

## Fluorescence *In Situ* Hybridization (FISH) for Myelodysplastic Syndromes

### Background

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell malignancies characterized by ineffective hematopoiesis and varying amounts of dysplasia in one or more of the hematopoietic lineages (erythroid, myeloid and/or megakaryocytic).<sup>1</sup> Establishing a diagnosis of a myelodysplastic syndrome requires correlation of clinical features, morphologic features and cytogenetic data.

Recurring cytogenetic abnormalities in MDS include monosomy of chromosome 5, deletions on chromosome 5q, monosomy 7, deletions on chromosome 7q, deletions on chromosome 20q, and trisomy 8. The identification of any of these abnormalities in bone marrow or peripheral blood using metaphase cytogenetic analysis or FISH studies assists in the diagnosis and prognostic assessment of myelodysplastic syndromes. These abnormalities, however, are not specific for myelodysplastic syndromes, as they may also occur in acute myeloid leukemia or in other malignancies. FISH data must therefore always be interpreted in the context of all of the clinical and pathologic findings in a given case.

Interphase fluorescent *in situ* hybridization (FISH) studies increase the rate of detection of myelodysplasia-associated abnormalities compared to metaphase cytogenetics alone, especially in cases with suboptimal metaphase cytogenetic studies.<sup>2</sup>

### Clinical Indications

Cleveland Clinic Laboratories offer FISH studies to detect -5/5q-, -7/7q-, 20q- and/or +8. Peripheral blood or bone marrow specimens or cytogenetic cell pellets are suitable for analysis.

### Interpretation

Reference ranges are established based on analysis of normal control peripheral blood and bone marrow samples. Patient samples are classified as positive for a given abnormality when the percentage of nuclei with an abnormal signal pattern exceeds the reference range.

### Limitations of the Assay

False negative results may occur when the neoplastic cells represent a small population of cells (less than the cutoff percentages listed above). A normal result with this assay does not exclude the presence of other karyotypic abnormalities that are not targeted by these probes. The evaluation of suspected myelodysplasia should therefore also always include standard metaphase cytogenetic analysis.

### Methodology

FISH analysis is performed on gravity preparations of peripheral blood, bone marrow, or cytogenetic cell pellets. Cells are hybridized with a series of six probes described below. Probes are combined into three probe mixtures, each performed on a separate slide. Two hundred cells are evaluated for each probe using supervised, automated signal enumeration.

#### Probe set 1:

A two-color probe set is used to detect abnormalities of chromosome 5. One 190 kb probe, labeled in red (PlatinumBright 550), hybridizes to the HTERT locus on chromosome 5p15. A second, 645 kb probe, labeled in green (PlatinumBright 495), hybridizes to the EGR1 locus on chromosome 5q31.

**Probe set 2:**

A two-color probe set is used to detect abnormalities of chromosome 7. The CEP7 probe, labeled in green (Spectrum Green) hybridizes to the chromosome 7 centromere. A second, 200kb probe, labeled in red (Spectrum Orange), hybridizes to the LSI D7S486 locus at chromosome 7q31.

**Probe set 3:**

A two-color probe set is used to detect abnormalities of chromosome 8 and/or chromosome 20. The CEP8 probe, labeled in green (Spectrum Green) hybridizes to the chromosome 8 centromere. A second, 170kb probe, labeled in red (Spectrum Orange), hybridizes to the LSI D20S108 locus at chromosome 20q12.

**References**

1. Brunning R.D., Orazi A, Germing U, *et al.* Myelodysplastic syndromes In: Swerdlow SH, Campo E, Harris NL, *et al.* World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2008;87-88.
2. Coleman FJ, Theil KS, Tubbs RS, *et al.* Diagnostic yield of bone marrow and peripheral blood testing in clinically suspected myelodysplastic syndromes and/or acute myeloid leukemia: a prospective analysis of 433 cases. *Am J Clin Pathol.* 2011;135:915-20.

**Test Overview**

<b>Test Name</b>	Fluorescence <i>in situ</i> hybridization (FISH) for Myelodysplastic Syndromes
<b>Reference Range</b>	Normal pattern (disomic) for each locus
<b>Methodology</b>	Interphase fluorescence <i>in situ</i> hybridization
<b>External Specimen Requirements</b>	Testing Volume/Size: 8 mL; Type: Blood; Tube/Container: EDTA (Lavender); Transport Temperature: Refrigerated. — OR — Testing Volume/Size: 8 mL; Type: Blood; Tube/Container: Sodium heparin (Green); Transport Temperature: Ambient. — OR — Testing Volume/Size: 8 mL; Type: Bone marrow; Tube/Container: Sodium heparin (Green); Transport Temperature: Ambient.
<b>Billing Code</b>	84379
<b>CPT Code</b>	88367(x6)
<b>Related Tests</b>	Chromosome Analysis, Hematological Malignancy, Bone Marrow

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