III. MATERIALS AND METHODS

A. Subjects

The patient group consisted of 178 subjects, 70 males and 108 females, with the age range from 10 to 64 years. The median age of onset of combined Graves' disease patients was 31 years (Fig 4). All patients were unrelated northern Thai subjects from upper northern provinces of Thailand (Chiang Mai, Chiang Rai, Lamphun, Lampang, Nan, Phrae, Phayao). They were diagnosed and treated at the Thyroid Clinic of the Maharaj Nakorn Chiang Mai Hospital between February, 1994 to August, 1994.

Diagnosis of Graves' disease was based on clinical assessment of hyperthyroidism. All patients had diffuse toxic goiter, mostly without nodule. Some also had exophthalmopathy and/or pretibial myxedema. A few experienced periodic paralysis, myopathy or myasthenia gravis. Fifty-six percent of the patients experienced relapse within 12 months or more of antithyroid drug withdrawal and the remaining 44% had clinical remission. Some are still recieving first course anti-thyroid treatment. Patients with disease relapse were divided into 3 groups as follows:

1. Clinical relapsing group. Patients were classified on the basis of clinical evaluation only.
2. Laboratory proven group. Patients with clinical relapse were confirmed by abnormally high level of serum thyroid hormone.
3. Exacerbated group. This group consisted of patients with mild disease but still requiring an antithyroid drug. After the peroid of treatment by a maintenance dose, the disease recurred and they required an additional dose.

Diagnosis of patients who were presented with atypical clinical manifestations was confirmed by the detection of anti-microsomal and anti-thyroglobulin antibodies in their sera. Both of these autoimmune antibodies were detected by using hemagglutination technique. The titer of 1:100 or more was considered as positive.

The patients were born in the upper northern provinces of Thailand. The ancestor were also born in northern Thailand and did not marry other race at least 3 generations. Burmese, Chinese and Hill tribe groups were excluded.

The control group included 118 unrelated blood donors from Blood Bank Unit and hospital personnel of Maharaj Nakorn Chiang Mai Hospital. The control group were

already HLA typed by the same method in the previous project (Wongkuttiya, 1994, Kunachiwa, 1995).

B. Preparation of genomic DNA

Genomic DNA was prepared by the salting-out method (Miller et al., 1988). Ten ml of peripheral venous blood was added with 50 µl of 0.5 M EDTA in a 50 ml centrifuge tube and centrifuged at 1,000 x g (Model K, International Equipment Company, USA) for 10 min at room temperature. Plasma was removed without interfering the buffy coat and stored at 4°C for subsequent determination of anti-microsomal antibody and anti-thyroglobulin antibody. The remaining red cells were lysed by adding the lysing solution (10 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 10 mM NaCl) up to 50 ml and mixing throughly. Following complete lysis of red cells, white blood cells were pelleted by centrifuging at 2,200 x g for 10 min at room temperature. If there are many pelleted ghost red cells, the lysing step was repeated once by adding another 30 ml of lysing solution and centrifuging exactly for 5 min. The supernatant was discarded and effort was made to remove the remaining ghost red cell from white cell pellet with Pasteur pipette. White cell pellet was resuspended completely with 5 ml of nucleic lysis buffer (10 mM Tris-HCl, pH 8.0, 400 mM NaCl, 2 mM EDTA). Fifty µl of 10% SDS and 50 µl of proteinase K solution (10 mg/ml) were added in order and the solution was incubated at 55 °C overnight. After the incubation, the mixture was added with 1.25 ml of saturated NaCl to precipitate the remaining proteins and was left standing at 4 °C for 20 min. The solution was spun at 2,200 x g for 25 min to pellet the proteins. The supernatant was transferred to a new 50 ml centrifuge tube and was added with 2 volumes of absolute ethanol. The genomic DNA precipitate was generally visible after mixing. It was then washed with 70% ethanol twice, dehydrated in 95% ethanol, let dried at room temperature and resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). The DNA solution was kept at room temperature for 2 days and then at 4 °C until use. Concentration of DNA was measured by UV light absorption at 260 nm. The quality of the genomic DNA was checked by running 500 ng of genomic DNA on 0.7% agarose gel and comparing with the phage lambda DNA.

