Clin. Lab. 2021;67:XXX-XXX

SHORT COMMUNICATION

Evaluation of LabType-SSO HLA Typing for HLA-A, -B, -C, -DRB1, and -DQB1 loci

Minjeong Nam [1], Kiwook Jung [1], Eun Youn Roh [1, 2], Sue Shin [1, 2], Kyoung Un Park [1, 3], Eun Young Song [1]

[1] *Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea*
[2] *Department of Laboratory Medicine, Seoul National University Boramae Medical Center Seoul, Korea*
[3] *Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Gyeonggi, Korea*

SUMMARY

Background: **For HLA genotyping, PCR sequence-specific oligonucleotide (SSO) methods using the Luminex platform are widely used. We evaluated the performance of LabType-SSO (One Lambda, USA) in Koreans.**
Methods: **LabType-SSO were performed on 50 residual DNA samples analyzed by sequence-based typing (SBT) for all HLA-A, -B, -C, -DRB1, and -DQB1 alleles with gene frequency > 0.1% in Koreans.**
Results: **The LabType-SSO results were in complete agreement with SBT at the 2-digit level. For 4-digit level, 9 HLA-A alleles, 1 HLA-B allele, 3 HLA-C alleles, neither HLA-DRB1 nor -DQB1 allele showed ambiguous results for assignment of most probable types considering HLA gene frequency in Koreans. In addition, two cases of DQB1*04:01 allele were incorrectly assigned to DQB1*04:02.**
Conclusions: **LabType-SSO tests showed accurate assignment of 2-digit level and LabType-SSO HLA-DRB1 test showed correct 4-digit most probable HLA type. The tests can be useful as intermediate resolution typing for solid organ transplantation.**
(Clin. Lab. 2021;67:xx-xx. DOI: 10.7754/Clin.Lab.2020.190930)

Correspondence:
Eun Young Song
Department of Laboratory Medicine
Seoul National University College of Medicine
101 Daehang-no Jongno-gu
Seoul 03080
Republic of Korea
Phone: +82 2-2072-2548
Fax: +82 2-747-0359
Email: eysong1@snu.ac.kr

Short Communication accepted May 25, 2020

KEY WORDS

HLA, Koreans, Luminex, PCR-SSO, organ transplantation

INTRODUCTION

For successful solid organ transplantation, it is well known that well-matched genotype at the HLA-A, -B, -DRB1 loci ensured a good prognosis [1]. In addition, donor-specific antibodies (DSA) for not only donor HLA-A, -B, and -DRB1 but also HLA-C and -DQB1 can affect graft outcome, and those DSA can have "allele-specific" reactions [2-4]. Thus, accurate identification of those loci via high resolution typing is needed; however, PCR sequence-based typing (PCR-SBT) is expensive and laborious [5].
Recently, PCR sequence-specific oligonucleotide (PCR-SSO) methods using the Luminex platform with intermediate resolution have been widely used [6]. At pres-

ent, two commercial kits, LIFECODES HLA-SSO (Immucor Transplant Technology, Stamford, CT, USA) and LabType-SSO HLA typing (One Lambda Inc., Canoga Park, CA, USA) are available [7]. In the previous studies, evaluation of LIFECODES HLA-A, -B, -DRB1, and DQB1 typing [8,9] and LabType-SSO HLA-A, -B, and -DRB1 typing from cord blood [10] were reported. However, there was no study evaluating LabType-SSO HLA-C and -DQB1 test. We aimed to investigate the performance of LabType-SSO for HLA-A, -B, -C, -DRB1 and DQB1 loci from peripheral blood.

MATERIALS AND METHODS

Subjects

Fifty residual DNA samples analyzed by PCR-SBT for each of HLA-A, -B, -C, -DRB1, and -DQB1 loci from Jan 2017 to Dec 2018 in Seoul National University Hospital were included. We included all alleles with gene frequency of more than 0.1% in Koreans [11] (Table 1). This study was conducted under the approval of the Institutional Review Board of Seoul National University Hospital (IRB No. 1908-120-1056).

