MAYO CLINIC LABORATORIES

Test Definition: CILPF

Congenital Infantile Leukemia, FISH, Tissue

Overview

Useful For

Detecting a neoplastic clone associated with the common chromosome abnormalities and classic rearrangements seen in infant patients with leukemia using tissue specimens

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
_PADD	Probe, +1	No, (Bill Only)	No
_PB02	Probe, +2	No, (Bill Only)	No
_PB03	Probe, +3	No, (Bill Only)	No
_PBCT	Probe, +2	No, (Bill Only)	No
_IL25	Interphases, <25	No, (Bill Only)	No
_I099	Interphases, 25-99	No, (Bill Only)	No
_I300	Interphases, >=100	No, (Bill Only)	No

Testing Algorithm

This test includes a charge for application of the first probe set (2 fluorescence in situ hybridization [FISH] probes) and professional interpretation of results. Additional charges will be incurred for all reflex probes performed. Analysis charges will be incurred based on the number of cells analyzed per probe set. If no cells are available for analysis, no analysis charges will be incurred.

This FISH test allows different combinations of probes to be utilized based on the patient's age and clinical question, per client request.

All probes marked with an asterisk* will be performed as reflex testing, without notification, if the corresponding region was disrupted or potentially disrupted. Patients found to have a MYC rearrangement will be reflexed to the break-apart BCL6 and BCL2 probe sets. Patients found to have 3 copies of KAT6A will be reflexed with D8Z2/MYC.

The FISH panel for patients **younger than 3 months** includes testing for the following abnormalities using the FISH probes listed:
11q23 rearrangement, MLL (KMT2A)
*t(4;11)(q21;q23) AFF1/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-
t(8;16), [M4,M5], KAT6A/CREBBP
*D8Z2/MYC for trisomy 8
t(1;22), [M7], RBM15/MKL1
+13/+21, 13q14 and 21q22

If no classic abnormalities are observed, other FISH probes may be offered by the laboratory.

The FISH panel is dependent on the reason for testing and the patient's diagnosis (acute myeloid leukemia [AML], B-cell acute lymphoblastic leukemia [ALL], or T-cell ALL).

The initial FISH panel for patients **3 months to 18 months of age** with AML includes testing for the following abnormalities:
11q23 rearrangement, MLL (KMT2A)
*t(4;11)(q21;q23) AFF1/MLL
*t(9;11)(p22;q23) MLLT3/MLL
*t(10;11)(p12;q23) MLLT10/MLL
*t(11;19)(q23;p13.1) MLL/ELL

If an MLL disruption is not identified, the following secondary AML FISH probes will be evaluated:
inv(16), [M4, Eos], MYH11/CBFB
t(8;21), [M2], RUNX1T1/RUNX1
t(15;17), [M3], PML/RARA
12p13 rearrangement, ETV6 break-apart
*t(7;12)(q36;p13), MNX1/ETV6
t(8;16), [M4,M5], KAT6A/CREBBP
inv(16), GLIS2/CBFA2T3
11p15.4 rearrangement, NUP98 break-apart
t(1;22), [M7], RBM15/MKL1

The initial FISH panel for patients **3 to 18 months of age** with B-cell ALL includes testing for the following abnormalities:
11q23 rearrangement, MLL (KMT2A)
*t(4;11)(q21;q23) AFF1/MLL
*t(9;11)(p22;q23) MLLT3/MLL
*t(10;11)(p12;q23) MLLT10/MLL
*t(11;19)(q23;p13.3) MLL/MLLT1

If an MLL disruption is not identified, the following secondary panel of B-cell ALL FISH probes will be evaluated:
+9/9p-, CDKN2A/D9Z1
t(9;22) BCR/ABL1 fusion
-17/17p-, TP53/D17Z1
t(1;19)(q23;p13), PBX1/TCF3 fusion
Hyperdiploidy, +4,+10,+17: D4Z1/D10Z1/D17Z1
t(12;21)(p13;q22), ETV6/RUNX1 fusion and iAMP21
*12p13 rearrangement, ETV6 break-apart
14q32 rearrangement, IGH break-apart
8q24.1 rearrangement, MYC
*3q27 rearrangement, BCL6 break-apart
*18q21 rearrangement, BCL2 break-apart

If a classic B-cell ALL abnormality is not identified in the first 11 probes analyzed, the following tertiary panel of B-cell

ALL FISH probes will be evaluated, 9p24.1 rearrangement, JAK2.

The initial FISH panel for patients **3 to 18 months of age** with T-cell ALL includes testing for the following abnormalities:
11q23 rearrangement, MLL (KMT2A)
*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;p13.3) MLL/MLLT1
*t(11;19)(q23;p13.1) MLL/ELL

If an MLL disruption is not identified, the following secondary panel of T-cell ALL FISH probes will be evaluated:
+9/9p-, CDKN2A/D9Z1
t(9;22) BCR/ABL1
-17/17p-, TP53/D17Z1
t(5;14), TLX3/BCL11B
7q34 rearrangement, TRB
*t(7;10) - TRB/TLX1
14q11.2 rearrangement, TRAD
*t(10;14) - TLX1/TRAD
t(10;11), MLLT10/PICALM
1p33 rearrangement, TAL1/STIL

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.

