Application Note

Reliable and fast detection of B-cell clonality in lymphoproliferations

Jean Benhattar

Institute of Pathology, Centre Hospitalier Universitaire Vaudois (CHUV) and University of Lausanne, Lausanne, Switzerland

DNA fragments amplified using multiplex PCR that was developed to detect immunoglobulin and T-cell receptor gene rearrangements were resolved using the QIAxcel® system. Accurate size determination enabled identification of the type of B-cell clonality in lymphoproliferation.

Introduction

Lymphoma is a cancer of the lymphatic or the immune system (1). The World Health Organization (WHO) lists 43 different forms of lymphoma, including well-known Hodgkin's and several non-Hodgkin lymphomas (2). Research on lymphoid malignancies relies on histomorphological, cytomorphological, and immunohistological methods and evaluations.

The vast majority of lymphomas (>98%) exhibit rearrangements in immunoglobulin (Ig) and/or T-cell receptor (TCR) genes. Because of the clonal origin, all cells of a lymphoma exhibit identical gene rearrangements, and, therefore, monoclonality in lymphoid tissue suggests a malignant disease. Polyclonality, in contrast, indicates normal B-cell maturation (3). Thus, clonal gene rearrangement assessments are of great importance for understanding lymphoid malignancies.

In the BIOMED-2 European collaborative project, multiplex PCR assays were developed and standardized to study such rearrangements. By detecting rearrangements in the in IGH and IGK genes using multiplex PCR, it is possible to identify virtually all B-cell proliferations of clonal origin (3).

Here, we describe the use of the QIAxcel system for fast and reliable capillary electrophoresis analysis to identify the clonality of B-cells in lymphoproliferative tissues.

Materials and methods

Detection of B-cell clonality was performed using BIOMED-2 primer sets for IGH (IGH-FR3 and IGH-FR2) and IGK (IGK-A and IGK-B) gene rearrangements (3). DNA was extracted from archival formalin-fixed, paraffin-embedded tissue sections.

PCR amplification (40 cycles) was performed in duplicate with 10–100 ng of extracted DNA in a total volume of 20 µl. PCR products were denatured at high temperature (5 minutes at 95°C) before being subjected to rapid renaturation at low temperature (1 hour at 4°C) to enhance the formation of multiple heteroduplexes. Homoduplexes exhibit the same migration rate during electrophoretic separation, whereas, heteroduplexes exhibit different migration rates, allowing monoclonal lymphoproliferations to be distinguished from polyclonal lymphoproliferations.

▷

PCR products were analyzed using the QIAxcel system with the QIAxcel DNA Screening Kit and the QX Alignment Marker 15 bp/450 bp. Electrophoretic separation was performed using 2 kV separation voltage and 700 second separation time. Alignment marker and samples were injected with an injection time of 35 seconds at 2 kV and 3 kV, respectively.

Results and discussion

Results of the analysis of 5 samples (S1–S5) are shown in Figure 1. The presence and size of fragments was accurately determined using BioCalculator Software.

B-cell monoclonality was observed for the S1, S4, and S5 samples. B-cell oligoclonality was detected for the S3 sample. For sample S5a and S5b, taken at different times during the course of the disease, the same monoclonal rearrangements were observed for the IGH and IGK genes. (Table 1).

