Test Definition: BALPF

B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Pediatric, FISH, 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 pediatric patients

As an adjunct to conventional chromosome studies for pediatric 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
BALPB	Probe, Each Additional (BALPF)	No, (Bill Only)	No

Testing Algorithm

This test includes a charge for the probe application, analysis, and professional interpretation of results for 11 probe sets (23 individual fluorescence in situ hybridization [FISH] probes). Additional charges will be incurred for all reflex or additional probe sets performed.

The initial (diagnostic) pediatric/young adult B-cell acute lymphoblastic leukemia (B-ALL) FISH panel 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 or +4,+10,+17, D4Z1/D10Z1/D17Z1
t(12;21)(p13;q22) or iAMP21, ETV6/RUNX1
14q32 rearrangement, IGH 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
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
7p-, IKZF1/CEP7

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

When an *IGH* or *CRLF2* rearrangement is identified, appropriate reflex testing will be performed using the CRLF2/IGH probe set will be considered at the laboratory's discretion to identify a potential t(X;14)(p22.33;q32) or t(Y;14)(p11.32;q32) cryptic translocation. Laboratory discretion may be influenced by available karyotype or other FISH results.

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 or absence of a potential rearrangement 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 rearrangement 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 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 30 years of age or younger.

This test is intended to be ordered when the entire B-ALL fluorescence in situ hybridization (FISH) panel is needed for a **pediatric** 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 31 years of age or older, this test will be canceled and automatically reordered by the laboratory as BALAF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Adult, 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, Pediatric, Varies.

If either (or both) AMLPF / Acute Myeloid Leukemia (AML), Specified FISH, Varies; or TALPF / T-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Pediatric, FISH, Varies, is (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 MYC, break-apart ABL2, break-apart PDGFRB, break-apart JAK2, break-apart ABL1, and IKZF1/cep7. If an abnormality is identified that would result in reflex testing in BALPF, the same reflex testing will be performed in the BALMF. This cancellation is necessary to avoid duplicate testing. Probes for CDKN2A/D9Z1, ABL1/BCR,

break-apart MLL, TP53/D17Z1 will still be performed as part of the pediatric 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-cell acute lymphoblastic lymphoma, 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 test should both be performed. If there is limited specimen available, this test only 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 an opposite sex bone marrow transplant, 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 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 *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 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 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*	All ages	Unfavorable

	rearrangement		
	-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
2. Moorman AV. The clinical relevance of chromosomal and genetic abnormalities in B-cell precursor acute lymphoblastic leukemia. Blood Rev. 2012;26:123-135
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. 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, ETV6, IGH*, *MYC, 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* gene is detected. If a *MYC* gene region separation is identified, break-apart *BCL2* and *BCL6* will be evaluated using a dual-color BAP strategy probe. 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 x 23, 88275 x 11, 88291 x 1-FISH Probe, Analysis, Interpretation; 11 probe sets
88271 x 2, 88275 x 1-FISH Probe, Analysis; each additional probe set (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
BALPF	Pediatric ALL (B-cell), FISH	102099-9

Result ID	Test Result Name	Result LOINC® Value
609548	Result Summary	50397-9
609549	Interpretation	69965-2
609550	Result Table	93356-4
609551	Result	62356-1
GC068	Reason for Referral	42349-1
GC069	Specimen	31208-2
609552	Source	31208-2
609553	Method	85069-3
609554	Additional Information	48767-8
609555	Disclaimer	62364-5
609556	Released By	18771-6