LabType-SSO on the Luminex platform

HLA typing by LabType-SSO (One Lambda Inc., Canoga Park, CA, USA) was performed for HLA-A, -B, -C, -DRB1, and -DQB1 loci according to manufacturer's recommendation on 50 DNA samples. Genomic DNA was extracted from peripheral blood by using QuickGene-Mini 80 DNA isolation system (Fujifilm, Tokyo, Japan) and preserved at -70°C. DNA was amplified by master mix containing biotinylated primers, dNTPs, buffer, and *tag* polymerase under the following conditions: 3 minutes at 96°C for one cycle; each 20 seconds at 96°C, 60°C, and 72°C for 5 cycles; 10 seconds at 96°C, 15 seconds at 60°C, and 20 seconds at 72°C for 30 cycles; 10 minutes at 72°C for 1 cycle, and then maintained at 4°C. PCR products checked on 2% agarose gel were incubated with different microsphere beads at 60°C for 15 minutes. After labeling microsphere beads with streptavidin-conjugated phycoerythrin (SAPE), acquisition of hybridized beads and measurement of fluorescent intensity were performed by LABScan3D™ (One Lambda, USA). HLA alleles were subsequently assigned by HLA Fusion™ software. For ambiguous results, most probable types were assigned considering HLA gene frequencies of > 0.1% in Koreans [11].

Sequence-based typing (SBT)

SBT was performed by AlleleSEQR® HLA-A, -B, -C, -DRB1, and -DQB1 (GenDx, Uttecht, Netherlands). The HLA loci were amplified using 0.1 μL DNA *taq* polymerase (Roche, Basel, Switzerland), PCR pre-mixture containing each primer mix for individual HLA loci, dNTP, and $MgCl_2$ on a PCR thermal cycler (Applied Biosystems, CA, USA). PCR was performed with denaturation at 95°C for 10 minutes, amplification for 36 cycles at 96°C for 20 seconds, at 60°C for 30 seconds, and 72°C for 3 minutes. Amplicons treated with 2 μL ExoSAP-IT (Exonuclease I and Shrimp Alkaline Phosphatase) to remove residual PCR primers and dNTPs were utilized as a sequencing template. After adding 8 μL sequencing mixtures, the sequencing condition was as follows: denaturation at 95°C for 10 minutes and amplification for 25 cycles at 96°C for 20 seconds, 50°C for 30 seconds, and 60°C for 2 minutes. The sequencing product was loaded onto the ABI 3500xl system (Applied Biosystems, Foster City, CA, USA) after being purified by ethanol precipitation. HLA alleles were assigned by using SBTengine (Genome Diagnostics, Utrecht, Netherlands) based on IPD-IMGT/HLA database (release version 3.36.0) [12] and Korean HLA allele frequency [11].

RESULTS

Fifty DNA samples which included 25 HLA-A, 43 HLA-B, 29 HLA-C, 29 HLA-DRB1, and 16 HLA-DQB1 alleles showing more than 0.1% of Korean allele frequency (Table 1) were assigned by PCR-SSO and were compared with the result of PCR-SBT at the 2- and 4-digit level, respectively.

Among all 250 tests, 228 tests required no cutoff adjustment of beads, 17 tests needed 1 adjustment, 5 tests needed 2 adjustments, and 1 test needed 5 adjustments. Among a total of 10,075 beads groups reactions (82, 97, 60, 73, and 91 beads groups for each HLA-A, -B, -C, -DRB1, and -DQB1 on 50 samples, respectively), 32 beads group (0.32%) showed false reactions (19 false positive and 13 false negative reactions). The beads group 66 of HLA-DQB1 showed false positive reactions five times (Table 2). However, after adjustment, the PCR-SSO results were in complete agreement with PCR-SBT for all alleles at the 2-digit level.

Regarding 4-digit level, 9 (36%) out of 25 HLA-A alleles, 1 (2.3%) out of 43 HLA-B alleles, 3 (10.3%) out of 29 HLA-C alleles, and no allele from HLA-DRB1 and DQB1 showed ambiguous types assigned with two or more alleles, even after considering the most probable HLA types in Koreans (Table 3). In addition, two samples with HLA-DQB1*04:01 were incorrectly assigned to DQB1*04:02 due to false negative reactions of beads group 6.

DISCUSSION

HLA genes are not only associated with autoimmune and infectious disease, but also critically important for selection of donor for solid organ or hematopoietic stem cell transplantation (HSCT) [13-15]. PCR-SSO HLA tests using the Luminex platform are widely used for solid organ transplantation or screening of an identical donor among recipient's siblings in HSCT due to their

Table 1. Analyzed HLA-A, -B, -C, -DRB1, and -DQB1 alleles* (n = 500).