C. DNA amplification

C. 1 PCR primer pairs for amplification

The second exons of the HLA-DQA1, HLA-DQB1, HLA-DRB1 genes were amplified by using polymerase chain reaction, using primers as recommended by the 11th International Histocompatibility Workshop (Table 3). Only one pair each of specific primers was employed for the amplification of HLA-DQA1 and HLA-DQB1 genes. For the HLA-DRB1 gene, a first set of primers allowed the amplification of the region corresponding to the amino acid positions 3th to 93th of all DRβ1 haplotypes. In order to determined the specific subgroups of this locus, additional group specific primer pairs were employed in the second round (Table 3). The DRBAMP-2 and DRBAMP-B primers were used for DR2 group specific amplification whereas the DRBAMP-4 and DRBAMP-B primers amplified the DR52 associated DRB1 group.

C. 2 Polymerase chain reaction

Approximately 500 ng of the genomic DNA was added into the pre PCR mixture which contained Taq DNA polymerase buffer (10 mM Tris-HCl, pH 8.4 at 25°C, 50 mM KCl), dNTPs (2 mM each of dATP, dCTP, dGTP and TTP), 2.5 mM $MgCl_2$, 25 pmol of each primer and 1 unit of Taq DNA polymerase (Promega Corporation, USA). The mixture was thoroughly mixed and overlaid with 2 drops of mineral oil to prevent condensation of samples. The PCR conditions, according to the 11th reference protocol, are shown in Table 4. Following the amplification, the relative quantity of amplified DNA was checked by electrophoresis on a 1.5% agarose gel. The size of the product was determined by comparing its mobility with those of HaeIII-digested Phi X 174 DNA fragments.

D. Dot blot hybridization

D. 1 Dot spotting of DNA

Approximately 50 ng of amplified DNA were spotted onto a prewetted nylon membrane filters (Micron Separation Inc., USA) by using the Dot Blotter (Schleicher &

Schuell, USA). The number of the membrane filter dotted depended upon the number of sequence-specific oligonucleotide (SSO) probes employed. Before dotting, the DNA samples were diluted in distilled water to equalize the total volume of DNA being blotted. The DNA spots were let dried at room temperature, denatured by soaking in 0.4 N NaOH for 5 min and neutralized in 10x SSPE (1.5 M NaCl, 0.1 M NaH_2PO_4, pH 7.4, 10 mM EDTA) for 10 min. Both sides of the membranes were then illuminated with a 254 nm-UV light source to 0.51 Joule/cm^2 to fix the DNA onto the membrane. Membranes were kept at 4 °C after being dried at room temperature.

D. 2 Labeling of the oligonucleotide probes

D. 2.1 Sequence-specific oligonucleotide (SSO) probes

We employed 10, 17, and 24 SSO probes for the typing of HLA-DQA1 alleles, HLA-DQB1 alleles and HLA-DRB1 gene, respectively. These probes were recommended by the 11th International Histocompatibility Workshop. Recently, many new alleles were reported (Marsh and Bodmer, 1995) which were not covered by the Workshop probes. Two new probes were then designed and employed for the typing a new HLA-DQA1*0502 allele. They are shown in Tables 5, 6, 7, 8 and 9.

D. 2.2 3'-end labeling with DIG-ddUTP

Before employing in the hybridization reaction, the SSO probes were added at the 3' terminus with a digoxigenin-conjugated dideoxyuridine triphosphate (DIG-ddUTP) by using DIG-oligonucleotide 3' end labeling kit (Boehringer Mannheim Biochemica, Germany). One hundred picomols of each SSO probe were mixed with the tailing buffer (0.14 mM sodium cacodylate, 30 mM Tris-HCl, pH 7.2, 10 mM $CoCl_2$), 1 mM DIG-11-ddUTP, 1 mM DTT and 20 units of terminal deoxynucleotidyl transferase. The mixture was incubated at 37 °C for 15 min. and then stored at -20 °C until use.

