Test Definition: FOXOF

FOXO1 (13q14), ARMS, FISH, Ts

Overview

Useful For

Supporting the diagnosis of alveolar rhabdomyosarcomas (ARMS) when used in conjunction with an anatomic pathology consultation

Aiding in the diagnosis of ARMS when reverse transcriptase-PCR results are equivocal or do not support the clinical picture

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
_PBCT	Probe, +2	No, (Bill Only)	No
_PADD	Probe, +1	No, (Bill Only)	No
_PB02	Probe, +2	No, (Bill Only)	No
_PB03	Probe, +3	No, (Bill Only)	No
_IL25	Interphases,	No, (Bill Only)	No
_I099	Interphases, 25-99	No, (Bill Only)	No
_I300	Interphases, >=100	No, (Bill Only)	No

Testing Algorithm

This test does not include a pathology consult. If a pathology consultation is requested, PATHC / Pathology Consultation should be ordered and the appropriate FISH test will be ordered and performed at an additional charge.

This test includes a charge for application of the first probe set (2 FISH probes) and professional interpretation of results. Additional charges will be incurred for all reflex probes performed. Analysis charges will be incurred based on the number of cells analyzed per probe set. If no cells are available for analysis, no analysis charges will be incurred.

Method Name

Fluorescence In Situ Hybridization (FISH)

NY State Available

Yes

Specimen

Specimen Type

Tissue

Advisory Information

If a pathology consultation is desired, order PATHC / Pathology Consultation.

Shipping Instructions

Advise Express Mail or equivalent if not on courier service.

Necessary Information

A reason for referral and pathology report are required in order for testing to be performed. Send information with specimen. Acceptable pathology reports include working drafts, preliminary pathology or surgical pathology reports.

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Tissue

Preferred: Tissue block

Collection Instructions: Submit a formalin-fixed, paraffin-embedded (FFPE) tumor tissue block. Blocks prepared with alternative fixation methods may be acceptable; provide fixation method used.

Acceptable: Slides

Collection Instructions: Four consecutive, unstained, 5-micron thick sections placed on positively charged slides, and 1 hematoxylin and eosin-stained slide.

Forms

If not ordering electronically, complete, print, and send an Oncology Test Request (T729) with the specimen.

Specimen Minimum Volume

Two consecutive, unstained, 5-micron thick sections placed on positively charged slides, and 1 hematoxylin and eosin (H and E)-stained slide

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Tissue	Ambient (preferred)		
	Refrigerated		

Clinical and Interpretive

Clinical Information

Rhabdomyosarcomas are a heterogeneous group of malignant tumors showing skeletal muscle differentiation. They can be divided into 3 subtypes: alveolar, embryonal, and pleomorphic. The rarer alveolar rhabdomyosarcomas (ARMS) are seen in older children, are more likely to occur in limbs, and are associated with higher stage disease and an unfavorable prognosis.

The alveolar form consists of 2 variants; classic and solid. The classic form is characterized by small round cells with dark hyperchromatic nuclei containing distinct nucleoli, held together by strands of intercellular collagen, thereby creating a cellular architecture resembling the alveolar spaces of the lungs. The solid form is characterized by a

similar cellular morphology but without the formation of alveolar spaces. ARMS are also members of the small round cell tumor group that includes synovial sarcoma, lymphoma, Wilms tumor, Ewing sarcoma, and desmoplastic small round cell tumor.

Most cases of ARMS (75%) are associated with a t(2;13)(q35;q14), where a chimeric gene is formed from the rearrangement of the *PAX3* gene on chromosome 2 and the *FOXO1(FKHR)* gene on chromosome 13. A small subset of ARMS patients (10%) are associated with a variant translocation, t(1;13)(q36;q14), involving the *PAX7* gene of chromosome 1 and the *FOXO1* gene. Detection of these transcripts by RT-PCR (ARMS / Alveolar Rhabdomyosarcoma by Reverse Transcriptase PCR [RT-PCR]), which allows specific identification of the t(2;13) and t(1;13), has greatly facilitated the diagnosis of ARMS tumors. FISH analysis (using the *FOXO1* probe) adds the ability to detect variant *FOXO1* rearrangements not detectible by PCR, and will often yield results when the quality of the available RNA is poor or the PCR results are equivocal.

Reference Values

An interpretive report will be provided.

Interpretation

A neoplastic clone is detected when the percent of cells with an abnormality exceeds the normal cutoff for the *FOXO1* FISH probe.

A positive result suggests rearrangement of the *FOXO1* gene region at 13q14 and is consistent with a subset of alveolar rhabdomyosarcomas (ARMS).

