

Multiple Myeloma (MM) by FISH

DETECTION OF PROGNOSTICALLY SIGNIFICANT GENOMIC ABERRATIONS IN MULTIPLE MYELOMA (MM) BY FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Test Highlights

- CD138 magnetic microbeads are used to sort plasma cells, which are then analyzed by FISH with a panel of probes to evaluate patients with multiple myeloma (MM).
- FISH is more sensitive than conventional cytogenetics in detecting genomic aberrations.
- This test accurately detects prognostically important genomic abnormalities in MM and is suitable for widespread use.

Clinical and Technical Background

- Multiple myeloma (MM) is a B-cell malignancy characterized by abnormal growth of plasma cells and is associated with paraprotein production and osteolytic bone lesions.
- MM primarily affects middle-aged to elderly patients.
- Incidence is three to nine cases per 100,000 per year.
- African-Americans and males are affected more often than Caucasians and females.¹
- MM is an incurable disease, and effective therapeutic approaches are urgently required for patients with MM at different risk groups.
- Standard prognostic factors include serum β 2-microglobulin, C-reactive protein (CRP), bone-marrow plasma-cell morphology, and plasma-cell proliferation (plasma-cell labeling index).²⁻³ These factors are independently associated with the prognosis of patients with MM. Recently, considerable interest has arisen in characterizing genomic markers to establish prognostic models that allow a better estimation of a patient's prognosis.
- Chromosomal abnormalities are among the most important prognostic parameters for patients with MM. However, conventional karyotyping has been hampered by the slow growth of MM cells in cell culture, and chromosomal abnormalities are often missed by this technique. Furthermore, earlier-stage myeloma cells are still dependent upon their microenvironment for growth. This hampers their growth in culture and contributes to the failure to detect chromosome abnormalities by metaphase analysis. The detection of abnormal metaphase cells is also indicative of stroma-independent growth and may be associated with advanced disease.^{4,6}
- Fluorescence in situ hybridization (FISH) detects chromosomal aberrations in both actively dividing cells and interphase nuclei.
- Certain chromosomal abnormalities have been associated with favorable and unfavorable prognostic groups in MM.⁴⁻¹⁰
 - Chromosome abnormalities associated with an unfavorable risk include deletion 13 or aneuploidy by conventional karyotyping (on metaphase chromosomes); t(4;14) or t(14;16) or t(14;20) by FISH, deletion 17p13 (p53) by FISH, gain of the 1q region including the CKS1B locus (detectable by FISH or metaphase chromosomes) and hypodiploidy.
 - Chromosome abnormalities associated with a favorable risk include the absence of unfavorable risk genetics and presence of hyperdiploidy, t(11;14) or t(6;14) by FISH. Hyperdiploidy

most commonly includes gains of chromosomes 15, 9, 3, 19, 11, 7, 21 and 5 (listed in order of frequency).¹¹

- Our group has developed a relatively simplified processing protocol targeting both normal and abnormal plasma cells, together with an MM FISH panel designed to detect the genomic aberrations with proven clinical significance. This approach utilizes commercially available probes for FISH analysis on CD138+ cells and reliably detects the prognostically significant genomic aberrations, thus allowing clinicians to assess the biological risk of disease progression in patients with MM.

Indications for Ordering

- FISH testing for MM is indicated in individuals who have been diagnosed with MM based on bone-marrow (BM) cell characteristics of morphology, cytochemical staining, and immunophenotype.
- FISH testing serves as a screen to prognostically stratify the risk of MM patients.

Methodology and Interpretation

- CD138 microbeads isolate human plasma cells. CD138 is expressed on normal and malignant plasma cells, but not on circulating B-cells, T-cells, and monocytes. Cell isolation is performed according to the manufacturer's protocol.
- CD138+ sorted BM cells are analyzed by FISH using a set of commercially available FISH probes specific for CKS1B (1q21), *IGH* (14q32), p53 (17p13.1), *IGH/CCND1* [t(11;14)], 11q13/15q22/9q34 (for ploidy analysis of chromosomes 11, 15 and 9). These probes constitute the basic MM FISH panel. If an *IGH* rearrangement is detected that does not involve *CCND1*, additional FISH experiments will be performed to detect a t(4;14) or t(14;16) using the *IGH/FGFR3* and *IGH/MAF* probes. Hybridization and detection of hybridization signals are performed according to the manufacturer's protocols.
- At least two technologists score the same case.
- 200 nuclei are evaluated for each probe.
- Bone marrow samples from 20 individuals without apparent hematological diseases and with normal karyotypes are used as controls to determine the cutoff value for normal variation of the probe patterns.

Additional Ordering Notes:

- A sodium-heparin (green-top) tube with 3–4 mL of bone marrow is required. Samples should be stored at room temperature and transported to the laboratory within 24 hours of draw.
- If insufficient bone marrow is available or the sorting process does not yield enough CD138+ cells, FISH will be performed on unsorted cells. However, this will decrease the sensitivity of the assay for low-level abnormalities.

Limitations

This probe panel only detects prognostically important imbalances (gain or loss of DNA) and rearrangements in the chromosomes of interest. Chromosome alterations outside the regions complementary to these FISH probes will not be detected.

References

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Test Information

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Multiple Myeloma Panel by FISH

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.