MAYO CLINIC LABORATORIES

Test Definition: AMLAF

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

Overview

Useful For

Detecting a neoplastic clone associated with recurrent chromosome abnormalities seen in adult patients with acute myeloid leukemia (AML) or other myeloid malignancies

An adjunct to conventional chromosome studies in patients with AML

Evaluating specimens in which standard cytogenetic analysis is unsuccessful

Reflex Tests

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

Testing Algorithm

This test includes a charge for the probe application, analysis, and professional interpretation of results for 4 probe sets (8 individual fluorescence in situ hybridization [FISH] probes). Additional charges will be incurred for all reflex or additional probe sets performed.

The initial panel includes testing for the following abnormalities using the probes listed:
inv(16), [M4, Eos], MYH11/CBFB
t(8;21), [M2], RUNX1T1/RUNX1
t(15;17), [M3], PML/RARA
11q23 rearrangement, [M0-M7], MLL (KMT2A)

Based on the results from the initial panel, if testing was ordered concurrently with a chromosomal study (CHRBM / Chromosome Analysis, Hematologic Disorders, Bone Marrow or CHRHB / Chromosome Analysis, Hematologic Disorders, Blood), testing will be held pending the results of the chromosome test. If the chromosome results are complete and informative, only appropriate secondary FISH probes will be selected and performed. If testing was NOT ordered concurrently with a chromosomal study each of the secondary probes will be performed. The secondary panel includes testing for the following abnormalities using the probes listed:
t(6;9), [M2,M4], DEK/NUP214
inv(3) or t(3;3), [M1,2,4,6,7], RPN1/MECOM
-5/5q-, D5S630/EGR1
-7/7q-, D7Z1/D7S486
17p-, TP53/D17Z1
t(9;22), ABL1/BCR

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 absence of RPN1/MECOM and RUNX1/RUNX1T1 fusion, when an extra MECOM signal and an extra RUNX1 signal are identified, reflex testing using the MECOM/RUNX1 probe set will be performed to identify a potential t(3;21)(q26.2;q22) rearrangement.

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 to identify a potential t(1;3)(p36;q21).

In the absence of MYH11/CBFB fusion, when an extra CBFB signal is identified, reflex testing will be performed using the CBFB break-apart probe set to evaluate for the presence or absence of an *CBFB* rearrangement.

In the absence of PML/RARA fusion, when an extra or atypical RARA signal is identified, testing using the 5'RARA/3'RARA rearrangement probe set will be performed to identify a potential variant translocation involving *RARA*. example: t(17;var)(q21;?).

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.

The following algorithms are available in Special Instructions:
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 older than 30 years of age.

This test is intended for instances when the entire AML fluorescence in situ hybridization (FISH) panel is needed for an **adult** patient.
-If this test is ordered on a patient 30 years of age or younger, this test will be canceled and automatically reordered by the laboratory as AMLPF / Acute Myeloid Leukemia, FISH, Pediatric, Varies.
- If this test is ordered and the laboratory is informed that the patient 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 limited AML FISH probes are preferred, order AMLMF / Acute Myeloid Leukemia, Specified FISH, Varies.

At follow-up, targeted AML FISH probes can be evaluated based on the specific abnormalities identified in the diagnostic study. Order AMLMF / Acute Myeloid Leukemia (AML), Specified FISH, Varies panel. and request specific probes or abnormalities.

For testing paraffin embedded tissue samples from patients with 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 (if available) 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. 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:

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.

Acceptable
Specimen Type: Blood
Container/Tube:

Preferred: Yellow top (ACD)
Acceptable: Green top (heparin) or lavender top (EDTA)
Specimen Volume: 6 mL
Collection Instructions: Invert several times to mix blood.

Forms
If not ordering electronically, complete, print, and send a Hematopathology/Cytogenetics Test Request (T726) 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
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), and abnormalities of the *MLL* (*KMT2A*) gene at 11q23. The most common genes juxtaposed with *MLL* through translocation events in AML include *MLLT3*- t(9;11), *MLLT4*)- t(6;11), *MLLT10*- t(10;11), and *ELL*- t(11;19p13.1).

Other recurrent chromosome abnormalities associated with AML include inv(3) or t(3;3), t(6;9) and t(9;22). In addition, AML can also evolve from myelodysplasia (MDS). Thus, the common chromosome abnormalities associated with MDS can also be identified in AML, which include: -5/5q-, -7/7q-, and 17p-. 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) and *MLL* rearrangements.

Fluorescence in situ hybridization (FISH) analysis of nonproliferating (interphase) cells can be used to detect the

common diagnostic and prognostic chromosome abnormalities observed in patients with AML. When recurrent translocations or inversions are identified, FISH testing can also be used to track response to therapy.

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 existing clinical and pathologic information.

Fluorescence in situ hybridization (FISH) is not a substitute for conventional chromosome studies because the latter detects many 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. Each probe set was evaluated to confirm the probe set detected the abnormality it was designed to detect.

Clinical Reference
1. Swerdlow SH, Campo E, Harris NL, et al, eds: WHO Classification of Tumour of Haematopoietic and Lymphoid Tissues. 4th ed. IARC Press; 20172.
2. 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

Performance

Method Description
This test is performed using commercially available and laboratory-developed probes . Deletion or monosomy of chromosomes 5, 7, and 17 are detected using enumeration strategy probes. Rearrangements involving *ABL1, MLL (KMT2A), 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), inv(16), t(8;21), t(15;17), t(6;9), t(8;16), t(3;21), t(1;3), t(1;22), t(9;22) 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 Regional Manager. 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. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
88271 x8, 88275 x4, 88291 x1- FISH Probe, Analysis, Interpretation; 4 probe sets
88271 x2, 88275 x1- FISH Probe, Analysis; each additional probe set (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
AMLAF	Adult AML, FISH	In Process

Result ID	Test Result Name	Result LOINC® Value
609518	Result Summary	50397-9
609519	Interpretation	69965-2
609520	Result Table	93356-4
609521	Result	62356-1
GC059	Reason for Referral	42349-1

GC060	Specimen	31208-2
609522	Source	31208-2
609523	Method	85069-3
609524	Additional Information	48767-8
609525	Disclaimer	62364-5
609526	Released By	18771-6