Original article

External proficiency testing in HLA typing for kidney transplantation in Thailand from 2009 to 2020

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Abstract:

Introduction: *HLA-A, -B, and -DRB1 typing between donors and patients is important in deceased donor kidney transplantation. The Organ Donation Centre and the National Blood Centre, Thai Red Cross Society, have conducted national proficiency testing to assess the laboratory performance of HLA typing and to verify the accuracy and reliability of test results since 2001.* ***Objective:*** *This retrospective study aimed to analyze the performance and improvement of HLA typing among participating laboratories from 2009 to 2020.* ***Materials and Methods:*** *From 2009 to 2020, 96 blood samples were sent to 9 participating laboratories requested to perform HLA-A, -B, and -DRB1 typing using routine reagents and techniques. The HLA typing results were returned, compiled and assessed. A summary of the HLA antigens was obtained from the results reported by 3 reference laboratories and confirmed by DNA sequencing. Discrepant typing results from each laboratory were analyzed.* ***Results:*** *The most common errors were found in HLA-B typing (9.89%), followed by HLA-A (5.73%), and HLA-DRB1 typing (5.73%). The discrepant results frequently occurred in the HLA-B*15 allele group (B62 and B75); A*11, A*69 and DRB1*14, due to their high polymorphism compared with other HLA alleles. From the years 2018 to 2020, correct HLA-A, -B, and -DRB1 typing results were obtained from all laboratories confirming the gradual improvement of laboratory performance during this study period.* ***Conclusion:*** *Participation in the national proficiency testing in HLA typing would be useful to assess laboratory performance and improvement. This could ensure that laboratory performance remains reliable and accurate among different laboratories in Thailand.*

Keywords : ● *HLA typing* ● *Proficiency testing* ● *Kidney transplantation*

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นิพนธ์ต้นฉบับ

การทดสอบความชำนาญ HLA สำหรับการปลูกถ่ายไตในประเทศไทย ตั้งแต่ปี พ.ศ. 2552 ถึง พ.ศ. 2563

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บทคัดย่อ

บทนำ *การตรวจชนิดของ HLA-A, -B และ -DRB1 ระหว่างผู้บริจาคไตและผู้ป่วยมีความสำคัญในการปลูกถ่ายไตจากผู้บริจาคไตสมองตาย ศูนย์รับบริจาคอวัยวะและศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย ได้เริ่มดำเนินโครงการการทดสอบความชำนาญของการตรวจ HLA เพื่อประเมินประสิทธิภาพห้องปฏิบัติการ และเพื่อตรวจสอบความถูกต้องและความน่าเชื่อถือของผลการทดสอบตั้งแต่ปี พ.ศ. 2544* ***วัตถุประสงค์*** *การศึกษาย้อนหลังนี้มีวัตถุประสงค์เพื่อวิเคราะห์ประสิทธิภาพและการปรับปรุงการตรวจ HLA ในห้องปฏิบัติการที่เข้าร่วมโครงการตั้งแต่ปี พ.ศ. 2552 ถึง พ.ศ. 2563* ***วัสดุและวิธีการ*** *ตั้งแต่ปี พ.ศ. 2552 ถึง พ.ศ. 2563 ได้ส่งตัวอย่างเลือดจำนวน 96 ตัวอย่างไปยังห้องปฏิบัติการที่เข้าร่วมโครงการ 9 แห่ง โดยให้ตรวจ HLA-A, -B และ -DRB1 โดยใช้น้ำยาและเทคนิคที่ใช้ในงานตรวจประจำ ได้รวบรวมผลการตรวจ HLA ที่ส่งกลับและประเมินสรุปชนิดแอนติเจนของ HLA ที่ได้จากผลที่รายงานโดยห้องปฏิบัติการอ้างอิง สามแห่งและตรวจยืนยันโดยการหาลำดับดีเอ็นเอ อีกทั้งทำการวิเคราะห์ผลการตรวจที่ผิดพลาดจากแต่ละห้องปฏิบัติการ* ***ผลการศึกษา*** *ข้อผิดพลาดที่พบบ่อยที่สุดคือ การตรวจ HLA-B (9.89%) ตามด้วย HLA-A (5.73%) และ HLA-DRB1 (5.73%) ผลตรวจแอนติเจนที่ไม่ตรงกันส่วนใหญ่พบในกลุ่มอัลลีล HLA-B * 15 (B62 และ B75); A*11, A*69 และ DRB1*14 เนื่องจากมีความหลากหลายสูงเมื่อเทียบกับอัลลีลของ HLA ชนิดอื่น ตั้งแต่ปี พ.ศ. 2561 ถึง พ.ศ. 2563 ผลการตรวจ HLA-A, -B และ -DRB1 จากห้องปฏิ บัติการให้ผลถูกต้องทั้งหมด ซึ่งช่วยยืนยันการปรับปรุงประสิทธิภาพของห้องปฏิบัติการอย่างต่อเนื่องในช่วงการศึกษานี้* ***สรุป*** *การมี ส่วนร่วมในการทดสอบความชำนาญระดับชาติในการตรวจ HLA จะเป็นประโยชน์ในการประเมินประสิทธิภาพและการปรับปรุงห้องปฏิบัติการ เพื่อให้มั่นใจได้ว่า การตรวจของห้องปฏิบัติการในประเทศไทย มีประสิทธิภาพ มีความน่าเชื่อถือและมีความแม่นยำ*