A negative result suggests *FOXO1* gene rearrangement is not present, but does not exclude the diagnosis of alveolar rhabdomyosarcomas (ARMS).

Cautions

This test is not approved by the US Food and Drug Administration and it is best used as an adjunct to existing clinical and pathologic information.

Fixatives other than formalin (eg, Prefer, Bouin) may not be successful for FISH assays, however, nonformalin-fixed samples will not be rejected.

Paraffin-embedded tissues that have been decalcified are generally unsuccessful for FISH analysis. The pathologist reviewing the hematoxylin and eosin-stained slide may find it necessary to cancel testing.

Supportive Data

FISH analysis was performed on 42 formalin-fixed paraffin-embedded tissue samples, including 17 rhabdomyosarcomas and 25 noncancerous soft tissue control specimens (from various anatomic locations). The normal controls were used to generate a normal cutoff for this assay. Rearrangement of *FOXO1* was identified in 10 of 17 (59%) of alveolar rhabdomyosarcoma specimens.

Clinical Reference

1. Fletcher CDM, Unni K, Mertens F: World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. IARC: Lyon 2002, pp 150-152

2. Galili N, Davis RJ, Fredericks WJ, et al: Fusion of a fork head domain gene to *PAX3* in the solid tumor alveolar rhabdomyosarcoma. Nat Genet 1993 Nov;5(3):230-235

3. Nishio J, Althof PA, Bailey JM, et al: Use of a novel FISH assay on paraffin-embedded tissues as an adjunct to diagnosis of alveolar rhabdomyosarcoma. Lab Invest 2006 Jun;86(6):547-556

4. Ladanyi M. Bridge JA: Contribution of molecular genetic data to the classification of sarcomas. Hum Pathol 2000 May;31(5):532-538

5. Lae ME, Roche PC, Jin L, et al: Desmoplastic small round cell tumor: a clinicopathologic, immunohistochemical, and molecular study of 32 tumors. Am J Surg Pathol 2002 Jul;26(7):823-835

6. Barr FG: Gene fusions involving PAX and FOX family members in alveolar rhabdomyosarcoma. Oncogene 2001 Sep 10;20(40):5736-5746

Performance

Method Description

The test is performed using a commercially available *FOXO1* dual-color break-apart strategy probe (BAP). Formalin-fixed paraffin-embedded tissues are cut at 5 microns and mounted on positively charged glass slides. The selection of selection of tissue and the identification of target areas on the hematoxylin and eosin (H and E)-stained slide is performed by a pathologist. Using the H and E-stained slide as a reference, target areas are etched with a diamond-tipped etcher on the back of the unstained slide to be assayed. The probe set is hybridized to the appropriate target areas and 2 technologists each analyze 50 interphase nuclei each (100 total) with the results expressed as the percent abnormal of nuclei.(Unpublished Mayo method)

PDF Report

No

Day(s) and Time(s) Test Performed

Samples processed Monday through Sunday.

Results reported Monday through Friday: 8 a.m.-5 p.m.

Analytic Time

7 days

Maximum Laboratory Time

10 days

Specimen Retention Time

Slides and H&E used for analysis are retained by the laboratory in accordance to CAP and NYS requirements. Client provided paraffin blocks and extra unstained slides (if provided) will be returned after testing is complete.

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

This test was developed using an analyte specific reagent. Its performance characteristics were determined by Mayo

Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

88271 x 2, 88291-DNA probe, each (first probe set), Interpretation and report

88271 x 2-DNA probe, each; each additional probe set (if appropriate)

88271-DNA probe, each; coverage for sets containing 3 probes (if appropriate)

88271 x 2-DNA probe, each; coverage for sets containing 4 probes (if appropriate)

88271 x 3-DNA probe, each; coverage for sets containing 5 probes (if appropriate)

88274 w/modifier 52-Interphase in situ hybridization, <25 cells, each probe set (if appropriate)

88274-Interphase in situ hybridization, 25 to 99 cells, each probe set (if appropriate)

88275-Interphase in situ hybridization, 100 to 300 cells, each probe set (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
FOXOF	FOXO1 (13q14), ARMS, FISH, Ts	93807-6

Result ID	Test Result Name	Result LOINC Value
52211	Result Summary	50397-9
52213	Interpretation	69965-2
52389	Result	62356-1
CG752	Reason for Referral	42349-1
52214	Specimen	31208-2
52215	Source	31208-2
52216	Tissue ID	80398-1
52217	Method	49549-9
54592	Additional Information	48767-8
52824	Disclaimer	62364-5
52218	Released By	18771-6