Overview

Useful For

Evaluation of pediatric bone marrow and peripheral blood specimens by fluorescence in situ hybridization (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 fluorescence in situ hybridization (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

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

Testing Algorithm

This test is only performed on specimens from pediatric patients who are candidates for enrollment in Children's Oncology Group (COG) clinical trials and research protocols.

For diagnostic samples, all probes in the initial panel will be performed. The initial panel includes testing for the following abnormalities using the probes listed:

t(8;21), [M2], *RUNX1T1*/*RUNX1*

t(15;17), [M3], *PML/RARA*

11q23 rearrangement, [M0-M7], *MLL* (*KMT2A*)

inv(16), [M4, Eos], *MYH11*/*CBFB*

Based on the results from the initial panel, reflex testing may be performed to identify the following abnormalities:

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

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

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

17p-, *TP53*/D17Z1

t(9;22), *BCR*/*ABL1*

*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 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 COGMF / Acute Myeloid Leukemia (AML), FISH, Children's Oncology Group Enrollment Testing, varies 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

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

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, Childrenâ€™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.

Â 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:

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

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

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 recurrent chromosomal abnormalities have been identified in AML. 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 *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), -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 chromosome abnormalities observed in patients with AML. The abnormalities have diagnostic and prognostic relevance, and FISH 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).

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 fluorescence in situ hybridization 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

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 Jul;116(3):354-365

2. Swerdlow SH, Campo E, Harris NL, et al. eds: International Agency for Research on Cancer (IARC): World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues. IARC Press, Oxford University Press; 2017

3. Manola KN: Cytogenetics of pediatric acute myeloid leukemia. Eur J Haematol. 2009;83(5):391-405

Performance

Method Description

This test is only performed on specimens from pediatric patients being considered for enrollment in a Children's Oncology Group (COG) protocol and uses commercially available and laboratory-developed probes. Deletion or monosomy of chromosomes 5 and 7, and deletion or rearrangement of chromosome 17 are detected using enumeration strategy probes. Rearrangements involving *MLL* (*KMT2A*) 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(11;22), t(9;22), 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) Performed

Monday through Friday

Report Available

7 to 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)

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 ID	Test Order Name	Order LOINC Value
COGMF	COG, AML, FISH	In Process

Result ID	Test Result Name	Result LOINC Value
602276	Result Summary	50397-9
602277	Interpretation	69965-2
602278	Result Table	93356-4
602279	Result	62356-1
GC013	Reason for Referral	42349-1
GC014	Specimen	31208-2
602281	Source	31208-2
602282	Method	85069-3
602283	Additional Information	48767-8
602284	Disclaimer	62364-5
602285	Released By	18771-6