Technical Brief

T-Cell and B-Cell Clonality Using BIOMED-2 PCR Primers

Background Information
An assessment of T-cell or B-cell clonality is an important part of the evaluation of suspected lymphoproliferative disorders. Historically, Southern blot studies for T-cell receptor beta chain (TCRB) rearrangements have been considered to represent the gold standard for T-cell clonality evaluation while immunoglobulin heavy chain (IGH) and/or immunoglobulin kappa light chain (IGK) rearrangements have served this purpose for B-cell clonality. Southern blot studies, however, are labor-intensive and time-consuming for the laboratory and often are impractical for routine practice as they require fresh or frozen tissue and cannot be performed on formalin-fixed, paraffin-embedded (FFPE) material. PCR assays for T-cell receptor gamma chain (TCRG) and/or TCRB rearrangements and IGH or IGK rearrangements offer the ability to assess clonality from standard FFPE, but until recently, PCR studies have been limited by a higher false negative rate compared to Southern blot studies.

The BIOMED-2 multinational collaborative study developed and standardized multiplexed PCR primers that are capable of detecting clonal lymphocyte populations with a sensitivity that approaches that of Southern blot (1). Cleveland Clinic Laboratories now offers T-cell clonality and B-cell clonality assays using BIOMED-2 PCR primers.

The T-cell clonality assay employs primers for both TCRB and TCRG, a combination that has been shown to detect clonality in essentially 100% of T-cell prolymphocytic leukemias, T-cell large granular lymphocyte disorders, and peripheral T-cell lymphomas, unspecified, with somewhat lower rates reported in angioimmunoblastic T-cell lymphomas and anaplastic large cell lymphoma (2). Assays for only TCRB or only TCRG rearrangements may also be ordered, if desired.

The B-cell clonality assay employs primers for both IGH and IGK. This combination of IGH and IGK primers has been shown to detect approximately 98% of B-cell clonal populations compared to Southern blot (3). Assays for only IGH or only IGK rearrangements may also be ordered, if desired.

Clinical Indications
These assays are designed for detection of clonal T-cell or B-cell populations in suspected lymphoproliferative disorders using fresh, frozen, or FFPE tissue.

Interpretation
Results are reported as:
- Positive for a clonal population,
- Negative for a clonal population, or
- Indeterminate.

Limitations of the Assays
1. Results of clonality studies must be interpreted in the context of the clinical and histologic findings. Clonality is not equivalent to malignancy as physiologic clonal populations may be detected in some reactive conditions.
2. For optimal detection of T-cell clonality, the use of both TCRB and TCRG primers is recommended.
3. For optimal detection of B-cell clonality, the use of both IGH and IGK primers is recommended.
4. The detection of TCRB and/or TCRG rearrangements (for T-cell clonality) or IGH and/or IGK rearrangements (for B-cell clonality) cannot be used for lineage assignment, as some T- or B-cell lymphoproliferative disorders or acute myeloid leukemias may have detectable clonal rearrangements with these primers. False positive “pseudoclonal” results may sometimes be detected when few T- or B-cells are present in the tissue analyzed. Rare clonal T-cell or B-cell populations may not be detected using these primers.
Methodology

PCR is performed utilizing the standardized BIOMED-2 protocol (1).

PCR products are analyzed using capillary electrophoresis.

For T-cell clonality: Rearrangements of the TCRB locus are assessed using three sets of labeled multiplexed PCR primers. The first two tubes consist of forward primers targeting V variable segments and reverse primers targeting the J joining region. The third TCRB tube detects incomplete rearrangements using forward primers to the D diversity segments and reverse primers targeting the J joining regions. Rearrangements of the TCRG locus are assessed using two sets of multiplexed labeled PCR primers, each with forward primers for the V variable regions and reverse primers targeting the J joining region.