HLA-A	N	HLA-B	N	HLA-C	N	HLA-DRB1	N	HLA-DQB1	N
A*01:01	3	B*07:02	1	C*01:01	4	DRB1*01:01	4	DQB1*02:01	3
A*02:01	19	B*07:05	2	C*01:02	18	DRB1*03:01	3	DQB1*02:02	7
A*02:03	1	B*07:06	1	C*01:03	6	DRB1*03:02	1	DQB1*03:01	12
A*02:06	10	B*08:01	4	C*02:01	5	DRB1*04:01	1	DQB1*03:02	11
A*02:07	4	B*13:01	2	C*02:02	4	DRB1*04:03	2	DQB1*03:03	13
A*02:10	1	B*13:02	1	C*03:02	3	DRB1*04:04	2	DQB1*03:13	1
A*03:01	1	B*14:01	1	C*03:03	3	DRB1*04:05	10	DQB1*04:01	10
A*03:02	3	B*15:01	5	C*03:04	3	DRB1*04:06	3	DQB1*04:02	4
A*11:01	7	B*15:02	1	C*04:01	1	DRB1*04:07	1	DQB1*05:01	6
A*11:02	1	B*15:07	1	C*05:01	1	DRB1*04:10	1	DQB1*05:02	3
A*23:01	1	B*15:11	4	C*06:01	2	DRB1*07:01	9	DQB1*05:03	5
A*24:02	20	B*15:18	1	C*06:02	3	DRB1*08:02	2	DQB1*06:01	11
A*24:08	1	B*15:27	1	C*06:03	4	DRB1*08:03	10	DQB1*06:02	8
A*24:20	1	B*15:38	1	C*06:04	2	DRB1*09:01	11	DQB1*06:03	1
A*26:01	1	B*27:04	1	C*07:02	6	DRB1*10:01	1	DQB1*06:04	2
A*26:02	3	B*27:05	2	C*07:04	4	DRB1*11:01	1	DQB1*06:09	3
A*26:03	2	B*35:01	7	C*07:06	2	DRB1*12:01	4		
A*29:01	2	B*35:03	1	C*08:01	8	DRB1*12:02	6		
A*29:02	1	B*37:01	2	C*08:03	1	DRB1*13:01	1		
A*30:01	1	B*38:02	2	C*12:02	2	DRB1*13:02	5		
A*30:04	1	B*39:01	1	C*12:03	1	DRB1*14:03	1		
A*31:01	6	B*40:01	4	C*14:02	7	DRB1*14:04	1		
A*32:01	1	B*40:02	2	C*14:03	3	DRB1*14:05	4		
A*33:03	8	B*40:03	1	C*15:02	2	DRB1*14:06	1		
A*33:25	1	B*40:06	3	C*15:05	1	DRB1*14:07	1		
		B*42:01	1	C*15:11	1	DRB1*14:54	1		
		B*44:02	2	C*16:01	1	DRB1*15:01	8		
		B*44:03	6	C*17:01	1	DRB1*15:02	3		
		B*45:01	1	C*20:02	1	DRB1*16:02	2		
		B*46:01	7						
		B*48:01	2						
		B*50:01	1						
		B*51:01	9						
		B*51:02	1						
		B*51:13	1						
		B*52:01	3						
		B*54:01	5						
		B*55:02	2						
		B*55:04	1						
		B*57:01	1						
		B*58:01	2						
		B*59:01	1						
		B*67:01	2						

*** All HLA-A, -B, -C, -DRB1, and -DQB1 alleles with allele frequency > 0.1% were included.**

Table 2. Cutoff adjustments performed.

Loci	False positive beads group (n = 19)	False negative beads group (n = 13)
HLA-B	none	037 (1), 044 (1), 072 (1), 100 (1)
HLA-C	093 (1)	none
HLA-DRB1	087 (3)	047 (1), 048 (1), 070 (1)
HLA-DQB1	5 (1), 41 (6), 59 (1), 66 (5), 98 (2)	14 (1), 41 (1), 42 (1), 67 (1), 75 (1), 86 (1)

The number in parentheses indicates the number of samples which needed adjustments of cutoff value of each beads group.

Table 3. Ambiguous alleles at 4-digit level for HLA-A, -B, -C, -DRB1, and -DQB1 loci.