D. 3 Hybridization

The hybridization was performed according to the 11th International Histocompatibility Workshop Reference Protocol (Kimura, 1991). The dot-blotted

membranes were prehybridized with 0.1 ml/cm^2 of hybridization buffer (6x SSPE, 5x Denhardt's solution, 0.1% sarcosine natrium, 0.02% SDS) at 42 °C for 1 hour in a hybridization incubator (Model 400, Robbins Scientific, USA) to reduce non-specific binding. Labeled SSO probes were next added directly into the hybridization solution to the final concentration of 1 pmol/ml. The hybridization reaction was allowed to proceed for 2 hours. At the end of the hybridization, the hybridization solution was poured off; the filters were rinsed in 2x SSPE twice at room temperature for 10 min each. The membranes were then washed in tetramethylammonium chloride (TMAC) washing solution (50 mM Tris-HCl, pH 8.0, 3 M TMAC, 2 mM EDTA, 0.1% SDS) once at room temperature for 10 min and twice at 57 °C for 30 min each. TMAC was used in the washing step to reduce the influence of GC content on the hybridization behavior (Jacob et al., 1988). In the last step, the filters were rinsed with 2x SSPE at room temperature for 10 min.

E. Chemiluminescent detection

Hybridized SSO probes were detected by employing the DIG-chemiluminescent detection kit (Boehringer Mannheim Biochemica, Germany). Following the last hybridization step, the membranes were rinsed in buffer 1 (100 mM Tris-HCl, pH 7.2; 150 mM NaCl) for 5 min. The buffer 2 (buffer 1 containing 0.5% blocking reagent) was next added for 30 min to block non-specific binding of anti-DIG antibody to the membrane. Alkaline phosphatase-conjugated anti-DIG antibody diluted in buffer 2 (1:5,000-1:10,000) was then added over the membrane and agitated constantly for 30 min. Unreacted antibody was washed off with washing buffer (buffer 1 with 0.3% Tween 20) twice. Before the detecting step, the membranes were equilibrated in buffer 3 (100 mM Tris-HCl, pH 9.5; 100 mM NaCl; 50 mM $MgCl_2$) for 5 min. Bound antibody-enzyme conjugate was detected by adding chemiluminescent substrate (disodium3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1^{3,7}]decan}-4-yl) phenyl phosphate, CSPD) (Boehringer Mannheim Biochemica) diluted in buffer 3 (0.25 mM). The substrate solution was drained off. Membranes were put in the PhotoGene development folder (Life Technologies Inc., USA) and the edges were heat-sealed in such a way that there was no air bubble inside the bag. After incubating the membranes at 37°C for 15 min, they were exposed to X-ray film (Agfa Curix XP, Belgium) for 2-12 hours depending on the specific activity of each labeled SSO probe.

F. Dehybridization

After hybridization, the dotted membranes could be reused. The bounded SSO-antibody-enzyme complex was removed by immersing the membranes in dehybridization solution (0.2 N NaOH, 0.01% SDS) at 42 °C for 30 min with agitation. They were then neutralized in 2x SSC, pH 7.0 and kept in 2x SSPE at 4 °C until used.

G. Interpretation of the results and Statistical analysis

The association between HLA antigen and Graves' disease was determined by employing the statistical method after grading the signal from hybridization-detection step.

G. 1 Grading of the chemiluminescent signal

The intensity of the signal was graded as follows:

0	not done,	1	negative (definite),
2	negative (probable),	4	indefinite,
6	positive (probable),	8	positive (definite).

After grading, the assignment of allelic forms of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles was done according to the Tables 10, 11, 12, 13 and 14.

The frequency was derived in 2 forms: the antigen frequency and the allele frequency. The direct counting of the numbers of individual who carried a particular allelic form resulted in the antigen frequency. By contrast, when each allele was counted individually (heterozygotes were counted as 1 and homozygotes were counted as 2 for each allele), the results were expressed as the allele frequency. Both frquencies were in percentage according to the following formulas:

$$\text{Antigen frequency (Ag freq.)} = \frac{\text{(Number of positive individuals)} \times 100}{\text{Total number of individuals tested}}$$

$$\text{Allele frequency (Allele freq.)} = \frac{\text{(Number of positive allele of each allelic form)} \times 100}{\text{Total number of individuals tested} \times 2}$$

G. 2 Statistical analysis

G. 2.1 Relative risk

The relative risk (RR) was used to indicate how many times more frequent the disease is in the individuals positive for the antigen or allele than in individuals negative for the antigen or allele (Tiwari and Terasaki, 1985). It simply gives the relation between risks of individuals of different exposure status. In a 2 x 2 table, the letter a, b, c and d represent the number of persons in each cell.

