

Application Note

Safe and rapid HLA typing using the QIAxcel[®] system

Jean-Luc Sambucy

Merck Serono SA, Geneva, Switzerland

Using the QIAxcel system and data exchange with HLA genotyping software, PCR products from different HLA loci can be analyzed and typed in a few minutes. This method is simple and reliable and significantly reduces hands-on time compared to traditional methods in clinical research.

Introduction

Human leukocyte antigens (HLA) are proteins that are present on each cell surface and allow the immune system to recognize “self” from “foreign” (1). Each individual human exhibits approximately 8 HLA types. Since each type comprises hundreds of subtypes, the chance that 2 unrelated individuals will have identical HLA patterns is very low (2).

Although HLA typing is primarily used to match organ donors and recipients, it is also often used in biomedical research because HLA profiles are associated with a number of diseases.

For example, the presence of HLA-DR4 and HLA-DRB1 increases the risk of rheumatoid arthritis 7-fold (3). A correlation between HLA type and the resistance or susceptibility to certain infectious diseases has also been observed (4). Epstein-Barr virus requires HLA Class II-Dr (in addition to CD21) to penetrate cells (5). Other diseases associated with HLA types include autoimmune diseases, post-infectious arthritis, type I diabetes, autoimmune hepatitis, and several others (see Table 1).

Rapid and routine HLA typing of individual humans and populations will facilitate understanding of the role of HLA types with respect to disease.

Table 1. HLA types correlated with disease*

Disease	HLA loci	Reference
Autoimmune diseases	HLA-B27	6
Rheumatoid arthritis	HLA-DR4, HLA-DRB1, HLA-DRB1*0404	3
Post-infectious arthritis	HLA-B27	7
Type I diabetes	HLA-DR3, HLA-DR4, HLA-DQ	8
Autoimmune hepatitis	HLA-DRB1*1301, HLA-DR3	9
Infectious diseases	HLA-DR2	10
Malaria	HLA-B53	11
Cancer	HLA-G	12
Breast cancer	HLA DQB*03032, HLA DRB1*11	13
Epstein-Barr virus infection	HLA Class II	5

* This list is not complete and includes only prominent examples of HLA genotypes that correlate with a disease or are suspected to play a role in a disease.

Materials and methods

PCR products produced using the A-B-DR Combi Tray Kit (Olerup SSP AB) were analyzed using the QIAxcel system with the QIAxcel DNA Fast Analysis Kit and the DM150 method. QX Alignment Marker 15 bp/3 kb and QX DNA Size Marker 50 bp – 1.5 kb were included in the analysis. Electrophoretic separation was performed using 10 kV voltage and 150 s separation time. Samples were injected with an injection time of 10 s at 10 kV.

HLA types were determined after analysis using BioCalculator software and HLA genotyping software.

