Overview

Useful For
Evaluation of pediatric bone marrow and peripheral blood specimens by FISH probe analysis for classic rearrangements and chromosomal copy number changes associated with acute myeloid leukemia (AML) in patients being considered for enrolment in Children’s Oncology Group (COG) clinical trials and research protocols.

Highlights
Cytogenetic testing is important for the diagnostic and prognostic classification of pediatric neoplasia and it is a critical element for the enrollment of children into clinical trials affiliated with the Children’s Oncology Group (COG). For over 25 years, the Mayo Clinic Genomics Laboratory has served as one of a select number of laboratories in the United States approved by the COG for the conventional chromosome analysis and FISH analysis of pediatric bone marrow, peripheral blood, and tissue specimens. All enrollment-required elements of cytogenetic testing will be electronically submitted by the Mayo Clinic Genomics Laboratory within the guidelines of COG.

Reflex Tests

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Testing Algorithm
This test is only performed on specimens from pediatric patients who are candidates for enrollment in Children’s Oncology Group clinical trials and research protocols.

Indicate the subtype, as well as, which abnormalities need to be investigated from the following profile:

- t(8;21), [M2], RUNX1T1/RUNX1
- t(15;17), [M3], PML/RARA
- 11p15.4 rearrangement, NUP98
- 11q23 rearrangement, [M0-M7], MLL (KMT2A)
- inv(16), [M4, Eos], MYH11/CBFB
- +8, [M0-M7], D8Z2/MYC
t(6;9), [M2,M4], *DEK/NUP214*

inv(3) or t(3;3), [M1,2,4,6,7], *RPN1/MECOM*

t(8;16), [M4,M5], *MYST3/CREBBP*

t(3;5)(q25.32;q35.1), *MLF1/NPM1*

t(1;22), [M7], *RBM15/MKL1* *

-5/5q-, *D5S630/EGR1*

-7/7q-, *D7S486/D7Z1*

13q-, *D13S319/LAMP1*

17p-, *TP53/D17Z1*

20q-, *D20S108/20qter*

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**Note:** The *RBM15/MKL1* probe set will only be used to test patients with a suspected or confirmed diagnosis of M7 or to confirm a t(1;22) identified by chromosome analysis.

-When NUP98 rearrangement is identified, reflex testing using the *HOXA9/NUP98* probe set will be performed to identify a potential t(7;11)(p15;p15.4).

-When a 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/MLL*, t(9;11)(p22;q23) *MLLT3/MLL*, t(10;11)(p13;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*.

-When 3 copies of *MECOM* are observed with no fusion with *RPN1*, reflex testing using the *MECOM/RUNX1* probe set will be performed to identify a potential t(3;21)(q26.2;q22) rearrangement.

-When 3 copies of *RPN1* are observed with no fusion with *MECOM*, reflex testing using the *PRDM16/RPN1* probe set will be performed to identify a potential t(1;3)(p36;q21).

The following testing algorithm is recommended for patients with acute myeloid leukemia (AML):

-At diagnosis, AML FISH panel and/or conventional chromosome studies COGBM / Chromosome Analysis, Hematologic Disorders, Children's Oncology Group Enrollment Testing, Bone Marrow should be performed. If there is limited specimen available, only the COGFM / Acute Myeloid Leukemia (AML), FISH, Children's Oncology Group Enrollment Testing, Blood and Bone Marrow test will be performed. --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 AMLF / Acute Myeloid Leukemia (AML), FISH, Varies.

See [Acute Promyelocytic Leukemia: Guideline to Diagnosis and Follow-up](#) in Special Instructions
Special Instructions
- Acute Promyelocytic Leukemia: Guideline to Diagnosis and Follow-up

Method Name
Fluorescence In Situ Hybridization (FISH)

NY State Available
No

Specimen

Specimen Type
Varies

Advisory Information
This test is only performed on specimens from pediatric patients being considered for enrollment in a Children's Oncology Group (COG) protocol. For all other patients, order AMLF / Acute Myeloid Leukemia (AML), FISH, Varies.

For children in whom disease relapse or a secondary myeloid neoplasm is a concern and enrollment in a new COG protocol is being considered; order COGBM / Chromosome Analysis, Hematologic Disorders, Children’s Oncology Group Enrollment Testing, Bone Marrow.

