SPECIMEN : Leukemic Blood

FISH INVESTIGATION FOR : Bcr-Abl (t (9:22) Translocation)

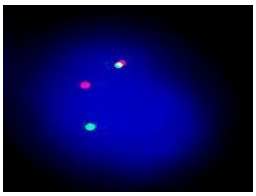
METHOD : Fluorescence in situ hybridization (FISH) was performed using fluorescent probes on cells obtained from un-stimulated cultures.The analysis was done on an Olympus BX43 fluorescent microscope with appropriate filters using the Applied Spectral Imaging Software.

PROBE(S) USED : ZytoLight SPEC BCR/ABL1 Dual Color Dual Fusion Probe

RESULTS :

Probe Name: BCR/ABL:t(9;22)

The probe hybridizes to chromosomes 22q11.2 (BCR gene – Green) and chromosomes 9q34 (ABL1 gene – Orange) which gives a normal pattern of two orange two green and a pattern of two fusion (yellow) , one orange and one green in t(9;22) positive cases. Apart from the signal patterns mentioned, other variant patterns may also be observed.

SIGNAL PATTERN	NO.OF CELLS	RESULT TYPE
1F1O1G/2O2G	20-180/200	Abnormal
	Interphase cell showing 1 Fusion ,1 Orange, 1 Green signals indicating BCR/ABL: Ph positive (9q deletion variant) status.	

O= orange (ABL signal);G= green (BCR signal);F=fusion(yellow) signal for BCR/ABL: Ph

INTERPRETATION : ISCN Nomenclature: nuc ish (ABL1,BCR)x2(ABL1 con BCRx1)[20/200]

FISH analysis revealed BCR/ABL:Ph positivity (9q deletion variant) in 10% of interphase cells analysed. (KIndly note that BCR ABL FISH pattern at diagnosis is not available)

The positivity is just above the cut off value for this pattern and could be due to reduction in abnormal clone or false positivity.

FISH RECOMMENDATION(S) : RQ-PCR for BCRABL1 IS testing is recommended for monitoring the response to treatment by reduction in the BCR/ABL1 clone size.

Cut-off: The cut off for numerical and structural abnormalities in normal individuals for BCR/ABL translocation is 1F1O1G pattern-9%, 2F1O1G pattern -2%.

Note:

- In case of a clinical suspicion of MPN, Jak2 V617F Reflex panel (Jak2 V617F, Jak2 Ex12, CALR Ex9, MPL W515/ S505) is indicated in Bcr/Abl negative cases.
- Chronic myeloid leukemia (also known as chronic myelogenous leukemia, CML) is a myeloproliferative neoplasm that originates in an abnormal pluripotent bone marrow (BM) stem cell or early progenitor cell and is defined by the presence of rearrangement of the BCR and ABL1 oncogenes, most commonly due to the reciprocal translocation t(9;22)(q34.1;q11.2).
- The chimeric BCR-ABL1 protein is a constitutively activated tyrosine kinase (TK) with a higher level of kinase activity when compared to the endogenous ABL1 kinase activity. The standard t(9;22) can be detected by conventional cytogenetic analysis/FISH in bone marrow or peripheral blood samples in more than 90% of CML patients.
- FISH is a molecular cytogenetic tool that uses fluorescently labeled DNA probes to detect the rearrangement of the BCR and ABL1 genes directly in either metaphase cells and/or interphase nuclei.
- Detection of the BCR/ABL1 fusion by FISH not only complements conventional chromosome analysis in disease diagnosis, follow-up and in monitoring treatment response until complete cytogenetic response is achienved, but also is very helpful in clarifying a variant, cryptic, or complex karyotype in CML patients.
- At the time of CML diagnosis, FISH can be used in parallel with cytogenetic analysis to confirm the t(9;22) and determine fusion signal patterns in bone marrow or peripheral blood samples. It is particularly important if the sample was suboptimal, or there were only few dividing cells in the cultured cells.
- Typical dual fusion signals (two fusions, one red and one green, 2F1R1G) are observed in 70-75 % cases. Atypical signal patterns are seen in approximately 25-30%cases. These include 1F1R2G, 1F2R1G and 1F1R1G representing deletions of the derivative 9 involving chromosome 9 sequences, chromosome 22 sequences, or both respectively; 3F1R1G usually representing gain of an additional Philadelphia chromosome (duplication of Ph associated with blast crisis) and 1F2R2G representing a three- or four-way variant translocation.
- FISH is also a very sensitive tool in monitoring treatment response and disease course by comparing the percentages of the BCR/ABL1 fusion positive leukemia cells because 200 or more interphase cells are examined as compared with 20 metaphase cells in conventional cytogenetic analysis. The specificity of metaphase FISH is for detection of the Philadelphia chromosome allows for easy identification of complex chromosomal rearrangements that mask the standard t(9;22).
- Disease can be monitored by BCR-ABL FISH cytogenetic testing until complete cytogenetic reponse is achieved (the assay with no longer detect the fusion gene), and then a sample for quantitative BCR-ABL testing should be sent for continued monitoring of disease, if the patient has the p190 or p210 isoform of the BCR-ABL fusion gene.
- CML treatments include therapy with a tyrosine kinase inhibitor (TKI) (eg, imatinib, nilotinib, or dasatinib) and allogeneic hematopoietic stem cell transplantation. TKI therapies have dramatically improved treatment outcomes in CML. Responses to TKI therapies are determined at the hematologic, cytogenetic, and molecular levels. Although these therapies induce durable responses in most patients with chronic-phase CML, resistance occasionally occur. The most common cause of this resistance is mutation in the ABL1 kinase domain. Laboratory tests that evaluate ABL1 kinase domain mutations are useful in selecting alternative treatments to cases with partial or poor response for standard tyrosine kinase therapies.

Disclaimer:

FISH is a rapid and precise molecular diagnostic technique that identifies only probe specific numerical and structural disorders. FISH cytogenetic testing is indicated as an initial evaluation for patients suspected to have CML or ALL and then for monitoring disease status, within the limits of the assay.

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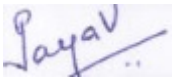

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