

## **Laboratory Tests for B- and T-cell Clonality based on *Immunoglobulin and T cell Receptor Gamma Gene Rearrangement***

The UNC Hospitals Molecular Genetics Laboratory performs polymerase chain reaction (PCR) of the immunoglobulin heavy chain (*IGH*), kappa light chain (*IGK*), and T cell receptor gamma (*TRG*) genes to assess clonality and lineage of lymphoid lesions.

### **Biology & Clinical Utility of Assays for B- and T- Cell Clonality**

*IGH* or *IGK* gene rearrangement is a sign of commitment to the B cell lineage, while *TRG* gene rearrangement characterizes T cell lineage. Lymphoid leukemias and lymphomas arise from a single transformed lymphocyte harboring a particular set of gene rearrangements, and that set of rearrangements is inherited by all tumor cell progeny. Every B cell malignancy harbors clonally rearranged IG genes that code for antibody specificity. Likewise, T cell tumors harbor clonally rearranged T cell receptor genes that code for antigen receptors. Malignant lymphomas and leukemias exhibit clonal gene rearrangement while benign, reactive lymphoid hyperplasias do not. In patient samples where diagnostic uncertainty remains after morphologic and immunophenotypic studies, gene rearrangement studies are often helpful in resolving whether lesions are polyclonal (reactive) or monoclonal (neoplastic). Because clonality is not always synonymous with malignancy, clinicopathologic correlation is required.

### **Laboratory Testing for *IGH*, *IGK*, or *TRG* gene rearrangement:**

Testing is performed on **blood** (3mL, EDTA), **bone marrow** (1ml, EDTA), **body fluids**, **paraffin blocks**, or **solid tissue** (fresh or stored frozen until analysis). DNA is extracted and PCR-amplified using primers targeting consensus variable and joining regions of the *IGH* and *IGK* (Biomed-2 primers from Invivoscribe) or *TRG* genes. Products are visualized by capillary gel electrophoresis to detect normally-distributed variably-sized products characterizing polyclonal lymphocytes, or a prominent product characterizing a clonal lymphoid population. Clones comprising as few as 10% of cells in the sample are detectable. Results are reported as presence or absence of a clonal lymphoid population, and the size of a clone is characterized as minor or major. If no clone is detected and if frozen tissue is available, additional testing by Southern blot analysis can be done by an outside laboratory to identify the approximately 10% of lymphoid neoplasms that are not detectable by this PCR assay.

### **References:**

1. Medeiros LJ, Carr J. Overview of the role of molecular methods in the diagnosis of malignant lymphomas. Archives of Pathology & Laboratory Medicine 1999;123:1189-207.
2. Krafft AE, Taubenberger JK, Sheng ZM, Bijwaard KE, Abbondanzo SL, Aguilera NS, Lichy JH. Enhanced sensitivity with a novel TCRgamma PCR assay for clonality studies in 569 formalin-fixed, paraffin-embedded (FFPE) cases. Molecular Diagnosis 1999;4:119-33.
3. JJM van Dongen, 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.
4. Cushman-Vokoun AM, Connealy S, Greiner TC. Assay design affects the interpretation of T cell receptor gamma gene rearrangements: comparison of the performance of a one-tube assay with the BIOMED-2-based TCRG gene clonality assay. J Mol Diagn 12:787, 2010.

### **Questions?**

Call the **Molecular Genetics Lab** at **(919) 966-4408** or Dr. Gulley at 843-4595.

E-mail [margaret\\_gulley@med.unc.edu](mailto:margaret_gulley@med.unc.edu)

Website= [http://labs.unchealthcare.org/directory/molecular\\_pathology/index\\_html](http://labs.unchealthcare.org/directory/molecular_pathology/index_html)