คำสำคัญ : ● *การตรวจชนิด HLA* ● *การทดสอบความชำนาญ* ● *การปลูกถ่ายไต*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2564;31:243-51.

Introduction

In Thailand, the Organ Donation Centre (ODC) of the Thai Red Cross Society (TRCS) is the principle organization to enroll patients with end-stage renal failure requiring kidney transplantation from deceased donors. The cooperation of the ODC-TRCS and the National Blood Centre (NBC-TRCS) for testing the compatibilities of ABO blood group and human leukocyte antigen (HLA) between donor and recipient has been conducted from 1997 until now. According to the kidney allocation criteria, the serum samples of wait-listed patients with the highest overall score from HLA mismatch, panel reactive antibody (PRA), renal waiting time, and patient age could be selected for HLA crossmatch with donor lymphocytes. Importantly, only patients with negative HLA crossmatch could be transplanted.[1]

In general, the standard complement-dependent cytotoxicity (CDC) test was formerly used for HLA-A, -B, and -DR typing. The CDC test has limitations such as unavailability of specific antiserum and low cell viability.[2] Presently, molecular techniques including polymerase chain reaction (PCR)-specific oligonucleotide probe (PCR-SSO), PCR-sequence-specific primers (PCR-SSP), and real-time PCR have been implemented for HLA-A, -B, and -DRB1 typing among donors and patients. Those techniques could provide accurate and reproducible testing results.[3-8] However, correct HLA typing results are extremely important for clinical decision making, especially regarding deceased-donor kidney transplantation outcomes.[9]

Because of the importance of HLA testing results, in 2001 the ODC-TRCS and NBC-TRCS conducted a national proficiency testing to assess the laboratory performance of HLA typing and to verify the accuracy and reliability of test results. The data of HLA typing results from 2001 to 2003 obtained from six participating laboratories were analyzed. HLA-A and -B typing were performed using CDC in most laboratories and HLA-DRB1 typing was performed using PCR-SSP and/or PCR-SSO. Discrepant results were commonly found in HLA-B testing (16.07%), followed by HLA-A testing (2.98%) and HLA-DRB1 testing (2.38%), respectively.[10]

The rapid increase in newly identified HLA-A and HLA-B alleles with different DNA sequences could encode proteins with similar serologic reactivity.[11,12] All participating laboratories have implemented different PCR techniques for HLA-A and HLA-B typing since 2009, and only one laboratory still performed HLA-A and -B typing using the CDC test in parallel. Moreover, three new laboratories also joined this national proficiency testing to participate in the external quality control (EQC) program as one part of laboratory accreditation. This retrospective study aimed to analyze the performance and improvement of HLA typing among participating laboratories from 2009 to 2020.

Materials and Methods

Altogether, nine HLA laboratories in Thailand, performing HLA typing for solid organ transplantation and stem cell transplantation both routinely and for research purposes. They included 1) Reference Laboratory Centre, NBC-TRCS, 2) Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 3) Histocompatibility & Immunogenetics Laboratory, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, 4) Department of Pathology, Phramongkutklao College of Medicine, 5) Immunology Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, 6) Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, 7) Blood Transfusion Centre, Faculty of Medicine, Khon Kaen University, 8) Blood Bank and Transfusion Medicine Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University and 9) HLA Laboratory, Blood Bank Section, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University.

