MAYO CLINIC LABORATORIES

Test Definition: AMLPF

Acute Myeloid Leukemia (AML), FISH, Pediatric, Varies

Overview

Useful For

This test **should not be used** to screen for residual acute myeloid leukemia (AML).

Useful at diagnosis for detecting recurrent common chromosome abnormalities in pediatric patients with AML

An adjunct to chromosome studies in patients with AML

Evaluating specimens in which chromosome studies are unsuccessful

Reflex Tests

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

Testing Algorithm

This test includes a charge for the probe application, analysis, and professional interpretation of results for 13 probe sets (26 individual fluorescence in situ hybridization [FISH] probes). Additional charges will be incurred for all reflex or additional probe sets performed. If no cells are available for analysis, no analysis charges will be incurred.

This test is performed as panel testing only and will be performed using the following analysis algorithm.

The **diagnostic** pediatric/young adult FISH panel includes testing for the following abnormalities using the FISH probes listed:
inv(16) or t(16;16), MYH11/CBFB
t(8;21), RUNX1T1/RUNX1
t(15;17), PML/RARA
11q23 rearrangement, MLL (KMT2A)
t(6;9), DEK/NUP214
inv(3) or t(3;3), RPN1/MECOM
t(8;16), KAT6A/CREBBP
t(1;22), RBM15/MKL1(MRTFA)
-5/5q-, D5S630/EGR1
-7/7q-, D7Z1/ D7S486
12p13 rearrangement, ETV6
inv(16), GLIS2/CBFA2T3
11p15.4 rearrangement, NUP98

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;16)(q23;p13.3) MLL::CREBBP, t(11;19)(q23;p13.1), MLL::ELL, or t(11;19)(q23;p13.3) MLL::MLLT1. In the event an 11q23 translocation is identified by chromosome analysis, only the targeted MLL reflex probe will be performed if applicable.

In the absence of *RPN1::MECOM* and *RUNX1::RUNX1T*1 fusion, when an extra MECOM signal and an extra RUNX1 signal are identified, reflex testing using the MECOM/RUNX1 probe set will be considered at the laboratory's discretion to identify a potential t(3;21)(q26.2;q22) rearrangement. Laboratory discretion may be influenced by available karyotype results.

In the absence of *RPN1::MECOM* fusion, when an extra RPN1 signal is identified, reflex testing using the PRDM16/RPN1 probe set will be considered at the laboratory's discretion to identify a potential t(1;3)(p36;q21). Laboratory discretion may be influenced by available karyotype results.

In the absence of *RPN1::MECOM* fusion, when an extra MECOM signal is identified, reflex testing using the break-apart MECOM probe set will be recommended at the laboratory's discretion to identify a potential variant translocation involving *MECOM*, t(3;var)(q26.2;?). Laboratory discretion may be influenced by available karyotype results.

In the absence of *MYH11::CBFB* fusion, when an extra CBFB signal is identified, reflex testing may be performed at the laboratory's discretion using the CBFB break-apart probe set to evaluate for the presence or absence of a potential variant translocation involving *CBFB*, t(16;var)(q22;?). Laboratory discretion may be influenced by available karyotype results.

In the absence of *PML::RARA* fusion, when an extra or atypical RARA signal is identified, testing using the RARA break-apart probe set may be performed at the laboratory's discretion to identify a potential variant translocation involving *RARA,* t(17;var)(q21;?). Laboratory discretion may be influenced by available karyotype results.

When an *ETV6* rearrangement is identified, reflex testing using the MNX1/ETV6 probe set will be considered at the laboratory's discretion to identify a potential t(7;12)(q36;p13). Laboratory discretion may be influenced by available karyotype results.

When a *NUP98* rearrangement is identified, reflex testing using the HOXA9/NUP98 probe set will be considered at the laboratory's discretion to identify a potential t(7;11)(p15;p15.4). Laboratory discretion may be influenced by available karyotype results.

In the absence of *RUNX1::RUNX1T1* fusion, when an extra RUNX1 signal is identified, reflex testing may be recommended at the laboratory's discretion using the RUNX1 break-apart probe set to evaluate for the presence or absence of a potential variant translocation involving *RUNX1,* t(21;var)(q22;?). Laboratory discretion may be influenced by available karyotype results.

For more information see:
Acute Promyelocytic Leukemia: Guideline to Diagnosis and Follow-up
Acute Leukemias of Ambiguous Lineage Testing Algorithm

Acute Myeloid Leukemia: Testing Algorithm

Special Instructions

- Acute Promyelocytic Leukemia: Guideline to Diagnosis and Follow-up
- Acute Leukemias of Ambiguous Lineage Testing Algorithm
- Acute Myeloid Leukemia: 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 acute myeloid leukemia (AML) who are 30 years of age or younger.

This test **should NOT be used** to screen for residual acute myeloid leukemia (AML).

Minimal residual disease (MRD) monitoring in patients with AML known to have either t(15;17) with PML::RARA fusion, inv(16) or t(16;16) with MYH11::CBFB fusion, or t(8;21) with RUNX1T1::RUNX1 fusion should be performed by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) and **NOT** by FISH testing.

It is recommended that MRD monitoring in AML patients be performed by AML-MRD Flow cytometry rather than fluorescence in situ hybridization (FISH) testing using individual FISH probe sets. This is particularly true for the deletion/monosomy probe sets (5 and 7) which have cutoffs that exceed 10% of nuclei.

If limited AML FISH probes are preferred, order AMLMF / Acute Myeloid Leukemia (AML), Specified FISH, Varies and request specific probes for targeted abnormalities.