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

Necessary Information
1. Provide a reason for referral with each specimen, as well as flow cytometry and/or a bone marrow pathology report and Children's Oncology Group (COG) protocol number. The laboratory will not reject testing if this information is not provided, but appropriate testing and interpretation may be compromised or delayed.

2. If a child has received an opposite sex bone marrow transplant prior to specimen collection for this protocol, convey this information to the laboratory.

Specimen Required
Submit only 1 of the following specimens:

Preferred:

Specimen Type: Bone marrow

Container/Tube: Green top (sodium heparin)

Specimen Volume: 1 to 2 mL

Collection Instructions:
Invert several times to mix bone marrow.

Acceptable:
Test Definition: COGMF
COG, AML, FISH

Specimen Type: Blood

Container/Tube: Green top (sodium heparin)

Specimen Volume: 6 mL

Collection Instructions:
Invert several times to mix blood.

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

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Clinical and 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 subtypes of AML have been recognized (termed AML-M0, M1, M2, M3, M4, M5, M6, and M7) based on the cell morphology and myeloid lineage involved.

In addition to morphology, several recurrent chromosomal abnormalities have been linked to specific subtypes of AML. The most common chromosome abnormalities associated with AML include t(8;21), t(15;17), inv(16), +8, t(6;9), t(8;16), t(1;22), t(9;22), t(3;5), and abnormalities of the \textit{MLL} (\textit{KMT2A}) gene at 11q23. The most common genes juxtaposed with \textit{MLL} through translocation events in AML include \textit{AFF1}- t(4;11), \textit{MLLT4}- t(6;11), \textit{MLLT3}- t(9;11), \textit{MLLT10}- t(10;11), \textit{CREBBP}- t(11;16), \textit{ELL}- t(11;19p13.1), and \textit{MLLT1}- t(11;19p13.3).

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), -5/5q-, -7/7q-, +8, 13q-, 17p-, 20q-, t(1;3), and t(3;21). In combination, the multiple 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: eg, inv(16), \textit{MLL} and \textit{NUP98} abnormalities.

\textit{FISH} analysis of nonproliferating (interphase) cells can be used to detect the common chromosome abnormalities observed in patients with AML. The abnormalities have diagnostic and prognostic relevance and this testing can also
be used to track response to therapy.

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 Children’s Oncology Group (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 intrachromosomal amplification of chromosome 21 (iAMP21). Occasionally, characterization of balanced or complex rearrangements presumed to involve critical genes (such as a kinase activating gene and a fusion partner) may be characterizable by Mate Pair sequencing (MTRBM / MatePair, Targeted Rearrangements, Hematologic, Varies); a clinically validated next generation sequencing technique developed at Mayo Clinic.

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.

Detection of an abnormal clone likely indicates a diagnosis of an acute myeloid leukemia of various subtypes.

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.

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 were evaluated to confirm each probe set detected the abnormality it was designed to detect.

Clinical Reference


Performance
Method Description
This test is only performed on specimens from pediatric patients being considered for enrolment in a Children’s Oncology Group (COG) protocol and uses commercially available and laboratory-developed probes. Deletion or monosomy of chromosomes 5, 7, 13, trisomy of chromosome 8 and deletion or rearrangement of chromosome 17 and 20 are detected using enumeration strategy probes. Rearrangements involving MLL (KMT2A) and NUP98 are detected using a dual-color break-apart (BAP) strategy probe. Dual-color, dual-fusion (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(11;22), t(9;22), t(3;5), t(1;22), and in reflex testing when rearrangements of the MLL gene are detected. For enumeration and BAP strategy probe sets, 200 interphase nuclei are scored; 500 interphase nuclei are scored when D-FISH probes are used. Two technologists analyze each probe set and all results are expressed as the percent abnormal nuclei. (Unpublished Mayo method)

PDF Report
No

Day(s) and Time(s) Test Performed
Specimens are processed Monday through Sunday.

Results reported Monday through Friday, 8 a.m.-5 p.m.

Analytic Time
7 days

Maximum Laboratory Time
10 days

Specimen Retention Time
4 weeks

Performing Laboratory Location
Rochester

Fees and 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 using an analyte specific reagent. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
88271 x 2, 88291-DNA probe, each (first probe set), Interpretation and report

88271 x 2-DNA probe, each; each additional probe set (if appropriate)

88271-DNA probe, each; coverage for sets containing 3 probes (if appropriate)
Test Definition: COGMF
COG, AML, FISH

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**

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