	Exposure		
Disease	Present	Absent	Total
Present (cases)	a	b	a+b
Absent (reference)	c	d	c+d
	a + c	b + d	

$$\text{Relative risk (RR)} = (a \times d) \div (b \times c)$$

The more relative risk value (>1), the greater association was detected. This means that the antigen or allele is associated with the disease (Tiwari et al, 1985). If the relative risk values less than 1, the weak association is indicated. The antigen or allele is less frequent in the patients. If the antigen or allele frequency of the particular allelic form of patients is equal to those of the control, the relative risk is 1. When both groups carry this allele, they have a equal chance to get sickness.

G. 2.2 Chi square test

Chi square test was employed in order to find the significant difference between the frequency of any allele in the patient and control groups. According to the Woolf's method (Woolf, 1955) and Haldane's modification (Haldane, 1955),

The sampling variance, V, of y is $V = 1/a + 1/b + 1/c + 1/d$

$$X = ad/bc$$

and w, the weight of y is

$$w = 1/V$$
$$y = \ln X$$
$$\chi^2_{1df} = wy^2$$

when a = numbers of positive individuals in patient group,
b = numbers of negative individuals in patient group,
c = numbers of positive individuals in control group,
d = numbers of negative individuals in control group.

When samples are small (≤ 5), the formula given by Haldane was used:

$$X = (2a+1)(2d+1)/(2b+1)(2c+1)$$

$$V = (1/a+1) + (1/b+1) + (1/c+1) + (1/d+1)$$

After the chi square was calculated. The probability value (p value) was obtained from the chi square table. The p value was then corrected by multiplying by the number of alleles tested at each locus. If the p value was less than 0.05, the difference was considered statistically significant.

G. 2.3 Etiologic fraction and preventive fraction

The etiologic fraction (EF) is defined as the proportion of cases, attributable to the exposure, out of the total number of cases developed in the total population (Green, 1982 b). If EF value is between 0 and 1, the hypothetical exposure per se is one of the causes of the disease. There is a positive association. In this case, the exposure means the specified HLA allele.

The preventive fraction (PF), analogous to the etiologic fraction, is defined as the proportion of cases prevented by the exposure. This measure may be obtained when an

association is negative (Green, 1982b). It is the proportion of a theoretical number of cases that would have developed in the combined population, if the exposure had not had the preventive effect.

According to Green (1982b):

$$EF = ER_c * \{(IDR-1)/IDR\} \quad (IDR \geq 1)$$

$$PE = \{(1- IDR)*ER_c\} / \{IDR*(1-ER_c)+ER_c\} \qquad (IDR \leq 1)$$

When ER_c is the exposure proportion among cases or the frequency of antigen/allele among the patients. It is simply calculated as a/(a+b) from the 2x2 table of the data in a case reference study (Green, 1982b).

$$IDR = ID_e/ID_o$$

$$ID_e = A/ N_e * \Delta t$$

$$ID_o = B/ N_o * \Delta t$$

when ID (incidence density) = the number of new (incident) cases in the age interval, divided by the observed person-time at risk in the age interval

IDR (incidence density ratio) = the ratio of two incidence densities, both referring to the age interval

e = refer to the category of exposed individuals
o = refer to the category of non-exposed individuals

A population of size N of person at risk, without the disease, may be divided in N_e, exposed individuals, and N_o, non-exposed individuals. During the period of time (Δt), A exposed individuals and B non-exposed individuals develop the disease.

$$IDR = (A \times N_o) \div (B \times N_e)$$

The ratio A/B is the ratio between exposed and non-exposed cases. Correspondingly, the ratio N_o/N_e is the exposure odds among the total candidate population for disease. Thus, IDR is estimated as (a x d)÷(b x c) which is the RR in the 2 x 2 table. IDR is similar to RR, if the disease is rare among the exposed as well as the non-exposed (Green, 1982a).

G. 2.4 Linkage disequilibrium

When two loci, A and B, on the same chromosome are in equilibrium with each other, the combination of alleles on a chromosome in a gamete follows the product rule of probability (Vogel and Motulsky, 1986). The proportion of combination of alleles will be the product of their population frequencies. When the population starts out with a deviation from this equilibrium, the expected frequency of combinations of alleles will be far below the observed frequency. According to Vogel and Motulsky (1986), if there is free combination between two alleles at two loci, their haplotype frequency should be :

$$\text{Haplotype frequency (h)} = p_a \times p_b$$

when p_a, p_b are the frequencies of two alleles, respectively.