Loci	Alleles	4-digit level ambiguity	N
HLA-A	A*02:01	*02:01, *02:07, *02:12, *02:15N, *02:36, *02:53N, *02:86, *02:251	20
	A*02:06	*02:06, *02:10, *02:28, *02:41, *02:61, *02:91	10
	A*02:07	*02:01, *02:07, *02:15N, *02:53N, *02:251	3
	A*02:10	*02:06, *02:10, *02:28, *02:41, *02:61	1
	A*11:01	*11:01, *11:02, *11:07	6
	A*11:02	*11:01, *11:02, *11:07	1
	A*24:02	*24:02, *24:05, *24:20, *24:21, *24:30, *24:37, *24:74, *24:75	21
	A*26:01	*26:01, *26:10, *26:32	2
	A*29:01	*29:01, *29:02	2
	A*29:02	*29:01, *29:02	1
	A*31:01	*31:01, *31:02, *31:11	3
	A*33:03	*33:03, *33:14, *33:15, *33:25	9
	A*33:25	*33:03, *33:14, *33:15, *33:25	1
HLA-B	B*07:05	*07:05, *07:06	2
	B*13:01	*13:01, *13:02	1
	B*15:01	*15:01, *15:28, *15:35	2
	B*35:01	*35:01, *35:64	7
	B*40:01	*40:01, *40:62	4
	B*40:02	*40:02, *40:05	1
	B*48:01	*48:01, *48:11	3
	B*51:01	*51:01, *51:58	1
	B*54:01	*54:01, *54:19	5
	B*55:02	*55:02, *55:19	2
	B*58:01	*58:01, *58:13	2
HLA-C	C*01:02	*01:02, *01:03, *01:11	15
	C*01:03	*01:02, *01:03, *01:11	1
	C*03:04	*03:04, *03:38, *03:46	10
	C*06:02	*06:02, *06:12, *06:27	6
	C*07:02	*07:02, *07:33N, *07:64, *07:102, *07:194, *07:271, *07:316, *07:322,	1
	C*07:06	*07:01, *07:06	3
	C*08:03	*08:03, *08:06	1
	C*15:02	*15:02, *15:03, *15:07, *15:13	1
	C*16:01	*16:01, *16:02	1
	C*17:01	*17:01, *17:03	1
HLA-DRB1	DRB1*04:03	*04:03, *04:51	2
	DRB1*04:05	*04:05, *04:45	10
	DRB1*07:01	*07:01, *07:03, *07:05, *07:07, *07:11, *07:13	9
	DRB1*09:01	*09:01, *09:04, *09:08	11
	DRB1*12:01	*12:01, *12:05, *12:06, *12:07, *12:08, *12:10, *12:17	4
	DRB1*12:02	*12:02, *12:12, *12:13	6
	DRB1*13:02	*13:02, *13:39	1
	DRB1*14:05	*14:05, *14:56	1
	DRB1*14:54	*14:01, *14:54	1
	DRB1*15:01	*15:01, *15:04	1
HLA-DQB1	† DQB1*04:01	*04:02	2

Underlined alleles represented the most probable type regarding allele frequency of more than 0.1% in Koreans.
Form "letters" indicated the alleles that remained ambiguous even after considering the most probable types in Koreans.
† Two cases of DQB1*04:01 allele were incorrectly assigned to DQB1*04:02.

higher throughput, lower cost, and low-to-intermediate resolution of typing [6]. In organ transplantation, assigning of most probable type at 4-digit level considering Korean HLA gene frequency are especially helpful to monitor allele-specific DSA or for epitope matching [14].

In this study, the portion of reactions modified by adjusting cutoff level was very low (0.32%, 32/10,075). Those false reactions were easily noticeable because they were not critical for HLA type assignment and did not cause ambiguity in 2-digit level assignment. For 4-digit level, most HLA-B alleles (excluding B*13:01),

all DRB1 alleles, and most DQB1 alleles (excluding *04:01) showed agreement with the most probable types in Koreans (allele frequency > 0.1%). In renal transplantation, DSA against HLA-DRB1 is more crucial than DSA against HLA-DQB1 [16]. In our previous study, LIFECODES HLA typing kit, the other PCR-SSO HLA test using the Luminex platform on the market, showed complete agreement with most probable type on HLA-B and -DQB1 loci at 4-digit level in Koreans [8,9]. However, LIFECODES HLA-DRB1 typing kit showed ambiguous results on DRB1*04 allelic groups (8.7%, 11 out of 126 tests) at 4-digit level [8], which can result in difficulty in assignment of DRB1*04 allele-specific DSA, which was completely resolved in LabType-SSO HLA-DRB1 kit in this study. False assignment of 2 DQB1*04:01 alleles to DQB1*04:02 caused error in assigning most probable type in Koreans. It might be a rarity of DQB1*04:01 allele in Caucasians compared to Koreans [11,17], which causes incomplete verification of beads group 6 which is critical for DQB1*04:01 assignment. We should report neither HLA-DQB1*04:01 nor DQB1*04:02 as the most probable type, and 2-digit level report is recommended for HLA-DQB1*04 until the beads group 6 is improved. In addition, ambiguity of LabType-SSO HLA-A locus was frequent, which might also be due to the rarity of some alleles such as HLA-A*02:06, *02:07, and *02:10 in Caucasians compared to Koreans [11, 18], which also needs some improvement for Koreans.

