphoma Genetic Testing Guidelines.

### Special Instructions

- B-Lymphoblastic Leukemia/Lymphoma Genetic Testing Guidelines
- 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 for instances when the entire B-ALL/LBL fluorescence in situ hybridization (FISH) panel is needed for an **adult** patient.

This test **should NOT be used** to screen for residual B-cell acute lymphoblastic leukemia/lymphoma (B-ALL/LBL).

If using FISH to monitor B-ALL patients, it is recommended to use individual (or limited) FISH probe sets.

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

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 30 years of age or younger AND is on a Children's Oncology Group 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 either or both AMLAF / Acute Myeloid Leukemia (AML), Specified, FISH, Adult, Varies; or TALAF / T-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Adult, FISH, Varies, are ordered concurrently with this test, the laboratory may cancel this test and automatically reorder as BALMF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Specified FISH, Varies with the following FISH probes: ETV6/RUNX1, PBX1/TCF3, 4/10/17, break-apart IGH, break-apart CRLF2, break-apart P2RY8, break-apart ABL2, and IKZF1/cep7. If an abnormality is identified that would result in reflex testing in BALAF, the same reflex testing will be performed in the BALMF. This cancellation is necessary to avoid duplicate testing. Probes for break-apart PDGFRB, break-apart JAK2, CDKN2A/D9Z1, ABL1/BCR, break-apart ABL1, break-apart MLL, TP53/D17Z1 will still be performed as part of the adult T-ALL FISH panel.

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. This cancellation is necessary to avoid duplicate testing as PHLDF probes are included within this test when appropriate.

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 both 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

1. A reason for testing and a flow cytometry and/or a 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/or interpretation may be compromised or delayed in some instances. If not provided, an appropriate indication for testing may be entered by Mayo Clinic Laboratories.
2. If the patient has received a bone marrow transplant from a person of an opposite sex, note this information on the request.

## 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 to 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

Whole 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 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. 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* fusion is 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, by conventional chromosome studies and/or fluorescence in situ hybridization (FISH) studies. B-ALL genetic subgroups are important to detect and can be critical prognostic markers. For example, a decision for early transplantation may be made if *BCR::ABL1* fusion, *KMT2A* rearrangement, iAMP21, or a hypodiploid clone is identified. In contrast, if *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 pediatric B-ALL panels to evaluate for Burkitt lymphoma. If a positive result is obtained, additional testing for the *BCL2* and *BCL6* gene regions may be considered.

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.

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

| Leukemia type | Cytogenetic change | Typical demographic | Risk category |
|---|---|---|---|
| B-acute | t(12;21)(p13;q22), *ETV6::RUNX1* | Pediatric | Favorable |

| | | | |
|---|---|---|---|
| lymphoblastic leukemia | 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.

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 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 in a targeted B-ALL 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 circulating malignant cells in the blood specimen (as verified by a hematopathologist).

### 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. 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;371(11):1005-1015. doi: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;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 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, *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 set 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)

### PDF Report
No

### Day(s) Performed
Monday through Friday

### Report Available
7 to 10 days

### Specimen Retention Time
4 weeks

### 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 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 | 102099-9 |

| 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 |