If the haplotype frequency (h) is higher than expected with free combination, there is linkage disequilibrium, which is symbolized as Δ (delta).

$$\Delta = \sqrt{d/N} - \sqrt{(b+d)x(c+d)/N^2}$$

when Δ = the linkage disequilibrium value, and a, b, c, d and N are given from the table as follows:

First antigen	Second antigen	+/+	+/-	-/+	-/-	Total
X	Y	a	b	c	d	N

(+ and - mean the positive and negative status for the antigen).

The haplotype frequency could be calculated by:

$$h_{XY} = p_X p_Y + \Delta_{XY}$$

The chi square is $\chi^2_{1df} = (ad-bc)\, N^2 / (a+b)(c+d)(a+c)(b+d)$.

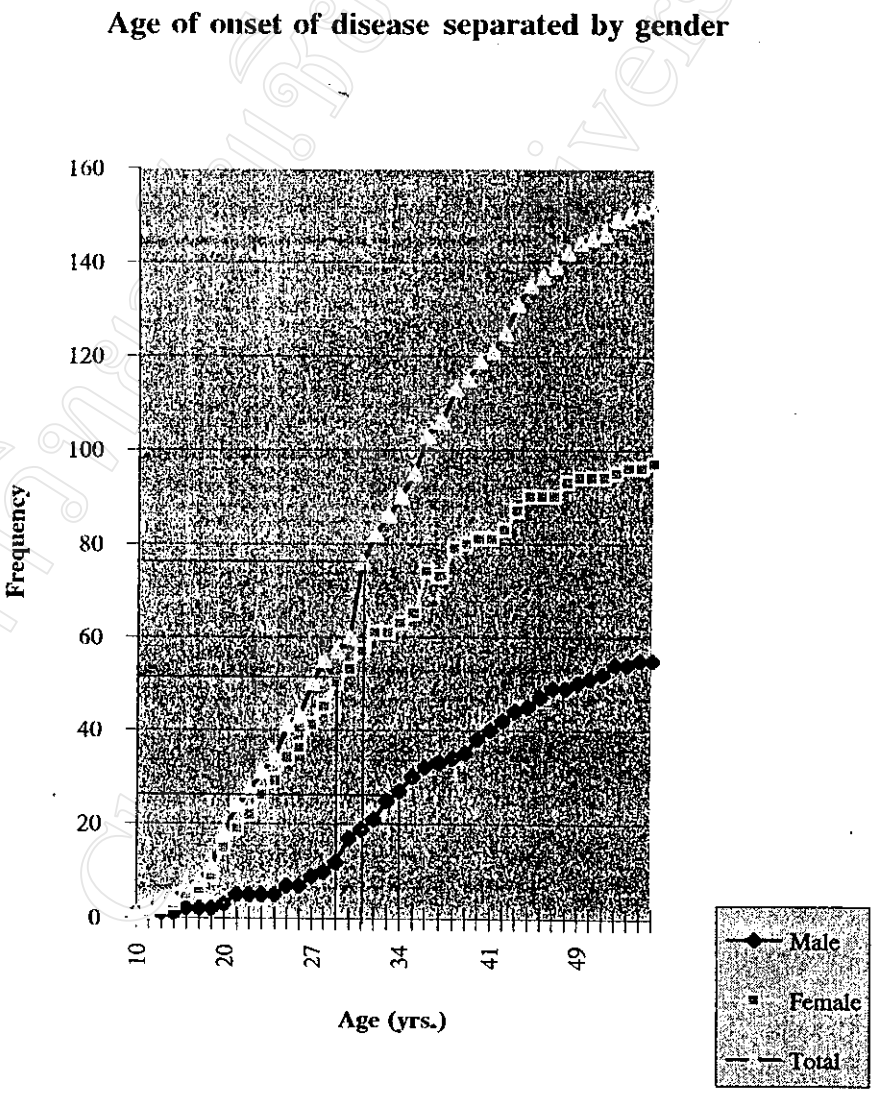
H. The new HLA-DRB1, HLA-DQA1 and HLA-DQB1 alleles and new probes

