MAYO CLINIC LABORATORIES

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 BCR::*ABL1*-like B-ALL in adult patients using a laboratory-designated probe set algorithm

As an adjunct to conventional chromosome studies in adult 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 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 is performed as panel testing only using the following analysis algorithm.

The **diagnostic** adult B-lymphoblastic leukemia (B-ALL) FISH panel includes testing for the following abnormalities using the FISH probes listed:
t(9;22)(q34;q11.2) or *BCR::ABL1* fusion, ABL1/BCR probe set
CRLF2 (Xp22.33) or (Yp11.32) rearrangement, CRLF2 break-apart probe set

If results for the initial panel are negative or demonstrate nonclassical abnormalities, the following 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, ABL2 break-apart probe set
t(5q32;var) or *PDGFRB* rearrangement, PDGFRB break-apart probe set
t(9p24.1;var) or *JAK2* rearrangement, JAK2 break-apart probe set
t(9q34;var) or *ABL1* rearrangement, ABL1 break-apart probe set

Finally, if results for the initial and secondary panels are negative or demonstrate nonclassical abnormalities, the following probe sets will be performed as a tertiary panel:
t(1;19)(q23;p13) or *TCF3::PBX1* fusion, PBX1/TCF3 probe set
Hyperdiploidy or +4,+10,+17, D4Z1/D10Z1/D17Z1 probe set
7p- or *IKZF1* deletion, IKZF1/CEP7 probe set
t(8;14)(q24.21;q32) or *IGH::MYC* fusion, MYC/IGH probe set
t(8q24.21;var) or *MYC* rearrangement, MYC break-apart probe set
t(11q23;var) or *KMT2A* rearrangement, KMT2A break-apart probe set
t(12;21)(p13;q22) or *ETV6::RUNX1* fusion or iAMP21, ETV6/RUNX1 probe set
t(14q32;var) or *IGH* rearrangement, IGH break-apart probe set

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, appropriate reflex testing with 1 or more dual-fusion FISH (D-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, AFF1/KMT2A probe set
t(6;11)(q27;q23) or *KMT2A::AFDN* ;fusion, AFDN/KMT2A probe set
t(9;11)(p22;q23) or *KMT2A::MLLT3* fusion, MLLT3/KMT2A probe set
t(10;11)(p12;q23) or *KMT2A::MLLT10* fusion, MLLT10/KMT2A probe set
t(11;19)(q23;p13.1) or *KMT2A::MLLT1* fusion, KMT2A/ELL probe set
t(11;19)(q23;p13.3) or *KMT2A::ELL* fusion, KMT2A/MLLT1 probe set

When an unbalanced *CRLF2* rearrangement is identified concurrently with an IGH rearrangement, testing using the CRLF2/IGH probe set will be considered to identify a potential t(X;14)(p22.33;q32) or t(Y;14)(p11.32;q32) cryptic translocation.

When a *MYC* rearrangement is identified, testing using 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 will be performed to identify a potential t(14;18)(q32;q21) or *IGH::BCL2* fusion.

Appropriate ancillary probes may be performed at the consultant's 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 aged 31 years 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-ALL/LBL. At follow-up, or if the patient clinically relapses, conventional cytogenetic studies (CHRBM / Chromosome Analysis, Hematologic Disorders, Bone Marrow) are useful to identify cytogenetic changes in the neoplastic clone or the possible emergence of a new therapy-related myeloid clone. Additionally, targeted B-ALL FISH probes can be evaluated based on the abnormalities identified in the diagnostic study.

If targeted 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 this test is ordered on a patient aged 30 years or younger, this test will be canceled and automatically reordered by the laboratory as BALFP / Pediatric B-Lymphoblastic Leukemia/Lymphoma panel, 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 either AMLFA / Adult Acute Myeloid Leukemia Panel, FISH, Varies or TALAF / T-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), FISH, Adult, 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 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, BCR::ABL1 fusion, break-apart ABL1, and break-apart KMT2A will still be performed as part of

the adult T-ALL FISH panel.

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

For testing paraffin-embedded tissue samples from patients with B-LBL, order BLBLF / B-Cell 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 fluorescence in situ hybridization panel should be performed. 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 must be provided. If this information is not provided, an appropriate indication for testing may be entered by Mayo Clinic Laboratories.
2. 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, but appropriate testing and interpretation may be compromised or delayed.
3. If the patient has received an opposite sex bone marrow transplant, note this information on the request.
4. If the patient has Down syndrome, 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 (sodium 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 (sodium 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

Bone marrow: 1 mL; Whole blood: 2 mL

Reject Due To

Fresh tissue	Reject

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 age 50. 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 the, *IGH::CRLF2* fusion is more commonly observed in patients with Down syndrome or of Hispanic descent.

Specific cytogenetic abnormalities are identified in most cases of B-ALL/LBL, by conventional chromosome studies 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 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 B-ALL with BCR::ABL1-like features 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/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 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(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 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-cell acute lymphoblastic leukemia 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). If no FISH signals are observed post-hybridization, the case will be released indicating a lack of FISH results.

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 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)

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