In conclusion, LabType-SSO kit showed exact 2-digit level assignment on all the HLA alleles with gene frequency > 0.1% in Koreans. For 4-digit level, all DRB1 alleles and most HLA-B and -DQB1 alleles were correctly assigned as most probable types considering gene frequency of > 0.1% in Koreans. In general, LabType-SSO HLA-A, -B, -C, -DRB1 and DQB1 kits can be successfully used for solid organ transplantation and donor screening among recipient's siblings in HSCT.

Acknowledgment:
We gratefully acknowledge the expert technical assistance of One Lambda.

Declaration of Interest:
The authors have no conflicts of interest to disclose.

References:

1. Opelz G, Döhler B. Effect of human leukocyte antigen compatibility on kidney graft survival: comparative analysis of two decades. Transplantation 2007;84:137-43 (PMID: 17667803).

2. Kosmoliaptsis V, Gjorgjimajkoska O, Sharples LD, et al. Impact of donor mismatches at individual HLA-A, -B, -C, -DR, and -DQ loci on the development of HLA-specific antibodies in patients listed for repeat renal transplantation. Kidney Int 2014;86:1039-48 (PMID: 24717292).

3. Leeaphorn N, Pena JRA, Thamcharoen N, Khankin EV, Pavlakis M, Cardarelli F. HLA-DQ mismatching and kidney transplant outcomes. Clin J Am Soc Nephrol 2018;13:763-71 (PMID: 29685925).

4. Muro M, González-Soriano MJ, Salgado G, et al. Specific "intra-allele" and "intra-broad antigen" human leukocyte antigen alloantibodies in kidney graft transplantation. Hum Immunol 2010;71: 857-60 (PMID: 20510320).

5. Dunn PP. Human leucocyte antigen typing: techniques and technology, a critical appraisal. Int J Immunogenet 2011;38:463-73 (PMID: 22059555).

6. Dunckley H. HLA typing by SSO and SSP methods. Methods Mol Biol 2012;882:9-25 (PMID: 22665226).

7. Testi M, Andreani M. Luminex-based methods in high-resolution HLA typing. Methods Mol Biol 2015;1310:231-45 (PMID: 26024639).

8. Roh EY, In JW, Shin S, Park KU, Song EY. Performance of LIFECODES HLA-A, -B, and -DRB1 typing kits using Luminex platform in Koreans. Clin Lab 2016;62:2057-63 (PMID: 28164515).

9. Roh EY, In JW, Shin S, Yoon JH, Park KU, Song EY. Performance of LIFECODES HLA-DQB1 typing kit using Luminex platform in Koreans. Ann Lab Med 2015;35:123-7 (PMID: 25553292).

10. Roh EY, Song EY, Chang JY, Yoon JH, Shin S. Evaluation of LABType® SSO HLA typing using the Luminex platform: cord blood registry typing for the Korean population. Clin Lab 2016; 62:1531-9 (PMID: 28164617).

11. In JW, Roh EY, Oh S, Shin S, Park KU, Song EY. Allele and haplotype frequencies of human leukocyte antigen-A, -B, -C, -DRB1, and -DQB1 from sequence-based DNA typing data in Koreans. Ann Lab Med 2015;35:429-35 (PMID: 26131415).

12. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh Steven GE. The IPD and IMGT/HLA database: allele variant databases. Nucleic Acids Res 2015;43:D423-31 (PMID: 25414341).

13. Ahn S, Choi HB, Kim TG. HLA and disease associations in Koreans. Immune Netw 2011;11:324-35 (PMID: 22346771).

14. Lim WH, Wong G, Heidt S, Claas FHJ. Novel aspects of epitope matching and practical application in kidney transplantation. Kidney Int 2018;93:314-24 (PMID: 29061333).

15. Mayor NP, Shaw BE. HLA typing-A case-based approach to donor selection. Adv Cell Gene Ther 2018;1:1-8. https://onlinelibrary.wiley.com/doi/full/10.1002/acg2.16

16. Song EY, Lee YJ, Hyun J, et al. Clinical relevance of pretransplant HLA class II donor-specific antibodies in renal transplantation patients with negative T-cell cytotoxicity crossmatches. Ann Lab Med 2012;32:139-44 (PMID: 22389881).

17. Sánchez-Velasco P, Leyva-Cobián F. The HLA class I and class II allele frequencies studied at the DNA level in the Svanetian population (Upper Caucasus) and their relationships to Western European populations. Tissue Antigens 2001;58:223-33 (PMID: 11782273).

18. Mack SJ, Tu B, Lazaro A, et al. HLA-A, -B, -C, and -DRB1 allele and haplotype frequencies distinguish Eastern European Americans from the general European American population. Tissue Antigens 2009;73:17-32 (PMID: 19000140).