For B-cell clonality: Rearrangements of the IGH locus are assessed using three sets of labeled multiplexed PCR primers. These tubes consist of forward primers targeting the IGH variable framework 1, framework 2, or framework 3 region with common reverse primers to the JH joining region. Rearrangements of the IGK locus are assessed using two sets of multiplexed labeled PCR primers. The first tube uses forward primers for the kappa variable regions and a reverse primer targeting the J joining region. The second tube uses forward primers to the kappa variable regions and an intron primer together with a reverse primer targeting the kappa locus deleting element.

References

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Reference Range</th>
<th>Specimen Requirements</th>
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</thead>
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| T-Cell Clonality Using BIOMED-2 PCR Primers | Negative for clonal rearrangement | External Specimen Requirements: 
Testing Volume/Size: 10 mm²
Type: Tissue, frozen
Tube/Container: Clean container
Note: Frozen tissue should be delivered to Surgical Pathology for accessioning and cutting.
Testing Volume/Size: 10 mm²
Type: Tissue, paraffin-embedded
Tube/Container: Clean container
Note: Paraffin-embedded tissue should be delivered to Surgical Pathology for accessioning and cutting.
Alternate Specimen Requirements:
Testing Volume/Size: 2 mL
Type: Bone marrow
Tube/Container: EDTA (Lavender)
Transport Temperature: Refrigerated
Testing Volume/Size: 2 mL
Type: Fluid, bone
Tube/Container: EDTA (Lavender)
Transport Temperature: Refrigerated
Note: Fluid must contain at least 3 million cells.
Testing Volume/Size: 8 mL
Type: Whole blood
Tube/Container: EDTA (Lavender)
Transport Temperature: Refrigerated
Testing Volume/Size: 5 mL
Type: Whole blood
Tube/Container: EDTA (Lavender)
Transport Temperature: Refrigerated
Testing Volume/Size: Other
Type: Extracted DNA
Tube/Container: Clean container
Transport Temperature: Frozen
Note: Volume/Size: 6µg |
| T-Cell Receptor Beta BIOMED-2 PCR         | Negative for clonal rearrangement | External Specimen Requirements: 
Testing Volume/Size: 10 mm²
Type: Tissue, frozen
Tube/Container: Clean container
Transport Temperature: Frozen
Note: Paraffin-embedded tissue should be delivered to Anatomic Pathology for accessioning and cutting.
Testing Volume/Size: 10 mm²
Type: Tissue, paraffin-embedded
Tube/Container: Clean container
Note: Paraffin-embedded tissue should be delivered to Anatomic Pathology for accessioning and cutting.
Alternate Specimen Requirements:
Testing Volume/Size: 2 mL
Type: Bone marrow
Tube/Container: EDTA (Lavender)
Transport Temperature: Refrigerated
Testing Volume/Size: 2 mL
Type: Fluid, body
Tube/Container: EDTA (Lavender)
Transport Temperature: Refrigerated
Note: Fluid must contain at least 3 million cells.
Testing Volume/Size: 8 mL
Type: Whole blood
Tube/Container: EDTA (Lavender)
Transport Temperature: Refrigerated |
| TCR-G (PCR)                              | Negative for clonal rearrangement | External Specimen Requirements: 
Testing Volume/Size: 10 mm²
Type: Tissue, frozen
Tube/Container: Clean container
Transport Temperature: Frozen
Note: Send specimen at -70°C on dry ice.
Testing Volume/Size: 10 mm²
Type: Tissue, paraffin-embedded
Tube/Container: Clean container
Note: Paraffin-embedded tissue should be delivered to Anatomic Pathology for accessioning and cutting.
Alternate Specimen Requirements:
Testing Volume/Size: 2 mL
Type: Bone marrow
Tube/Container: EDTA (Lavender)
Transport Temperature: Refrigerated
Testing Volume/Size: 2 mL
Type: Fluid, body
Tube/Container: EDTA (Lavender)
Transport Temperature: Refrigerated
Note: Fluid must contain at least 3 million cells.
Testing Volume/Size: 8 mL
Type: Whole blood
Tube/Container: EDTA (Lavender)
Transport Temperature: Refrigerated |

