Overview

Useful For
Supporting a diagnosis of well-differentiated liposarcoma/atypical lipomatous tumor

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 fluorescence in situ hybridization (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.

Reflex Tests

<table>
<thead>
<tr>
<th>Test Id</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>_PBCT</td>
<td>Probe, +2</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>_PADD</td>
<td>Probe, +1</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>_PB02</td>
<td>Probe, +2</td>
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<td>No</td>
</tr>
<tr>
<td>_PB03</td>
<td>Probe, +3</td>
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<td>No</td>
</tr>
<tr>
<td>_IL25</td>
<td>Interphases, &lt;25</td>
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<td>No</td>
</tr>
<tr>
<td>_I099</td>
<td>Interphases, 25-99</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>_I300</td>
<td>Interphases, &gt;=100</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Method Name
Fluorescence In Situ Hybridization (FISH)

NY State Available
Yes

Specimen

Specimen Type
Tissue

Necessary Information
1. A pathology report is required in order for testing to be performed. Acceptable pathology reports include working drafts, preliminary pathology or surgical pathology reports.
2. A reason for testing must be provided. If this information is not provided, an appropriate indication for testing may be entered by Mayo Clinic Laboratories.

Specimen Required
Submit only 1 of the following specimens:
Test Definition: MDM2F
MDM2 (12q15) Amp, FISH, Ts

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 1 of the following forms with the specimen:
- Oncology Test Request (T729)
- Cardiovascular Test Request (T724)

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

Specimen Minimum Volume
Two consecutive, unstained, 5- micron-thick sections placed on positively charged slides and 1 hematoxylin and eosin-stained slide.

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td>Ambient (preferred)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Refrigerated</td>
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</tbody>
</table>

Clinical & Interpretive

Clinical Information
Differential diagnosis of well-differentiated liposarcoma/atypical lipomatous tumor:
The histological discrimination of well-differentiated liposarcoma/atypical lipomatous tumor (WDL/ALT) from lipoma can be diagnostically challenging. However, standard cytogenetic identification of ring and giant rod chromosomes strongly support the diagnosis of WDL/ALT. These abnormal chromosomes are mainly composed of amplified sequences derived from chromosome bands 12q13-15, and contain several amplified genes including MDM2, CPM, CDK4, and TSPAN31. MDM2 is amplified in greater than 99% of WDL, and up to 30% of other types of sarcomas.

Differential diagnosis of osteosarcoma:
The histological discrimination of parosteal or low grade central osteosarcoma from other morphologically similar, but clinically distinct entities, can be difficult. Amplification of genomic material derived from chromosome 12q13-15, which contains several genes including MDM2, has been shown to be a recurrent finding in a large proportion (67-100%) of parosteal and central low-grade osteosarcomas. Therefore, the detection of MDM2 gene amplification by fluorescence in situ hybridization (FISH) may be a useful adjunct to support a diagnosis of low-grade central or parosteal osteosarcoma in the proper histopathologic context. Amplifications of 12q13-15 (including MDM2) are less common in conventional high-grade osteosarcoma, estimated to occur in approximately of 5% to 10% of tumors.

Reference Values
An interpretive report will be provided.
Interpretation

Differential diagnosis of well-differentiated liposarcoma/atypical lipomatous tumor:
A neoplastic clone is detected when the percent of cells with an abnormality exceeds the normal reference range for the $MDM2$ fluorescence in situ hybridization (FISH) probe (positive result). A positive result is consistent with amplification of the $MDM2$ gene locus (12q15) and supports the diagnosis of well-differentiated liposarcoma/atypical lipomatous tumor (WDL/ALT). A negative result is consistent with absence of amplification of the $MDM2$ gene locus (12q15). However, negative results do not exclude the diagnosis of WDL/ALT. Amplification varies in individual tumors and among different cells in the same tumor.

Differential diagnosis of osteosarcoma:
A positive result is consistent with amplification of the $MDM2$ gene locus (12q15) and supports the diagnosis of parosteal osteosarcoma or low-grade central osteosarcoma. A negative result indicates an absence of amplification of the $MDM2$ gene locus (12q15). However, negative results do not exclude the diagnosis of low-grade central osteosarcoma or parosteal osteosarcoma.

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 fluorescence in situ hybridization (FISH) assays; however non-formalin-fixed samples will not be rejected.

Paraffin-embedded tissues that have been decalcified may not be successful for FISH analysis. FISH studies will be attempted if sufficient tumor is present for analysis. However, if no FISH signals are observed post-hybridization, the case will be released indicating a lack of FISH results.

Supportive Data

Fluorescence in situ hybridization (FISH) analysis was performed on 10 formalin-fixed, paraffin-embedded, well-differentiated liposarcoma/atypical lipomatous tumors (WDL/ALT) tumor samples and 25 normal controls. Amplification of $MDM2$ was identified in the WDL/ALT samples and correlated with the CPM results. Amplification of $MDM2$ was not observed in any of the control samples tumors.

Clinical Reference

**Performance**

**Method Description**
This test is performed using commercially available *MDM2* (12q15) and chromosome 12 centromere (D12Z3) probes. Formalin-fixed, paraffin-embedded tissues are cut at 5 microns and mounted on positively charged glass slides. The 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 30 interphase nuclei (60 total) per probe set with the results expressed as a ratio *MDM2*:D12Z3 signals. (Unpublished Mayo method)

**PDF Report**
No

**Specimen Retention Time**
Slides and H and 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 & Codes**

**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 US Food and Drug Administration.

**CPT Code Information**
88271x2, 88291-DNA probe, each (first probe set), Interpretation and report
88271x2-DNA probe, each; each additional probe set (if appropriate)
88271x1-DNA probe, each; coverage for sets containing 3 probes (if appropriate)
88271x2-DNA probe, each; coverage for sets containing 4 probes (if appropriate)
88271x3-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)