Method Name
Fluorescence In Situ Hybridization (FISH)

NY State Available
Yes

Specimen

Specimen Type
Tissue

Ordering Guidance
This test is only performed on specimens from patients with acute leukemia who are 18 months of age or younger.

For testing bone marrow or blood specimens from patients with congenital infantile leukemia, order CILDF / Congenital Infantile Leukemia, Diagnostic FISH, Varies.

If this test is ordered on a patient older than 18 months of age and the reason for testing is B-cell or T-cell acute

lymphocytic leukemia (B-ALL or T-ALL), this test will be canceled and automatically reordered by the laboratory as BLBLF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma, FISH, Tissue or TLBLF / T-Cell Acute Lymphoblastic Leukemia/Lymphoma, FISH, Tissue.

If this test is ordered on a patient older than 18 months of age and the reason for testing is acute myeloid leukemia (AML), this test will be canceled and automatically reordered by the laboratory as MSTF / Myeloid Sarcoma, FISH, Tissue.

This test does not include a pathology consult. If a pathology consultation is requested, PATHC / Pathology Consultation should be ordered and the appropriate FISH test will be ordered and performed at an additional charge.

Shipping Instructions

Advise Express Mail or equivalent if not on courier service.

Necessary Information

A reason for testing and pathology report are required for testing to be performed. Send information with specimen. Acceptable pathology reports include working drafts, preliminary pathology or surgical pathology reports. The laboratory will not reject testing if this information is not provided, but appropriate testing and interpretation may be compromised or delayed. If this information is not provided, an appropriate indication for testing may be entered by Mayo Clinic Laboratories.

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Tissue
Preferred: Tissue block
Collection Instructions: Submit a formalin-fixed, paraffin-embedded (FFPE) tumor tissue block. Blocks prepared with alternative fixation methods may be acceptable; provide fixation method used.
Additional Information:
1. The paraffin embedded specimen can be from any anatomic location (skin, soft tissue, lymph node, etc.).
2. Bone specimens that have been decalcified will be attempted for FISH, with a success rate of approximately 50%.

Acceptable: Slides
Collection Instructions: 20 Consecutive, unstained, 5 micron-thick sections placed on positively charged slides, and 1 hematoxylin and eosin-stained slide.

Forms

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

Specimen Minimum Volume

15 consecutive, unstained, 5-micron-thick sections placed on positively charged slides, and 1 hematoxylin and eosin-stained slide.

Reject Due To

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

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

While pediatric leukemia is the most common malignancy affecting children, acute leukemia occuring prior to the age of 18 months (infant leukemia) or occuring within the first 3 months of life (congenital leukemia) are relatively rare in occurrence. The incidence of congenital and infant acute leukemia cases (through 12 months of age) is estimated at only 30 to 40 cases/million/year, with the majority comprising infant cases. Nearly all cases of congenital and infant acute leukemia represent either acute myeloid leukemia (AML) or B-cell acute lymphocytic leukemia/lymphoblastic lymphoma (B-ALL/LBL) with only very rare cases of T-cell-ALL/LBL identified in this age group.

Characteristic genetic abnormalities have been identified in both the congenital acute leukemia and infant acute leukemia setting, each with uniquely associated clinical-pathologic correlations. Rare but important patients with *KAT6A*/*CREBBP* translocations and congenital acute leukemia have been described with spontaneously remitting AML despite the lack of therapeutic intervention. In addition, transient abnormal myelopoiesis associated with Down syndrome is another common manifestation encountered in the neonatal setting that can be associated with the development of frank acute leukemia. In contrast, nearly 80% of infant acute leukemia cases are associated with MLL(*KMT2A*) translocation events with varying percentages of translocation partners based on an AML versus B-ALL/LBL presentation.

Due to the underlying genetic heterogeneity associated with both congenital and infant leukemia and the important prognostic, diagnostic, and occasional therapeutic targets identified, appropriate genetic characterization of this uncommon acute leukemia presentation is critical. These thorough fluorescence in situ hybridization (FISH) panels have been developed by Mayo Clinic Laboratories to interrogate the more common AML and B-ALL abnormalities associated with both congenital and infant acute leukemias. These FISH probes have been validated both in bone marrow/blood CILDF / Congenital Infantile Leukemia, Diagnostic FISH, Varies and in paraffin CILPF / Congenital Infantile Leukemia, FISH, Tissuesince a significant minority of these patient’s present clinically with isolated extramedullary (tissue) manifestations (ie, myeloid sarcoma).

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.

A positive result is not diagnostic for congenital or infantile leukemia but may provide relevant prognostic information.

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 existing clinical and pathologic information.

