MAYO CLINIC LABORATORIES

# Test Definition: AMLFP

Pediatric Acute Myeloid Leukemia Panel, FISH, Varies

## Overview

### Useful For

Detecting, at diagnosis, recurrent common chromosome abnormalities associated with acute myeloid leukemia (AML) in patients 30 years and younger using a laboratory-designated probe set algorithm

As an adjunct to conventional chromosome studies in patients with AML

Evaluating specimens in which chromosome studies are unsuccessful

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

### Reflex Tests

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| AMLBP | Probe, Each Additional (AMLFP) | No, (Bill Only) | No |

### Testing Algorithm

This test includes a charge for probe application, analysis, and professional interpretation of results for 11 probe sets (22 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(3) or t(3;3) or *GATA2::MECOM* fusion, GATA2/MECOM probe set
-5/5q-, D5S630/EGR1 probe set
t(6;9)(p22.3;q34) or *DEK::NUP214* fusion, DEK/NUP214 probe set
-7/7q-, D7Z1/D7S486 probe set
t(7;12)(q36;p13) or *MNX1::ETV6* fusion, MNX1/ETV6 probe set
t(8;16)(p11;p13) or *KAT6A::CREBBP* fusion, KAT6A/CREBBP probe set
t(8;21)(q21.3;q22) or *RUNX1::RUNX1T1* fusion, RUNX1T1/RUNX1 probe set
t(11p15;var) or *NUP98* rearrangement, NUP98 break-apart probe set
t(11q23;var) or *KMT2A* rearrangement, KMT2A break-apart probe set
t(15;17)(q24;q21) or *PML::RARA* fusion, PML/RARA probe set
inv(16) or t(16;16) or *CBFB::MYH11* fusion, MYH11/CBFB probe set
inv(16)(p13q24) or *CBFA2T3:: GLIS2* fusion, CBFA2T3/GLIS2 probe set

Appropriate ancillary probes may be performed at consultant discretion to render comprehensive assessment. Any additional probes used will have the results included within the final report and will be performed at an additional

charge. 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.

In the absence of *GATA2::MECOM* fusion, when an extra GATA2 signal is identified, testing using the PRDM16/GATA2 probe set to identify a potential t(1;3)(p36;q21) may be performed.

In the absence of *GATA2::MECOM* fusion, when an extra MECOM signal is identified, testing using the break-apart MECOM probe set to identify a potential variant translocation involving *MECOM*, t(3;var)(q26.2;?) may be performed.

When a *KMT2A* rearrangement is identified, testing with 1 or more dual-fusion (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;16)(q23;p13.3) or *KMT2A::CREBBP* fusion, KMT2A/CREBBP 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

In the absence of *PML::RARA* fusion, when an extra or atypical RARA signal is identified, testing using the RARA break-apart probe set to identify a potential variant translocation involving *RARA,* t(17;var)(q21;?) may be performed.

In the absence of *CBFB::MYH11* fusion, when an extra CBFB signal is identified, testing 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;?) may be performed.

In the absence of *RUNX1::RUNX1T1* fusion, when an extra RUNX1 signal is identified, testing 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;?) may be performed.

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.

If acute promyelocytic leukemia is probable and expedited PML/RARA results are needed, order AMLMF / Acute Myeloid Leukemia (AML), Specified FISH, Varies. If *PML*::*RARA* fusion is identified in AMLFP, the laboratory will automatically expedite analysis. Results will not be provided until the complete panel testing is finalized. The laboratory is unable to provide preliminary results.

This test **should NOT be used** to screen for residual 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 *CBFB*::*MYH11* fusion, t(8;21) with *RUNX1*::*RUNX1T1* fusion, or t(9;22) with *BCR*::*ABL1* fusion should be performed by quantitative reverse transcriptase polymerase chain reaction and **NOT** by fluorescence in situ hybridization (FISH) testing.

It is recommended that MRD monitoring in AML patients be performed by AML-MRD flow cytometry rather than 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 targeted 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 AMLFA / Adult Acute Myeloid Leukemia Panel, 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 (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) BALFP / Pediatric B-Lymphoblastic Leukemia/Lymphoma Panel, FISH, Varies or TALFP / Pediatric T-Lymphoblastic Leukemia/Lymphoma Panel, FISH, 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, GATA2/MECOM, DEK/NUP214, D5S630/EGFR1, D7Z1/D7S486, MNX1/ETV6, KAT6A/CREBBP, GLIS2/CBFA2T3, and NUP98 3'/5'. 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 KMT2A probe set will still be performed as part of either the pediatric B-ALL or T-ALL FISH panel.

For testing paraffin-embedded tissue specimens from patients with AML/myeloid sarcoma, order MSTF / Myeloid Sarcoma, FISH, Tissue. If a paraffin-embedded tissue specimen is submitted for this test, this test will be canceled and MSTF will be added and performed as the appropriate test.

## 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.

## 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 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 in original tube. **Do not aliquot.**

## Forms

If not ordering electronically, complete, print, and send an 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

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 *KMT2A* gene at 11q23. The most common genes juxtaposed with *KMT2A* through translocation events in AML include *AFDN*- t(6;11), *MLLT3*- t(9;11), *MLLT10*- t(10;11), and *ELL*-t(11;19p13.1).

Acute myeloid leukemia 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 *KMT2A* 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.

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).

### 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).

If no FISH signals are observed post-hybridization, the case will be released indicating a lack of FISH results.

### 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 fluorescence in situ hybridization (FISH) probes. Deletion or monosomy of chromosomes 5 and 7 are detected using enumeration strategy probes. Rearrangements involving *MECOM*, *NUP98, KMT2A*, *CBFB, RARA*, and *RUNX1* are detected using a dual-color break-apart (BAP) strategy probe set. Dual-color, dual-fusion (D-FISH) strategy probe sets are used to detect t(1;3), inv(3) or t(3;3), t(6;9), t(7;12), t(8;16), t(8;21), t(15;17), inv(16) or t(16;16), and in reflex testing when rearrangements of the

*KMT2A* 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
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
88271x22, 88275x11, 88291 x1-FISH Probe, Analysis, Interpretation; 11 probe sets
88271x2, 88275x1-FISH Probe, Analysis; each additional probe set (if appropriate)

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| AMLFP | Pediatric-AML panel, FISH | 102101-3 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 622391 | Result Summary | 50397-9 |
| 622392 | Interpretation | 69965-2 |
| 622393 | Result Table | 93356-4 |

| | | |
|---|---|---|
| 622394 | Result | 62356-1 |
| GC150 | Reason for Referral | 42349-1 |
| GC151 | Specimen | 31208-2 |
| 622395 | Source | 31208-2 |
| 622396 | Method | 85069-3 |
| 622397 | Additional Information | 48767-8 |
| 622398 | Disclaimer | 62364-5 |
| 622399 | Released By | 18771-6 |