

FISH for Sarcoma Translocation Testing

Background Information

Soft tissue sarcomas are a histologically and genetically heterogeneous group of tumors, accounting for approximately 1% of all adult malignancies. Many sarcomas show characteristic combinations of morphologic and immunophenotypic features, which, in concert with clinical information and tumor site, allow their appropriate classification. However, their histologic features frequently overlap or may not be apparent due to poor differentiation or limited sampling in small biopsies.

In recent years, molecular genetic findings have advanced the basic scientific and clinical understanding of sarcomas and have become increasingly important in their precise diagnostic classification. Detection of chromosomal translocations in neoplastic cells is one of the molecular characteristics that can serve as a highly precise tool in diagnosis. These chromosomal translocations result in specific gene fusions, and each gene fusion or its closely related variant is usually present in most cases of a given sarcoma subtype. Such highly specific genetic changes are known to exist in more than 10 different sarcoma subtypes, and their expanding utilization in diagnosis is similar to what has become the standard of care in the diagnosis of leukemias and lymphomas in the past decade.

Detection of translocation events in sarcomas provides an important objective tool for confirmation of sarcoma diagnosis and disease monitoring, and their demonstration is important when considering tumor-specific therapeutic approaches to clinical management.

Clinical Indications

In situ hybridization (FISH) testing is performed for chromosomal abnormalities in formalin-fixed, paraffin-embedded specimens of soft tissue neoplasms. A series of break-apart format FISH assays is currently available and includes testing for the following:

1. translocations involving the *EWSR1* gene at 22q12 (Ewing sarcoma, desmoplastic small round-cell tumor, clear-cell sarcoma, angiomatoid fibrous histiocytoma, soft tissue myoepithelioma and extraskelatal myxoid chondrosarcoma).
2. the *DDIT3* (*CHOP*) gene at 12q13 (myxoid/round cell liposarcoma).
3. the *FOXO1A* (*FKHR*) gene at 13q14 (alveolar rhabdomyosarcoma).
4. the *SYT* gene at 18q11.2 (synovial sarcoma).
5. the *FUS* gene at 16p11.2 (low-grade fibromyxoid sarcoma, sclerosing epithelioid fibrosarcoma, angiomatoid fibrous histiocytoma, myxoid liposarcoma).
6. the *ALK* gene at 2p23 (inflammatory myofibroblastic tumor).

Interpretation

Detection via FISH of amplification of the *MDM2* gene locus is also helpful in the differential diagnosis of soft tissue tumors of lipomatous derivation (see separate Technical Brief).

Optimal samples have a minimum of 40 tumor cells for analysis.

Positive test:

10% or > cells showing a break-apart configuration of the two probes included in each of the individual assays.

Negative test:

< 10% break-apart signals identified.

Methodology

FISH can be utilized as an ancillary test in conjunction with routine histologic assessment and immunohistochemical studies in the diagnosis of soft tissue neoplasms.

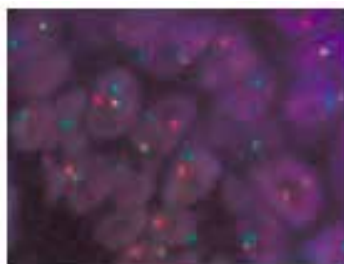
All FISH assays included in this panel utilize break-apart format probes and can be performed on routine sections from formalin-fixed, paraffin-embedded tissue. Fixation in solutions other than neutral buffered formalin compromises the quality of the assay and are not recommended.

Following cell conditioning, the slides are protease-digested, post-fixed in formaldehyde, washed and dehydrated.

Hybridization is performed using a selected pair of dual-color break-apart interphase FISH probes (Abbott Molecular, Vysis, Des Plaines, IL). Each probe kit includes two directly labeled probes flanking each of the following genes: *EWSR1* (22q12), *SYT* (18q11), *DDIT3* (*CHOP*) (12p13), *FUS* (16p11.2), *ALK* (2p23) and *FOXO1A* (*FKHR*) (13q14). The reagents have been validated as Analyte-Specific Reagents. The cells are analyzed using fluorescence microscopy.

Limitations of the Assay

False negative results may occur if less than 40 tumor cells are available for analysis. The assay has been developed for formalin-fixed tissue, and fixation in other solutions may compromise the assay results.



SYT gene translocation detected in a case of synovial sarcoma by fluorescence *in situ* hybridization (FISH), using a break-apart probe. The break-apart configuration (separation) of red and green signals in the tumor nuclei indicates a translocation involving the region of the SYT gene.

Recommended Reading

1. Zucman J, Melot T, Desmase C *et al.* Combinatorial generation of variable fusion proteins in the Ewing's family of tumors. *EMBO J.* 1993;12:4481.
2. Ladanyi M, Gerald W. Fusion of the *EWSR1* and *WT1* genes in desmoplastic small round cell tumor. *Cancer Res.* 1994;54:2837.
3. Clark J, Benjamin H, Gill S *et al.* Fusion of *EWSR1* gene to *CHN*, a member of the steroid/thyroid receptor gene superfamily, in a human myxoid chondrosarcoma. *Oncogene.* 1996;12:229.
4. Zucman J, Delattre O, Desmase C *et al.* *EWSR1* and *ATF-1* gene fusion induced by t(12;22) translocation in malignant melanoma of soft parts. *Nat Genet.* 1993;4:341.
5. Clark J, Rockques PJ, Crew AJ *et al.* Identification of novel genes, *SYT* and *SSX*, involved in t(X;18)(p11.2;q11.2) found in human synovial sarcoma. *Nat Genet.* 1994;7:502.
6. Rabbitts TH, Forster A, Larson R *et al.* Fusion of the dominant negative transcription regulator *CHOP* with a novel gene *FUS* by translocation t(12;16) in malignant liposarcoma. *Nat Genet.* 1993;4:175.
7. Galili N, Davis RJ, Fredericks WJ *et al.* Fusion of fork head domain (*FHFR*) gene to *PAX3* in the solid tumour alveolar rhabdomyosarcoma. *Nat Genet.* 1993;5:230.

Test Overview

Test Name	FISH for <i>EWSR1</i> (22q12)	FISH for <i>SYT</i> gene (18q11)	FISH for <i>DDIT3</i> (<i>CHOP</i>) gene (12p13)	FISH for <i>FOXO1A</i> (<i>FKHR</i>) gene (13q14)	FISH for <i>FUS</i> gene (16p11.2)	FISH for <i>ALK</i> gene (2p23)
Reference Range	10% or > cells showing a break-apart configuration of the two probes included in each of the individual assays					
Billing Code	82671	82787	83763	83763	83758	88844
CPT Code	88368 (x2)	88368 (x2)	88368 (x2)	88368 (x2)	88368 (x2)	88368 (x2)

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