MAYO CLINIC LABORATORIES

Test Definition: COGBF

B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Children's Oncology Group Enrollment Testing, FISH, Varies

Overview

Useful For

Evaluation of pediatric bone marrow and peripheral blood specimens by fluorescence in situ hybridization probe analysis for classic rearrangements and chromosomal copy number changes associated with B-cell acute lymphoblastic leukemia/lymphoma (B-ALL) and Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-like ALL) in patients being considered for enrollment in Children's Oncology Group (COG) clinical trials and research protocols

As an adjunct to conventional chromosome studies in performed in pediatric patients with B-ALL and Ph-like ALL being considered for enrollment in COG protocols

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
COGBB	Probe, Each Additional (COGBF)	No, (Bill Only)	No

Testing Algorithm

This test is only performed on specimens from pediatric patients being considered for enrollment in a Children's Oncology Group (COG) protocol.

The FISH panel includes testing for the following abnormalities using the fluorescence in situ hybridization (FISH) probes listed:
+9/9p-, CDKN2A/D9Z1
t(9;22) BCR/ABL1
11q23 rearrangement, MLL (KMT2A) break-apart
-17/17p-, TP53/D17Z1
t(1;19)(q23;p13), PBX1/TCF3
Hyperdiploidy, +4,+10,+17: D4Z1/D10Z1/D17Z1
t(12;21)(p13;q22), ETV6/RUNX1 fusion and iAMP21
14q32 rearrangement, IGH break-apart
t(Xp22.33;var) or t(Yp11.32;var), P2RY8 rearrangement
t(Xp22.33;var) or t(Yp11.32;var), CRLF2 rearrangement
8q24.1 rearrangement, MYC break-apart

If the FISH panel demonstrates normal or nonclassical abnormalities, BALPF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), FISH, Pediatric Varies panel will be performed.

The Philadelphia chromosome-like acute lymphoblastic leukemia (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, reflex testing will be performed to identify the translocation partner. Probes include identification of:
t(4;11)(q21;q23) AFF1/MLL
t(6;11)(q27;q23) MLLT4(AFDN)/MLL
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

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

In the absence of *BCR::ABL1* fusion, when an extra ABL1 signal is identified, reflex testing will be performed using the ABL1 break-apart probe set to evaluate for the presence or absence of an *ABL1* rearrangement.

In the absence of *ETV6::RUNX1* fusion, when an extra ETV6 signal is identified, reflex testing will be performed using the ETV6 break-apart probe set to evaluate for the presence or absence of an *ETV6* rearrangement.

If a *MYC* rearrangement is identified, both the BCL2 and BCL6 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

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 pediatric patients being considered for enrollment in a Children's Oncology Group (COG) protocol. If this test is ordered and the laboratory is informed that the patient is not on a COG protocol, this test will be canceled and automatically reordered by the laboratory as BALPF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), FISH, Pediatric Varies.

At follow-up, conventional cytogenetic studies (CHRBM / Chromosome Analysis, Hematologic Disorders, Bone Marrow) and targeted B-cell ALL fluorescence in situ hybridization (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.

Shipping Instructions
Advise Express Mail or equivalent if not on courier service.

Necessary Information
1. A reason for testing, a flow cytometry and/or a bone marrow pathology report, and a Children's Oncology Group (COG) registration number and protocol number 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. If not provided, an appropriate indication for testing may be entered by Mayo Clinic Laboratories.
2. If a patient has received an opposite sex bone marrow transplant prior to specimen collection for this protocol, 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: 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 aChildren's Oncology Group Test Request (T829) 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 B-cell lineage (B-ALL) and 15% are T-cell lineage (T-ALL). 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. For more than 25 years, the Mayo Clinic Genomics Laboratory has served as a Children's Oncology Group (COG) accredited laboratory for the performance of cytogenetic testing in pediatric patients being considered for enrollment in COG clinical trials and research. The laboratory is highly equipped to perform the time sensitive and critical cytogenetic testing necessary to assign risk stratification and facilitate enrollment in COG protocols.

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 (Ph-like ALL), 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.

Metaphase FISH confirmation of classic translocations that are cryptic and not visually detectable by chromosome analysis [ie, t(12;21) associated with *ETV6/RUNX1* fusion] is performed, as required by COG, and is included as part of the electronic case submission by the Mayo Clinic Genomics Laboratory to COG for central review.

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-cell acute lymphoblastic leukemia	t(12;21)(p13;q22), *ETV6(TEL)/RUNX1(AML1)*	Pediatric	Favorable
	Hyperdiploidy	Pediatric	Favorable
	t(1;19)(q23;p13.3), *PBX1/TCF3*	Pediatric	Intermediate
	t(9;22)(q34;q11.2), *BCR/ABL1*	Pediatric/adult	Unfavorable
	iAMP21, *RUNX1*	Pediatric	Unfavorable

	del(9p), *CDKN2A(p16)*	All ages	Unknown
	t(11q23;var), *MLL*	All ages	Unfavorable
	t(4;11)(q21;q23), *AFF1(AF4)/MLL*	All ages	Unfavorable
	t(6 ;11)(q27;q23), *MLLT4(AFDN)/MLL*	All ages	Unfavorable
	t(9;11)(p22;q23), *MLLT3(AF9)/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(ENL)*	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(Xp22.3;var) or t(Yp11.32;var), *P2RY8*	All ages	Unfavorable
	-17/17p-, *TP53*	All ages	Unfavorable
	t(8q24.1;var), *MYC*	Pediatric/ adolescent/ young adult	
	Complex karyotype (> or =4 abnormalities)	Adult	Unfavorable
	Low hypodiploidy/near triploidy	Adult	Unfavorable
	Near-haploid/hypodiploid	All ages	Unfavorable
	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 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 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. For each probe set a series of chromosomally abnormal specimens was evaluated to confirm each probe set detected the abnormality it was designed to detect.

Clinical Reference

1. Moorman AV, Harrison CJ, Buck GAN, 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
2. Moorman AV: The clinical relevance of chromosomal and genetic abnormalities in B-cell precursor acute lymphoblastic leukemia. Blood Rev. 2012 May;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 Sep 11;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 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, 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 break-apart (BAP) strategy 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 2, 88275, 88291-FISH Probe, Analysis, Interpretation; 1 probe set
88271 x 2, 88275-FISH Probe, Analysis; each additional probe set (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
COGBF	COG, ALL (B-cell), FISH	102100-5

Result ID	Test Result Name	Result LOINC® Value

602296	Result Summary	50397-9
602297	Interpretation	69965-2
602298	Result Table	93356-4
602299	Result	62356-1
GC019	Reason for Referral	42349-1
GC020	Specimen	31208-2
602301	Source	31208-2
602302	Method	85069-3
602303	Additional Information	48767-8
602304	Disclaimer	62364-5
602305	Released By	18771-6