Forty-eight HLA-DRB1 alleles, 15 HLA-DQB1 alleles and 8 HLA-DQA1 alleles can be differentiated by the Eleventh International Histocompatibility Workshop Oligotyping method (Kimura et al., 1991). In an update for new HLA class II sequences (Marsh and Bodmer, 1995), 13 alleles of HLA-DQA1 locus, 25 of HLA-DQB1 locus and 135 of HLA-DRB1 locus were reported. Thus when compared with the previous compilation, there were 5, 20 and 87 new alleles of HLA-DQA1, HLA-DQB1 and HLA-DRB1 genes, respectively. These new alleles are shown in Table 2, but this did not include new alleles with only base substitution which did not change the amino acid sequence.

Table 2 The new alleles of HLA-DQA1, HLA-DQB1 and HLA-DRB1 genes, Update June 1995

New HLA-DQA1 allele	New HLA-DQB1 allele	New HLA-DRB1 allele		
0104	0202	0104	1108	1319
			1109	1320
0502	0304	0303	1110	1321
0503	0305	0304	1111	1322
		0305	1112	
	0606		1113	1409
	0607	0412	1114	1410
	0608	0413	1115	1411
	0609	0414	1116	1412
		0415	1117	1413
		0416	1118	1414
		0417	1119	1415
		0418	1120	1416
		0419	1121	1417
		0420		1418
		0421	1203	1419
		0422		1420
			1306	1421
		0805	1307	
		0806	1308	1504
		0806	1309	1505
		0809	1310	
		0810	1311	1603
		0811	1312	1604
			1314	1605
		1105	1315	1606
		1106	1316	
		1107	1317	

Figure 4 The Age of onset of disease of Graves' patients separated by gender



*the median age of onset of disease in Graves' patients is 31 years (Male = 34, Female = 29)

Table 3 HLA-DQA1, HLA-DQB1 and HLA-DRB1 generic and group specific primer pairs

HLA loci	PCR primer pairs for amplification.	Position of ampification	Length of product (bp)
HLA-DQA1	DQAAMP-A 5'-ATGGTGTAAACTTGTACCAGT-3' DQAAMP-B 5'-TTGGTAGCAGCGGTAGAGTTG-3'	32-260	229
HLA-DQB1	DQBAMP-A 5'-CATGTGCTACTTCACCAACGG-3' DQBAMP-B 5'-CTGGTAGTTGTGTCTGCACAC-3'	39-252	214
HLA-DRB1 generic primers	DRBAMP-A 5'-CCCCACAGCACGTTTCTTG-3' DRBAMP-B 5'-CCGCTGCACTGTGAAGCTCT-3'	8-279	274
Specific primer DR2-DRB1	DRBAMP-2 5'-TTCCTGTGGCAGCCTAAGAGG-3' DRBAMP-B same as in the generic amplification	19-279	261
DR52 associated group-DRB1	DRBAMP-3 5'-CACGTTTCTTGGAGTACTCTAC-3' DRBAMP-B same as in the generic amplification	14-279	266

Table 4 Conditions of PCR for HLA-DQA1, HLA-DQB1, HLA-DRB1 generic and HLA-DRB1 group specific typing

PCR condition	Denaturation	Annealling	Extention	cycle	Final extention
Generic PCR					
HLA-DQA1	94°C, 30 sec	56°C, 1 min	72°C, 1.5 min	30	72°C, 8.5 min
HLA-DQB1	96°C, 30 sec	57°C, 1 min	72°C, 2 min	30	72°C, 8.5 min
HLA-DRB1	96°C, 30 sec	57°C, 1 min	72°C, 2 min	30	72°C, 8.5 min
HLA-DRB group-specific PCR					
DR2-DRB1	96°C, 30 sec	61°C, 30 sec	70°C, 2 min	30	70°C, 8.5 min
DR52 ass.-DRB1	96°C, 30sec	61°C, 30 sec	70°C, 2 min	30	70°C, 8.5 min