Results

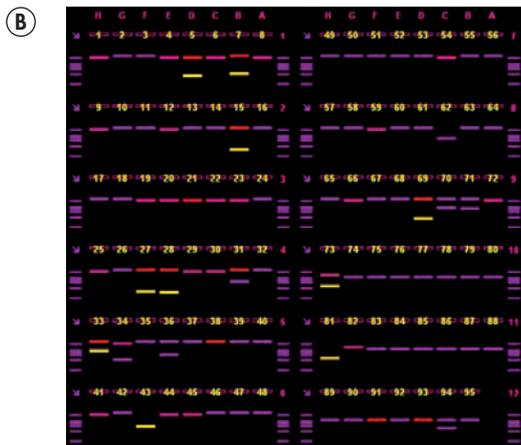
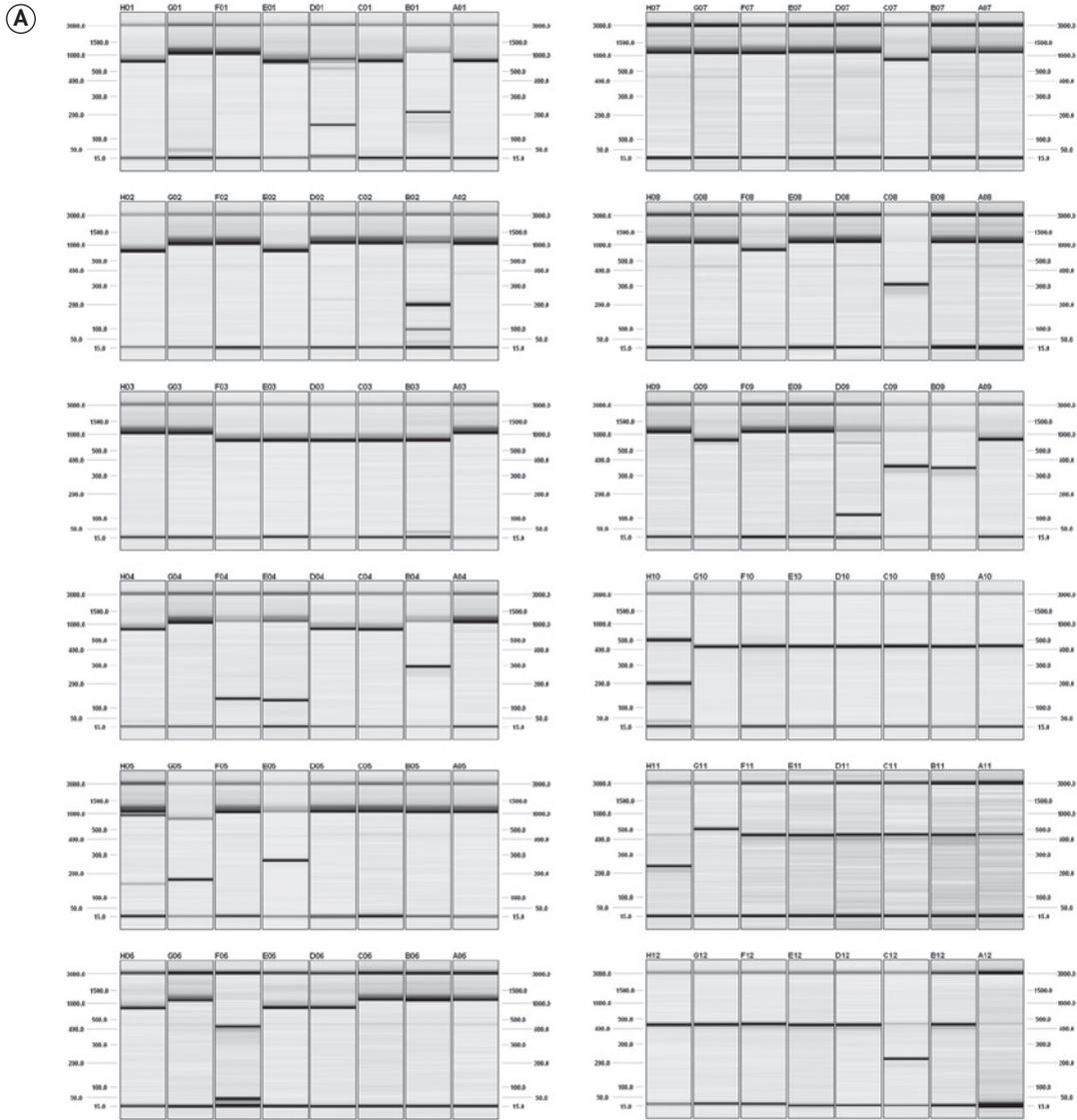
Three different types of HLA typing kits are available — sequence-specific oligonucleotides (SSO), sequencing-based typing (SBT), and sequence-specific primers (SSP) — and these are used in combination with different detection systems. For gel electrophoresis, sequence-specific primer kits are used. These kits are primarily dedicated to analysis of the A, B, C, DR, DQ, and DP loci of polymorphic HLA genes.

Figure 1 presents the results of analysis of 96 samples for one person. Separation of 12 samples was completed on the QIAxcel in 3 minutes using the QIAxcel DNA Fast Analysis Kit. Ready-to-run gel cartridges allow 96 samples to be processed automatically without manual intervention, thus reducing handling errors. The QIAxcel system minimizes hands-on time significantly compared to traditional methods. Electrophoresis and data acquisition are fully automated. Since results obtained using the QIAxcel system are compatible with HLA genotyping software, data can be imported and exported easily, and HLA genotypes are obtained within a few minutes.

Since the QIAxcel capillary electrophoresis system uses only minute amounts of DNA through electrokinetic injection, the samples are retained for downstream procedures.