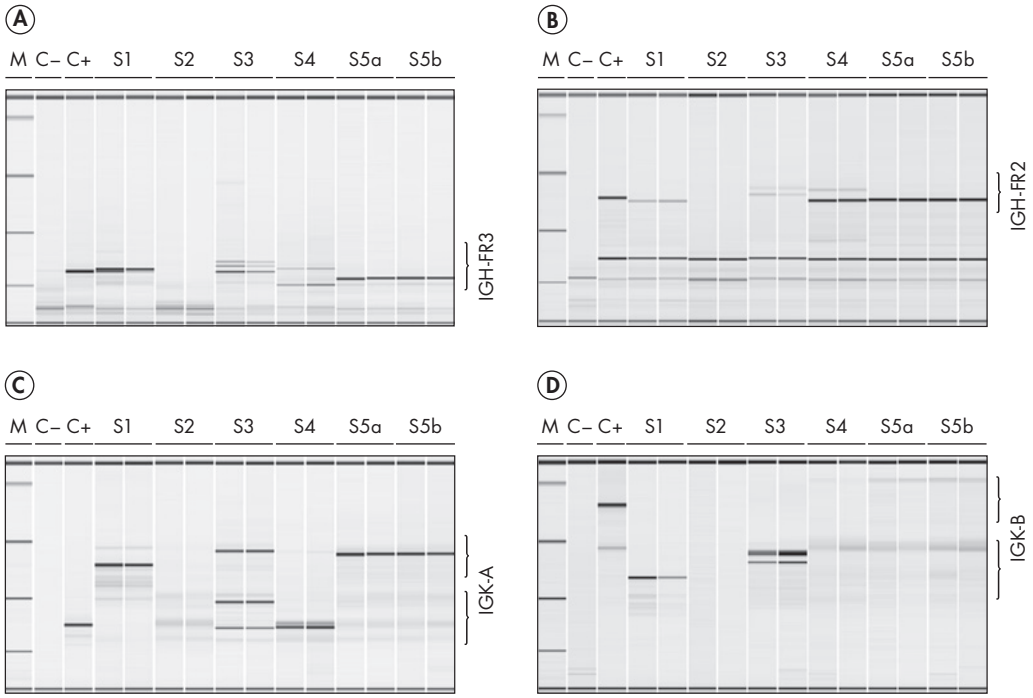

Figure 1. Identification of gene rearrangements in proliferating B-cells. DNA from lymphoid tissues was amplified using primers developed in the BIOMED-2 study and analyzed using the QIAxcel system with the QIAxcel DNA Screening Kit. **M**: size marker; **C–**: negative control; **C+**: positive control. **S1–S5b**: duplicate samples of multiplex PCR products. Primers were chosen to detect rearrangements in FR3 (**A**) and FR2 (**B**) VD FR regions of the IGH gene as well as the IGK-A (**C**) and IGK-B (**D**) genes.

Table 1. Observed IGH and IGK gene rearrangements

Gene	S1	S2	S3	S4	S5a	S5b
IGH-FR3	+	–	+ *	+	+ †	+ †
IGH-FR2	+	–	+	+	+ †	+ †
IGK-A	+	–	+ *	+	+ †	+ †
IGK-B	+	–	+ *	–	–	–

Monoclonal gene rearrangement present (+) or absent (–).

* Oligoclonality.

† The same monoclonal gene rearrangements were observed in both S5 samples.

Conclusions

- The QIAxcel capillary electrophoresis system is an optimal tool to resolve amplification products from multiplex PCR samples. Since even very small differences in fragment size could be resolved, it was possible to unambiguously detect immunoglobulin gene rearrangements. This allowed us to determine whether a lymphoid tissue was of monoclonal, oligoclonal, or polyclonal origin.
- Fast and accurate detection of gene rearrangement in B-cells facilitates the understanding of the development of malignancies during maturation of B-cells.
- The QIAxcel system processes samples in batches of 12 and allows analysis of up to 96 samples without manual intervention. Results can be displayed as a gel-like image as well as an electropherogram. The size and concentration data can be exported as a Microsoft® Excel® table.
- QIAxcel capillary electrophoresis uses electrokinetic injection of minute quantities of DNA, allowing the samples to be retained for downstream procedures, such as sequencing.

References

1. Parham, P. (2009) *The Immune System*. 3rd ed. New York: Garland Science.
2. Jaffe, E.S. et al. eds (2001) *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: IARC Press.
3. van Dongen, J.J.M. et al. (2003) 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 **17**, 2257.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device, including computer, and QIAxcel ScreenGel Software; 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Fast Analysis Kit (3000)	QIAxcel DNA Fast Analysis Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX DNA Size Marker 50 bp – 1.5 kb, QX Alignment Marker 15 bp/3 kb, 12-Tube Strips	929008
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104

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