Test items

For each year in the study (2009 to 2020), eight blood samples collected in citrate phosphate dextrose were distributed to participating laboratories. Samples were delivered four times yearly. Each time, two unknown

samples were shipped from the ODC-TRCS to the participants by express mail service and each participant received them within 3 days. The participants were instructed to handle these samples as part of their routine work and returned the HLA-A, -B, and -DR typing results by surface mail or fax within one month. Late results were excluded from the analysis.

Test performed

Each laboratory was requested to perform HLA-A, -B, and -DRB1 typing using routine reagents and techniques. Techniques used include PCR-SSO (N = 6), PCR-SSP (N = 2), and PCR-SSO and PCR-SSP (N = 1). A result form was developed and used to record the individual laboratory test results and the techniques used. The HLA low resolution typing results obtained from all participating laboratories were compiled and assessed. For each test result, a summary of the HLA antigens was obtained from the results reported by three reference laboratories and confirmed by DNA sequencing. Then, regarding the correct HLA typing results, the individual laboratory performance, and a code summary of all participant results were shared with all participants, enabling them to have an inter-laboratory comparison. In addition, at the end of each year, a summary of each laboratory performance was reported to the laboratory director. This allowed them to be aware of the capacity building required for laboratory personnel and equipment, reagents and technique used.

To educate and increase the awareness of all members; a workshop was set up at the end of each year. All participants were invited free of charge and the lectures on suitable topics were provided. Causes of discrepant results and any difficulties found during the program were discussed and clarified by the organizing team. Additionally, a certificate of attendance was given to each participating laboratory.

Statistical analysis

Descriptive analysis of HLA-A, -B, and -DRB1 typing results obtained from all participating laboratories was performed and the discrepant results were expressed in number and percentages.

Results

During the period of this study (2009 to 2020), nine HLA laboratories sequentially participated in a national proficiency testing in HLA typing. All laboratories submitted the HLA-A, -B, and -DRB1 genotyping results before the due date. The EQC blood samples were sent to participating laboratories four times yearly. The frequencies of HLA-A, -B, and DRB1 alleles in 96 EQC samples are summarized in Table 1. Samples with HLA-A*25, -B*81, and -B*45 were excluded during this study. According to the deceased donor kidney allocation criteria of the ODC-TRCS, HLA serological-equivalent match between donors and wait-listed patients was used to evaluate well-matched patients. The data of HLA typing results in deceased kidney donors matched with wait-listed patients by serological equivalent are shown in Table 2. The discrepant results from each laboratory were identified when comparing the results with the serological equivalent of HLA-A, - B, and -DRB1 typing from reference laboratories.

For the discrepant results of HLA-A typing found in 96 samples, 11 errors (5.73%) were reported from 2009 to 2015, as shown in Table 3. Both common and rare HLA-A alleles A*11, A*23, A*66, A*69 and A*74 were found from 2009 to 2013, and 2015. From 2016 to 2020, no discrepant results in HLA-A typing were observed.

Regarding HLA-B typing results, 19 errors (9.89%) occurred from 2009 to 2017, as shown in Table 3. The most common errors were found in seven samples with HLA-B*15 alleles to identify B62, B63, B75, B76, and B77 antigens in 2012 and 2014 to 2017. Other errors were found in B35, B60, B61, B41, B42, B46, B48, B51, B55, and B59. From 2018 to 2020, no discrepant results in HLA-B typing were observed.

For HLA-DRB1 typing results, discrepant results were found in 11 samples (5.73%) from 2009 to 2011 and 2013 to 2015. Discrepant results were commonly found in three samples with DRB1*14 (DR14), followed by DRB1*04 (DR4), DRB1*07 (DR7), DRB1*08 (DR8), DRB1*11 (DR11), DRB1*13 (DR13), and DRB1*15 (DR15), respectively. From 2016 to 2020, no discrepant results in HLA-DRB1 typing were observed.

