Lymphoma (Aggressive) Panel by FISH

Indications for Ordering
- Aid in diagnosis of aggressive large B-cell lymphoma with intermediate features between Burkitt lymphoma and diffuse large B-cell lymphoma (DLBCL)
- Confirmation of suspected double hit lymphoma

Test Description
Fluorescence in situ hybridization (FISH)
- FISH probes include
  - MYC
  - IGH
  - BCL2
  - BCL6

Tests to Consider
Typical Testing Strategy
- Lymph node biopsy with morphologic and immunohistochemical evaluation
- Leukemia/lymphoma phenotyping by flow cytometry
- In aggressive B-cell lymphomas with a high proliferation index and/or with unusual morphologic, phenotypic, or clinical features
  - Tests for MYC, BCL2, and BCL6

Panel Tests
- Aggressive B-Cell Lymphoma Reflex Panel by FISH, Tissue 3001495
  - Formalin-fixed, paraffin-embedded (FFPE) tissue specimens
- If MYC (8q24) Gene Rearrangement by FISH is positive, then IGH-BCL2 Fusion, t(14;18) by FISH will be added
- If IGH-BCL2 Fusion, t(14;18) by FISH is negative, then BCL6 (3q27) Gene Rearrangement by FISH will be added

Lymphoma (Aggressive) Panel by FISH 2002650
- Bone marrow (BM) or whole blood specimens; other specimens may be acceptable
  - FFPE and frozen specimens unacceptable

Individual Tests
- FFPE tissue specimens
  - MYC (8q24) Gene Rearrangement by FISH 3001300
    - Detects all MYC rearrangements, including t(8;14), t(2;8), and t(8;22)
- IGH-MYC (8;14) by FISH 3001299
- IGH-BCL2 Fusion, t(14;18) by FISH 3001298
- BCL6 (3q27) Gene Rearrangement by FISH 3001311

Related Tests
- Leukemia/Lymphoma Phenotyping Evaluation by Flow Cytometry 3001780
  - Aids in diagnosis of hematopoietic neoplasms
- Chromosome FISH, Interphase 2002298
  - Specific probes must be requested
    - MYC break apart, BCL2, BCL6
  - Fresh tissue specimens only
- Chromosome Analysis, Bone Marrow 2002292
  - Diagnosis, prognosis, and monitoring of lymphoma in BM
- Chromosome Analysis, Solid Tumor 2002296
  - May identify additional, useful cytogenetic abnormalities in tissues that are not targeted by FISH assays

Disease Overview
Prognostic Issues
- B-cell lymphomas with two recurrent chromosomal breakpoint aberrations are referred to as double hit lymphomas and are classified as high-grade B-cell lymphomas with MYC and BCL2 and/or BCL6 (WHO 2016)
  - Usually involve MYC oncogene in association with BCL2, less often with BCL6
  - Lymphomas with three translocations (usually MYC/BCL2/BCL6) are referred to as triple hit lymphomas
    - Rare
  - Important to identify these lymphomas in diagnostic evaluation of morphologically aggressive lymphomas
    - They are highly resistant to standard chemotherapy
      - Poor outcome independent of regimen intensity or inclusion of rituximab
    - Individuals have shortened survival compared with those having Burkitt lymphoma or international prognostic index (IPI)-matched DLBCL
### Breakpoints Used to Identify Double Hit or Triple Hit Lymphomas

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Break apart MYC</th>
<th>BCL2</th>
<th>BCL6</th>
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<tbody>
<tr>
<td>Locus</td>
<td>8q24</td>
<td>18q21</td>
<td>3q27</td>
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**Biology**
- Accelerator of cell proliferation
- Apoptosis inhibitor
- Transcription modifier

**Cytogenetics**
- Any MYC translocation
- BCL2/IGH – t(14;18) (q32;q21)
- BCL6 most commonly has a non-IGH translocation partner – BCL6 (3q27)
- Uncommon partner – BCL6/IGH [t(3;14)(q27;q32)]

**Specific lymphomas associated with translocation**
- Burkitt lymphoma
- DLBCL
- Aggressive B-cell lymphoma not otherwise specified (NOS)
- Follicular lymphoma
- DLBCL
- Follicular lymphoma
- High grade lymphomas (rare)

### Test Interpretation

**Results**
- Abnormal – t(14;18)(q32;q21) (IGH/BCL2 translocation) or other rearrangements involving BCL6 and/or MYC detected
  - Presence of two or more translocations is associated with poor prognosis in mature B-cell lymphomas
  - Single rearrangements can provide diagnostic and/or prognostic information in the appropriate context
- Normal – t(14;18)(q32;q21) (IGH/BCL2 translocation) or other rearrangements involving BCL6 or MYC not detected

**Limitations**
- Interpretation of results requires correlation with morphology and immunophenotype
- MYC and/or BCL2 overexpression can be due to other mechanisms not detected by this test
- Chromosome alterations outside probe region are not detected