This test is intended for instances when the entire AML FISH panel is needed for a **pediatric** patient.

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 AMLAF / Acute Myeloid Leukemia (AML), FISH, Adult, 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 (COG) protocol, this test will be canceled and automatically reordered by the laboratory as COGMF /

Acute Myeloid Leukemia (AML), Children's Oncology Group Enrollment Testing, FISH, Varies.

If either (or both) BALPF / B-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), Pediatric, FISH, Varies or TALPF / T-Cell Acute Lymphoblastic Leukemia/Lymphoma (ALL), FISH, Pediatric, Varies, is ordered concurrently with this test, the laboratory may cancel this test and automatically reorder as AMLMF / Acute Myeloid Leukemia (AML), Specified FISH, Varies with the following FISH probes: RUNX1T1/RUNX1, PML/RARA, MYH11/CBFB, RPN1/MECOM, DEK/NUP214, D5S630/EGFR1, D7Z1/D7S486, break-apart ETV6, KAT6A/CREBBP, GLIS2/CBFA2T3, break-apart NUP98, and RBM15/MKL1. If an abnormality is identified that would result in reflex testing in this test, the same reflex testing will be performed in the AMLMF. This cancellation is necessary to avoid duplicate testing. The break-apart MLL probe set will still be performed as part of either the pediatric B-ALL or T-ALL FISH panel.

For testing paraffin-embedded tissue samples from patients with AML/myeloid sarcoma, order MSTF / Myeloid Sarcoma, FISH, Tissue.

Shipping Instructions

Advise Express Mail or equivalent if not on courier service.

Necessary Information

A reason for testing and a flow cytometry and/or a bone marrow pathology report are requested 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.

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 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 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
Acute myeloid leukemia (AML) is one of the most common adult leukemias, with almost 10,000 new cases diagnosed per year. AML also comprises 15% of pediatric acute leukemia and accounts for the majority of infant (<1 year old) leukemia.

Several recurrent chromosomal abnormalities have been identified in AML with associated clinical significance. The most common chromosome abnormalities associated with AML include t(8;21), t(15;17), inv(16) or t(16;16), and abnormalities of the *MLL* (*KMT2A*) gene at 11q23. The most common genes juxtaposed with *MLL* through translocation events in AML include *MLTT4(MLLT4)*- t(6;11), *MLLT3*- t(9;11), *MLLT10*- t(10;11), and *ELL*- t(11;19p13.1).

AML can also evolve from myelodysplasia (MDS). Thus, the common chromosome abnormalities associated with MDS can also be identified in AML, which include: inv(3) or t(3;3), -5/5q-, -7/7q-. Overall, the recurrent chromosome abnormalities identified in patients with AML are observed in approximately 60% of diagnostic AML cases.

Conventional chromosome analysis is the gold standard for identification of the common, recurrent chromosome abnormalities in AML. However, some of the subtle rearrangements can be missed by karyotype, including inv(16) or t(16;16) and *MLL* rearrangements.

Fluorescence in situ hybridization analysis of nonproliferating (interphase) cells can be used to detect the common diagnostic and prognostic chromosome abnormalities observed in patients with AML.

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 myeloid leukemia clone 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 existing clinical and pathologic information.

Fluorescence in situ hybridization (FISH) is not a substitute for conventional chromosome studies since only the common acute myeloid leukemia (AML) abnormalities are evaluated by the FISH panel and a chromosome analysis can also identify abnormalities associated with other hematological disorders that would be missed in a targeted AML 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 myeloblasts 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 at least 25 normal specimens. In addition, each probe set was evaluated in a blinded fashion to confirm the probe set detected the abnormality it was designed to detect.

Clinical Reference

1. Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetics classification in acute myeloid leukemia: determination of prognostic significance or rare recurring chromosomal abnormalities among 5879 younger adult patients treated in the United Kingdom Research Council trials. Blood. 2010;116(3):354-365
2. Swerdlow SH, Campo E, Harris NL, et al. eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. IARC Press; 2017
3. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-447. doi:10.1182/blood-2016-08-733196
4. Pollyea DA, Bixby D, Perl A, et al. Acute Myeloid Leukemia, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19(1):17-27. doi:10.6004/jnccn.2021.0002

Performance

Method Description

This test is performed using commercially available and laboratory-developed probes. Deletion or monosomy of chromosomes 5 and 7 are detected using enumeration strategy probes. Rearrangements involving *MLL* (*KMT2A*), *NUP98, ETV6, CBFB,* and *RARA* 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 inv(3) or t(3;3), inv(16) or t(16;16), t(8;21), t(15;17), t(6;9), t(8;16), t(3;21), t(1;3), t(1;22), t(7;11), t(7;12), and in reflex testing when rearrangements of the

MLL gene are detected. For enumeration and BAP strategy probe sets, 100 interphase nuclei are scored; 200 interphase nuclei are scored when D-FISH probes are used. All 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
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
88271x26, 88275x13, 88291 x1-FISH Probe, Analysis, Interpretation; 13 probe sets
88271x2, 88275x1-FISH Probe, Analysis; each additional probe set (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
AMLPF	Pediatric AML, FISH	102103-9

Result ID	Test Result Name	Result LOINC® Value
609528	Result Summary	50397-9
609529	Interpretation	69965-2
609530	Result Table	93356-4

609531	Result	62356-1
GC062	Reason for Referral	42349-1
GC063	Specimen	31208-2
609532	Source	31208-2
609533	Method	85069-3
609534	Additional Information	48767-8
609535	Disclaimer	62364-5
609536	Released By	18771-6