Table 1 Frequencies of HLA-A, -B and -DRB1 alleles in 96 EQC samples (2009-2020)

HLA-A allele	Total	HLA-B allele	Total	HLA-DRB1 allele	Total
A*01	7	B*07	7	DRB1*01	7
A*02	34	B*08	2	DRB1*03 (DR17)	11
A*03	3	B*13	5	DRB1*03 (DR18)	2
A*11:01	33	B*14:01 (B64)	1	DRB1*04	22
A*11:02	8	B*14:02 (B65)	1	DRB1*07	18
A*23	3	B*15:01 (B62)	5	DRB1*08	6
A*24	31	B*15:02 (B75)	6	DRB1*09	11
A*26	9	B*15:08 (B75)	1	DRB1*10	9
A*29	8	B*15:12 (B76)	2	DRB1*11	14
A*30	6	B*15:13 (B77)	7	DRB1*12	23
A*31	2	B*15:17 (B63)	6	DRB1*13	11
A*32	6	B*15:21 (B75)	1	DRB1*14	21
A*33	21	B*15:25 (B62)	3	DRB1*15	28
A*34	3	B*15:32 (B62)	2	DRB1*16	9
A*66	1	B*15:58 (B62)	1		
A*68	9	B*18	3		
A*69	5	B*27:02 (B27)	1		
A*74	3	B*27:04 (B27)	11		
		B*27:05 (B27)	9		
		B*27:06 (B27)	7		
		B*27:07 (B27)	3		
		B*27:61 (B27)	1		
		B*35	4		
		B*38	3		
		B*39	1		
		B*40:01 (B60)	16		
		B*40:02 (B61)	3		
		B*40:06 (B61)	4		
		B*41	4		
		B*42	2		
		B*44	7		
		B*46	9		
		B*47	1		
		B*48	2		
		B*49	1		
		B*50	2		
		B*51	10		
		B*52	9		
		B*53	1		
		B*54	2		
		B*55	4		
		B*56	1		
		B*57	6		
		B*58	12		
		B*59	1		
		B*67	2		

Table 2 HLA typing results among kidney donors matched with wait-listed patients by serological equivalent

HLA -A, -B and -DR typing results between donor and patient					
Donor	**Matched patient**	**Donor**	**Matched patient**	**Donor**	**Matched patient**
A1	A1	B7	B7, B703, B81	DR1	DR1, DR103
A2	A2, A203, A210	B8	B8	DR2	DR2, DR15, DR16
A3	A3	B13	B13	DR3	DR3, DR17, DR18
A10	A10, A66	B14	B14, B64, B65	DR4	DR4
A11	A11, A11.1, A11.2	B15	B15, B75, B76, B77	DR5	DR5, DR11, DR12
A19	A19, A74	B18	B18	DR6	DR6, DR13, DR14, DR1403/4
A23	A23	B21	B21, B4005	DR7	DR7
A24	A24, A2403	B22	B22, B54	DR8	DR8
A25	A25	B27	B27	DR9	DR9
A26	A26	B35	B35	DR10	DR10
A28	A28, A68, A69	B38	B38	DR11	DR11, DR5, DR12
A29	A29	B39	B39, B3901, B3902	DR12	DR12, DR5, DR11
A30	A30	B40	B40, B61	DR13	DR13, DR6, DR14
A31	A31	B41	B41	DR14	DR14, DR6, DR13, DR1403 /4
A32	A32	B42	B42	DR15	DR15, DR2, DR16
A33	A33	B44	B44	DR16	DR16, DR2, DR15
A34	A34	B46	B46	DR17	DR17, DR3, DR18
A66	A66, A10	B47	B47	DR18	DR18, DR3, DR17
A68	A68, A28	B48	B48		
A69	A69, A28	B49	B49		
A74	A74, A19	B50	B50, B4005		
		B51	B51, B5102, B5103		
		B52	B52		
		B53	B53		
		B54	B54, B22		
		B55	B55		
		B56	B56		
		B57	B57		
		B58	B58		
		B59	B59		
		B60	B60		
		B61	B61, B40		
		B62	B62		
		B63	B63		
		B64	B64, B14		
		B65	B65, B14		
		B67	B67		
		B70	B70, B71, B72		
		B71	B71, B70		
		B72	B72, B70		
		B75	B75, B15		
		B76	B76, B15		
		B77	B77, B15		
		B81	B81, B7, B703		

Table 3 Discrepancy in HLA-A, -B and -DRB1 typing results reported by participating laboratories from 2009 to 2017