Fixatives other than formalin (eg, Prefer, Bouin's) may not be successful for fluorescence in situ hybridization (FISH) assays. Although FISH testing will not be rejected due to non-formalin fixation, results may be compromised.

Paraffin-embedded tissues that have been decalcified may be unsuccessful for FISH analysis. FISH studies will be attempted if sufficient tumor is present for analysis. The pathologist reviewing the hematoxylin and eosin-stained slide may find it necessary to cancel testing. If no FISH signals are observed post-hybridization, the case will be released indicating a lack of FISH results.

Supportive Data

For each probe set, blinded fluorescence in situ hybridization (FISH) analysis was performed on 20 to 25 normal paraffin-embedded, formalin-fixed tissue controls and between 2 and 20 paraffin-embedded, formalin-fixed tissue samples from patients diagnosed with B-cell lymphoblastic leukemia or lymphoma. Results from the 25 controls were used to generate the normal cutoff values.

Clinical Reference

1. Swerdlow SH, Campo E, Harris NL, et al, eds: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. IARC Press; 2017
2. Tomizawa D: Recent progress in the treatment of infant acute lymphoblastic leukemia. Pediatr Int. 2015;57(5):811-819. doi: 10.1111/ped.12758
3. Inaba H, Zhou Y, Abla O, et al: Heterogeneous cytogenetic subgroups and outcomes in childhood acute megakaryoblastic leukemia: a retrospective international study. Blood. 2015;126(13):1575-1584. doi: 10.1182/blood-2015-02-629204
4. Coenen EA, Zwaan CM, Reinhardt D, et al: Pediatric acute myeloid leukemia with t(8;16)(p11;p13), a distinct clinical and biological entity: a collaborative study by the International-Berlin-Frankfurt-Munster AML-study group. Blood. 2013;122(15):2704-2713. doi: 10.1182/blood-2013-02-485524

Performance

Method Description

This test is performed using commercially available and laboratory-developed probes. Gain or loss of chromosomes 4, 8, 10, 13, 17, and 21 are detected using enumeration strategy probes. Deletion of the *CDKN2A* locus on chromosome 9 and *TP53* on chromosome 17 are detected using enumeration strategy. Rearrangements involving genes involving *MLL* (*KMT2A*), *NUP98, ETV6, MYC, JAK2, IGH, TAL1/STIL, TRB,* and *TRAD* are detected using a dual-color break-apart (BAP) strategy probe. 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. Dual-color, dual-fusion fluorescence in situ hybridization (D-FISH) strategy probe sets are used to detect inv(16), t(8;21), t(15;17), t(8;16), t(1;22), t(7;12), t(9;22), t(12;21), t(1;19), t(5;14), t(9;22), t(10;11), and in reflex testing when a rearrangement of the *MLL, TRB*, *TRAD* gene region is observed. Amplification of *ABL1* (9q34) is detected using a D-FISH probe strategy.

Paraffin-embedded tissues are cut at 5 microns and mounted on positively charged glass slides. The selection of tissue and the identification of target areas on the hematoxylin and eosin (H and E)-stained slide are performed by a

pathologist. Using the H and E-stained slide as a reference, target areas are etched with a diamond-tipped etcher on the back of the unstained slide to be assayed. For each probe set, the probes are hybridized to the appropriate target areas and 2 technologists each analyze 50 interphase nuclei (100 total) per probe set with the results 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
Slides and hematoxylin and eosin used for analysis are retained by the laboratory in accordance to CAP and NYS requirements. Client provided paraffin blocks and extra unstained slides (if provided) will be returned after testing is complete.

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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
88271 x2, 88291-DNA probe, each (first probe set), interpretation and report
88271 x2-DNA probe, each; each additional probe set (if appropriate)
88271-DNA probe, each; coverage for sets containing 3 probes (if appropriate)
88271 x 2-DNA probe, each; coverage for sets containing 4 probes (if appropriate)
88271 x 3-DNA probe, each; coverage for sets containing 5 probes (if appropriate)
88274 w/modifier 52-Interphase in situ hybridization, <25 cells, each probe set (if appropriate)
88274-Interphase in situ hybridization, 25 to 99 cells, each probe set (if appropriate)
88275-Interphase in situ hybridization, 100 to 300 cells, each probe set (if appropriate)

LOINC® Information

Test Definition: CILPF

Congenital Infantile Leukemia, FISH, Tissue

Test ID	Test Order Name	Order LOINC® Value
CILPF	Congenital Infantile Leuk, FISH, Ts	In Process

Result ID	Test Result Name	Result LOINC® Value
614181	Result Summary	50397-9
614182	Interpretation	69965-2
614183	Result Table	93356-4
614184	Result	62356-1
GC095	Reason for Referral	42349-1
614185	Specimen	31208-2
614186	Source	31208-2
614187	Tissue ID	80398-1
614188	Method	85069-3
614189	Additional Information	48767-8
614190	Disclaimer	62364-5
614191	Released by	18771-6