**Test Ordering Information**
- Clearly indicate specimen source on sample label

**Billing Code**
- 87903
- 87965
- 81402

**CPT Code**
- 83891; 83898; 83900; 83901(x7); 83909(x5); 83912
- 83891; 83898; 83900; 83901(x3); 83909(x3); 83912
- 83891; 83900; 83901(x4); 83909(x2); 83912
## Test Overview

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Reference Range</th>
<th>Specimen Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-Cell Clonality Using BIOMED-2 PCR Primers</td>
<td>Negative for clonal rearrangement</td>
<td><strong>External Specimen Requirements:</strong> Testing Volume/Size: 10 mm² Type: Tissue Tube/Container: Clean container <strong>Note:</strong> May submit fresh, frozen or paraffin-embedded tissue <strong>Alternate Specimen Requirements:</strong> Testing Volume/Size: 2 mL Type: Bone marrow Tube/Container: EDTA (Lavender) Transport Temperature: Refrigerated <strong>Note:</strong> Frozen and fresh tissue should be delivered to Surgical Pathology for accessioning and cutting. Paraffin-embedded tissue should be delivered to Anatomic Pathology for accessioning and cutting. Testing Volume/Size: 2 mL Type: Fluid, body Tube/Container: EDTA (Lavender) Transport Temperature: Refrigerated <strong>Note:</strong> Fluid must contain at least 3 million cells. Testing Volume/Size: 8 mL Type: Whole blood Tube/Container: EDTA (Lavender) Transport Temperature: Refrigerated</td>
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<tr>
<td>Immunoglobulin Heavy Chain Using BIOMED-2 PCR Primers</td>
<td>Negative for clonal rearrangement</td>
<td><strong>External Specimen Requirements:</strong> Testing Volume/Size: 10 mm² Type: Tissue Tube/Container: Clean container Transport Temperature: Frozen <strong>Note:</strong> Frozen and fresh tissue should be delivered to Surgical Pathology for accessioning and cutting. Paraffin-embedded tissue should be delivered to Anatomic Pathology for accessioning and cutting. <strong>Alternate Specimen Requirements:</strong> Testing Volume/Size: 2 mL Type: Bone marrow Tube/Container: EDTA (Lavender) Transport Temperature: Refrigerated Testing Volume/Size: 8 mL Type: Blood Tube/Container: EDTA (Lavender) Transport Temperature: Refrigerated Testing Volume/Size: Other Type: Extracted DNA Tube/Container: EDTA (Lavender) Transport Temperature: Frozen <strong>Note:</strong> Volume/Size: 6 µg</td>
</tr>
<tr>
<td>Immunoglobulin Kappa Chain Using BIOMED-2 PCR Primers</td>
<td>Negative for clonal rearrangement</td>
<td><strong>External Specimen Requirements:</strong> Testing Volume/Size: 10 mm² Type: Tissue Tube/Container: Clean container Transport Temperature: Frozen <strong>Note:</strong> Frozen and fresh tissue should be delivered to Surgical Pathology for accessioning and cutting. Paraffin-embedded tissue should be delivered to Anatomic Pathology for accessioning and cutting. <strong>Alternate Specimen Requirements:</strong> Testing Volume/Size: 2 mL Type: Bone marrow Tube/Container: EDTA (Lavender) Transport Temperature: Refrigerated Testing Volume/Size: 8 mL Type: Blood Tube/Container: EDTA (Lavender) Transport Temperature: Refrigerated Testing Volume/Size: Other Type: Extracted DNA Tube/Container: Clean container Transport Temperature: Frozen <strong>Note:</strong> Volume/Size: 6 µg</td>
</tr>
</tbody>
</table>

### Test Ordering Information
- Clearly indicate specimen source on sample label
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### Billing Code
- 87904
- 87960
- 87954

### CPT Code
- 83891; 83898(x3); 83900; 83901(x2); 83909(x5); 83912
- 83891; 83898(x3); 83909(x3); 83912
- 83891; 83900; 83901(x4); 83909(x2); 83912

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