HLA		No. of sample	No. of discrepancies	Year reported								
Allele	Antigen			2009	2010	2011	2012	2013	2014	2015	2016	2017
HLA-A												
A*11:01	A11	33	2	1						1		
A*11:02	A11	8	1		1							
A*23	A23	3	2	1				1				
A*66	A66	1	1			1						
A*69	A69	5	4		1		1	2				
A*74	A74	3	1							1		
	Total		**11**	**2**	**2**	**1**	**1**	**3**	**-**	**2**	**-**	**-**
HLA-B												
B*15:01	B62	5	1						1			
B*15:02	B75	6	2						1	1		
B*15:12	B76	2	1							1		
B*15:13	B77	7	1									1
B*15:17	B63	6	1				1					
B*15:25	B62	3	1								1	
B*35	B35	4	1								1	
B*40:01	B60	16	1					1				
B*40:06	B61	4	1						1			
B*41	B41	4	2	2								
B*42	B42	2	1			1						
B*46	B46	9	1		1							
B*48	B48	2	1						1			
B*51	B51	10	1			1						
B*55	B55	4	2		1	1						
B*59	B59	1	1		1							
	Total		**19**	**2**	**3**	**3**	**1**	**1**	**4**	**2**	**2**	**1**
HLA-DRB1												
DRB1*04	DR4	22	1						1			
DRB1*07	DR7	18	2		1					1		
DRB1*08	DR8	6	2					1		1		
DRB1*11	DR11	14	1	1								
DRB1*13	DR13	11	1						1			
DRB1*14	DR14	21	3		1	1		1				
DRB1*15	DR15	28	1		1							
	Total		**11**	**1**	**3**	**1**	**-**	**2**	**2**	**2**	**-**	**-**

Discussion

The clinical applications of HLA typing include first, to allocate deceased donor kidneys to individuals on the kidney transplant waiting list; second, to find the HLA-matched donor with the patient for hematopoietic stem cell transplantation and third, to be used as a genetic marker in the diagnosis of HLA-associated diseases such as the association of HLA-B27 with ankylosing spondylitis.[1,9] For the deceased donor kidney allocation criteria, the matching results of HLA-A, -B, and -DR typing are needed among both donors and wait-listed patients to determine the appropriate priority for HLA crossmatch before kidney transplantation. Therefore, proficiency testing samples for HLA typing must be performed with the laboratory's regular patient workload, using routine testing reagents and techniques.

In this study, we reported the discrepant HLA-A, -B, and -DRB1 typing results found in 96 EQC samples sent to nine participating laboratories from 2009 to 2020. The numbers of participating laboratories were increased compared with the first report in 2004.[5] The HLA-A, -B, and -DRB1 genotyping results of the EQC samples were successfully returned within the due date from all participating laboratories. This would be useful to analyze the essential data for improvement.

For the selection of appropriate EQC samples to cover HLA -A, -B, and -DRB1 alleles in Thai populations, from 2009 to 2020, only three alleles including HLA-A*25 (A25), -B*81 (B81), and -B*45 (B45) could not be selected due to their scarcity in Thai and other populations.[5,9,14] The discrepancies in HLA-B typing were the most common (9.89%), similar to a related study in 2004.[10] Even though different molecular techniques were used in all laboratories, misidentification of HLA-B*15 alleles to clarify B62, B63, B75, B76, and B77 antigens was observed. This might have been due to the limitations of primers and/or probes used and misinterpretation by laboratory personnel. The HLA-B*15, especially B75 and B 62 antigens were commonly found among Thai donors and patients.[5,15,16] Moreover, misassignments in HLA-A and HLA -B typing by serology testing (7.1% and 22.5%) were found in proficiency testing when the typing results were compared with molecular testing.[17,18] Hence, appropriate selection of commercial reagents for HLA typing among Thai and other populations is needed.

Regarding HLA-A and -DRB1 typing results, the errors in both groups were 5.73%. The frequent discrepant results in HLA-A typing were found in A*11 and some rare alleles (A*23, A*66, A*69 and A*74). Moreover, the discrepant results in HLA-DRB1 typing were commonly found in DRB1*14, owing to its high polymorphism compared with other HLA-DRB1 alleles.[12] Interestingly, from 2018 to 2020, correct HLA-A, -B, and -DRB1 typing results were obtained from all laboratories. This finding confirms the gradual improvement of laboratory performance during this study period. The ability to accurately determine HLA -A, -B, and -DRB1 typing when the same material is examined could lead to improved graft function and patient survival.

Conclusion

This study confirmed that participating in the national external quality control program in HLA typing would be useful to assess laboratory performance and improvement. This could ensure that laboratory performance remains reliable and accurate among different laboratories in Thailand.

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