Conclusions

- The combination of the QIAxcel system and QIAxcel DNA Fast Analysis Kit is an optimal solution for fast PCR checking, enabling rapid, reproducible, and standardized analysis of multiple HLA loci. The QIAxcel system is, therefore, highly suited for HLA genotyping and examining the role of HLA genes in multiple diseases in clinical research.
- BioCalculator software provides automated data generation and data acquisition, using formats that allow data exchange with other analysis software.
- The QIAxcel system is a powerful and versatile tool for researching and determining HLA types. Analysis of PCR products using the QIAxcel system facilitates a better understanding of the role of highly polymorphic HLA genes in multiple diseases in clinical research.



C

Allele combinations		<input checked="" type="checkbox"/> summarised results
common	alleles	serological equivalents
DRB1*01	DRB1*07:01:01:01	0 DR1 DR7
DRB4		0
rare	combinations	serological equivalents
DRB1*01	DRB1*07	0 DR1, - DR7, Null, -

Figure 1. Routine automated HLA genotyping. HLA loci were amplified from 96 samples using the A-B-DR Combi Tray (Olerup SSP AB). **A.** PCR products were analyzed using the QIAxcel system. **B.** Analysis results were exported from BioCalculator software for further analysis using HLA genotyping software. **C.** HLA genotypes were obtained within a few minutes.

References

1. Marsh, S.G.E., Parham, P., and Barber, L.D. (2000) *HLA FactsBook*. San Diego: Academic Press.
2. Gerlach, J.A. (2002) Human lymphocyte antigen molecular typing: how to identify the 1250+ alleles out there. *Arch. Pathol. Lab. Med.* **126**:281.
3. Weyand, C.M., and Goronzy, J. (2000) Association of MHC and rheumatoid arthritis: HLA polymorphisms in phenotypic variants of rheumatoid arthritis. *Arthritis Res.* **2**:212.
4. Klein, J., and Sato, A. (2000) The HLA system. Second of two parts. *N. Engl. J. Med.* **343**:782.
5. Haan, K.M., Kwok, W.W., Longnecker, R., and Speck, P. (2000) Epstein-Barr virus entry utilizing HLA-DP or HLA-DQ as a coreceptor. *J. Virol.* **74**:2451.
6. Reveille, J.D., (2006) Major histocompatibility genes and ankylosing spondylitis. *Best Pract. Res. Clin. Rheumatol.* **20**:601.
7. Amital, H., et al. (2008) Role of infectious agents in systemic rheumatic diseases. *Clin. Exp. Rheumatol.* **26**:S27.
8. Chiu, H.K., and Palmer, J.P. (2004). Autoimmune diabetes: More than just one flavor? *J. Endocrinol. Invest.* **27**:480.
9. De Silva, S., and Neuberger, J. (2006) HLA and autoimmune hepatitis. *Liver Int.* **26**:509.
10. Selvaraj, P. (2000) Role of human leukocyte antigen (HLA) and non-HLA genes in susceptibility or resistance to pulmonary tuberculosis. *Ind. J. Tub.* **47**:133.
11. Sing, N., Agrawal, S., and Rastogi, A.K. (1997) Infectious diseases and immunity: special reference to major histocompatibility complex. *Emerging Infect. Dis.* **3**:41.
12. Shih, I.M. (2007) Application of human leukocyte antigen-G expression in the diagnosis of human cancer. *Hum. Immunol.* **68**:272.
13. Chaudhuri, S., et al. (2000) Genetic susceptibility to breast cancer: HLA DQB*03032 and HLA DRB1*11 may represent protective alleles. *Proc. Nat. Acad. Sci. USA* **97**:11451.

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 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

For SSP-based HLA tissue typing applications, the above products can be directly ordered from Olerup SSP (www.olerup-ssp.com). For this application, these products should be exclusively ordered from Olerup SSP in the following countries: Australia, Belgium, Brazil, Canada, China, Denmark, Finland, France, Germany, Italy, Japan, Korea, Luxembourg, Malaysia, Mexico, Netherlands, Norway, Singapore, Sweden, United Kingdom, and USA. In all other countries, the products can be ordered from Olerup SSP as well as from QIAGEN or QIAGEN's distributors.

For other applications of HLA typing, the products should be ordered from QIAGEN or QIAGEN's distributors.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Visit www.qiagen.com/HLA-typing and find out how automated gel electrophoresis can benefit your lab!

Trademarks: QIAGEN®, QIAxcel®, Sample to Insight®, ScreenGel™ (QIAGEN Group). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

© 2016 QIAGEN, all rights reserved. PROM.5216-002

Ordering www.qiagen.com/shop | Technical Support support.qiagen.com | Website www.qiagen.com