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

1. JJM VanDongen, AW Langerak, M Bruggermann *et al*. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*. 2003;17:2257-2317.
2. M Bruggermann, H White, P Gaulard *et al*. Powerful strategy for polymerase chain reaction-based clonality assessment in T-cell malignancies: Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. *Leukemia*. 2007;21:215-221.
3. PAS Evans, Ch Pott, PJTA Groenen *et al*. Significantly improved PCR-based clonality testing in B-cell malignancies by use of multiple immunoglobulin gene targets. Report of the BIOMED-2 Concerted Action BHM4-CT-98-3936. *Leukemia*. 2007;21:207-214.

Test Overview

Test Name	T-Cell Clonality Using BIOMED-2 PCR Primers	T-Cell Receptor Beta BIOMED-2 PCR	TCR-G (PCR)
Reference Range	Negative for clonal rearrangement	Negative for clonal rearrangement	Negative for clonal rearrangement
Specimen Requirements	**External Specimen Requirements:** Testing Volume/Size: 10 mm^2 Type: Tissue, frozen Tube/Container: Clean container *Note: Frozen tissue should be delivered to Surgical Pathology for accessioning and cutting.* Testing Volume/Size: 10 mm^2 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, 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	**External Specimen Requirements:** Testing Volume/Size: 10 mm^2 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 mm^2 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: 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*	**External Specimen Requirements:** Testing Volume/Size: 10 mm^2 Type: Tissue, frozen Tube/Container: Clean container Transport Temperature: Frozen *Note: Send specimen at -70°C on dry ice.* Testing Volume/Size: 10 mm^2 Type: Tissue, paraffin-embedded Tube/Container: Clean container Transport Temperature: Ambient **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	Clearly indicate specimen source on sample label	Clearly indicate specimen source on sample label
Billing Code	87903	87965	81402
CPT Code	81340+18342	81340	81342

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Test Overview

Test Name	B-Cell Clonality Using BIOMED-2 PCR Primers	Immunoglobulin Heavy Chain Using BIOMED-2 PCR Primers	Immunoglobulin Kappa Chain Using BIOMED-2 PCR Primers
Reference Range	Negative for clonal rearrangement	Negative for clonal rearrangement	Negative for clonal rearrangement
Specimen Requirements	**External Specimen Requirements:** Testing Volume/Size: 10 mm^2 Type: Tissue Tube/Container: Clean container *Note: May submit fresh, frozen or paraffin-embedded tissue* **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	**External Specimen Requirements:** Testing Volume/Size: 10 mm^2 Type: Tissue Tube/Container: Clean container Transport Temperature: Frozen *Note: 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.* **Alternate Specimen Requirements:** 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 *Note: Volume/Size: 6 µg*	**External Specimen Requirements:** Testing Volume/Size: 10 mm^2 Type: Tissue Tube/Container: Clean container Transport Temperature: Frozen *Note: 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.* **Alternate Specimen Requirements:** 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 *Note: Volume/Size: 6 µg*
Test Ordering Information	Clearly indicate specimen source on sample label	Clearly indicate specimen source on sample label	Clearly indicate specimen source on sample label
Billing Code	87904	87960	87954
CPT Code	81261+81264	81261	81264

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