

FISH for Myeloproliferative Neoplasms

Background Information

In the 2008 WHO classification system, myeloid malignancies are classified based on a combination of clinical, morphologic, phenotypic and molecular cytogenetic features. Within the category of myeloproliferative neoplasms, four entities are defined by specific recurrent cytogenetic abnormalities that may be identified by fluorescence in situ hybridization (FISH):

1. Chronic myeloid leukemia, *BCR/ABL1* positive: By definition, all cases of chronic myeloid leukemia contain a *BCR/ABL1* translocation. This abnormality is also found in a subset of cases of acute lymphoblastic leukemia.
2. Myeloid neoplasms with abnormalities of *PDGFRA*: These cases exhibit leukocytosis with prominent eosinophilia and a rearrangement of chromosome 4q12 that produces a *FIP1L1-PDGFR*A fusion gene. This rearrangement is cryptic by banded cytogenetics alone, emphasizing the importance of FISH analysis for diagnosis.
3. Myeloid neoplasms with abnormalities of *PDGFRB*: Myeloid neoplasms with *PDGFRB* translocations show distinctive features including eosinophilia and, in some cases, monocytosis. The most common abnormality is a t(5;12)(q33;p13) *ETV6/PDGFRB*, but more than 20 different translocation partner genes have been described. FISH is performed using a break-apart probe designed to detect *PDGFRB* translocations. Although these abnormalities are most often detectable by banded cytogenetics, the 2008 WHO recommends confirmation by a molecular method such as FISH, which improves both specificity and sensitivity of detection.
4. Myeloid neoplasms with abnormalities of *FGFR1*: Myeloid neoplasms with *FGFR1* abnormalities are heterogeneous, but are usually associated with peripheral eosinophilia. Some cases represent so-called “stem-cell leukemia/lymphoma” with clonally related myeloid and lymphoblastic tumors. The most common abnormality is t(8;13)(p12;q12) *FGFR1/ZNF198*, but numerous alternative

FGFR1 translocations have also been described. *FGFR1* translocations are detected using a break-apart probe strategy designed to detect any *FGFR1* translocation.

Clinical Indications

FISH studies are useful in the workup of suspected myeloproliferative neoplasms, especially those showing eosinophilia. Although isolated eosinophilia is an uncommon presentation of *BCR/ABL1* positive CML, sufficient morphologic overlap is possible between CML and the myeloid neoplasms presenting with eosinophilia to warrant exclusion of *BCR/ABL1* in these cases. FISH testing may be ordered for each probe set individually, or as the FISH for MPN panel that includes all four of these loci.

Interpretation

Patient samples are probed and 200 nuclei are scored. Results for each probe set are compared to reference ranges established for abnormal signal patterns in healthy controls. Results are reported as positive or negative for each abnormality. The percentage of abnormal nuclei identified and interpretation are provided.

Limitations of the Assays

False negative results may occur if the clonal cells are present below the limit of detection of FISH assays. These tests are not intended for minimal residual disease detection. Rarely, fusion genes may arise through insertion events that are cryptic by FISH.

Methodology

FISH can be performed on peripheral blood or bone marrow aspirate specimens. For *PDGFRB* and *FGFR1* probes, paraffin-embedded material is also acceptable. Hybridizations are performed using the appropriate probe sets and examined using fluorescence microscopy.

The FISH reagents employed include dual color, dual fusion (DF) probes that specifically identify a translocation (*BCR/ABL1*) and dual color break-apart (BA) probes that identify the presence of a translocation involving the gene of interest but do not specifically identify the translocation partner gene (*PDGFRB*, *FGFR1*). *PDGFRA* abnormalities are assessed using a tri-color (TC) rearrangement probe designed to detect *PDGFRA-FIP1L1* or variant *PDGFRA* abnormalities.

Probe	Abnormality	Type
BCR/ABL1	t(9;22)(q34;q11.2) <i>BCR/ABL1</i>	DF
PDGFRA	<i>FIP1L1-PDGFRA</i> fusion or variant 4q12 (<i>PDGFRA</i>) rearrangement	TC
PDGFRB	5q33 (<i>PDGFRB</i>) translocation	BA
FGFR1	8p12 (<i>FGFR1</i>) translocation	BA

References

1. Bain BJ, Gilliland DG, Horny H-P, et al. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1*. In: WHO Classification of Haematopoietic and Lymphoid Tissues. Swerdlow SH, Campo E, Harris NL, et al. (eds). IARC Press: Lyon 2008. Pp 68-73.
2. Anastasi J. The myeloproliferative neoplasms including the eosinophilia-related myeloproliferations associated with tyrosine kinase mutations: changes and issues in classification and diagnosis criteria. *Semin Diagn Pathol*. 2011;28:304-13.
3. Gotlib J. World Health Organization-defined eosinophilic disorders: 2014 update on diagnosis, risk stratification, and management. *Am J Hematol*. 2014;89:325-37.

Test Overview

Test Name	FISH for Myeloproliferative Neoplasms
Ordering Mnemonic	MPNFSH
Includes	FISH for <i>BCR/ABL1</i> ; FISH for <i>FGFR1</i> ; FISH for <i>PDGFRA</i> ; FISH for <i>PDGFRB</i>
Specimen Requirements	Testing Volume/Size: 5 mL; Type: Whole blood; Container: EDTA (Lavender); Transport Temperature: Ambient
Minimum Specimen Requirements	Volume/Size: 3 mL
Alternate Specimen Requirements	Testing Volume/Size: 5 mL; Type: Bone marrow; Container: EDTA (Lavender).~~OR~~ Testing Volume/Size: 5 mL; Type: Bone marrow; Container: Sodium heparin (Green).~~OR~~ Testing Volume/Size: 5 mL; Type: Whole blood; Container: Sodium heparin (Green).
Billing Code	89510
CPT Codes	88367(x4); 88368(x5)

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