Table 5 Sequences and specificities of DQA SSO probes

HLA-DQA1 SSO probes	Sequence (5'-3')	Specificity
DQA 2502	TGGCCAGTTCACCCATGA	HLA-DQA1*0103, *0201, *0601
DQA 3401	GAGATGAGGAGTTCTACG	HLA-DQA1*0101
DQA 3402	GAGATGAGCAGTTCTACG	HLA-DQA1*0102, *0103, *0501
DQA 3403	GAGACGAGCAGTTCTACG	HLA-DQA1*0401, *0601
DQA 4102	ACCTGGAGAAGAAGGAGA	HLA-DQA1*0103
DQA 5501	TCAGCAAATTTGGAGGTT	HLA-DQA1*0101, *0102, *0103
DQA 5502	TCCACAGACTTAGATTTG	HLA-DQA1*0201
DQA 5503	TCCGCAGATTTAGAAGAT	HLA-DQA1*03, *03012, *0302
DQA 5504	TCAGACAATTTAGATTTG	HLA-DQA1*0401, *0501, *0601
DQA 6903	ATCGCTGTCCTAAAACAT	HLA-DQA1*0501, *03012
DQA 0501	TTTGACCCGCAATTTGCA	HLA-DQA1*0201, *03, *0501, *0601
DQA 0502	TTTGACCGGCAATTTGCA	HLA-DQA1*0502

Table 6 Sequences and specificities of DQB SSO probes

HLA-DQB1 SSO probes	Sequence 5'-->3'	Specificity
DQB 2301	GACCGAGCTCGTGCGGGG	HLA-DQB1*0401
DQB 2302	AACGGGACCGAGCGCGTG	HLA-DQB1*03031, *0402
DQB 2601	CGGGGTCTGACCAGACAC	HLA-DQB1*0501, *0502,*05031, *05032
DQB 2603	CGTCTTGTGACCAGATAC	HLA-DQB1*0602, *0302, *03032
DQB 2604	CGTCTTGTAACCAGACAC	HLA-DQB1*0603, *0604
DQB 2606	CGTCTTGTAACCAGATAC	HLA-DQB1*0605
DQB 0504	TCCTGGAGGAGGACCGGG	HLA-DQB1*0504, *0401, *0402
DQB 3702	AGGAGGACGTGCGCTTCG	HLA-DQB1*0601
DQB 4501	GACGTGGAGGTGTACCGG	HLA-DQB1*0301, *0304
DQB 4901	GGTGTACCGGGCAGTGAC	HLA-DQB1*0501
DQB 5701	GCGGCCTGTTGCCGAGTA	HLA-DQB1*0501, *0604, *0605
DQB 5702	GCGGCCTAGCGCCGAGTA	HLA-DQB1*0502, *0504
DQB 5703	GGCGGCCTGACGCCGAGT	HLA-DQB1*05031, *0601
DQB 5704	GCGGCCTGATGCCGACTA	HLA-DQB1*05032, *0602, *0603
DQB 5705	GGCTGCCTGCCGCCGAGT	HLA-DQB1*0201
DQB 5706	GGCCGCCTGACGCCGAGT	HLA-DQB1*0301, *03031, *03032
DQB 5707	GGCCGCCTGCCGCCGAGT	HLA-DQB1*0302, *0304

Table 7 Sequences and specificities of DRB SSO probes for HLA-DRB1 generic group typing

HLA-DRB1 SSO probes	Sequence (5'-->3')	Specificity
DRB 1001	TAAGTTTGAATGTCATTT	HLA-DRB1*0101-3
DRB 1002N	CCTAAGAGGGAGTGTCAT	HLA-DRB1*1501, *1502, *1601, *1602
DRB 1003	GTACTCTACGTCTGAGTG	HLA-DRB1*0301, *0302, *1101-4, *1301-5, *1401-3, *1405
DRB 1004	GAGCAGGTTAAACATGAG	HLA-DRB1*0401-11
DRB 1005	AGAAATAACACTCACCCG	HLA-DRB1*1201-2, *1404, *0801-4
DRB 1006	TGGCAGGGTAAGTATAAG	HLA-DRB1*0701
DRB 1007	GAAGCAGGATAAGTTTGA	HLA-DRB1*0901
DRB 1008N	GACCAGGTTAAGTTTGAC	HLA-DRB1*1001

Table 8 Sequences and specificities of DRB SSO probes for HLA-DR52 associated DRB1 typing

HLA-DR52 SSO probes	Sequence 5'-->.3'	Specificity
DRB 1003	GTACTCTACGTCTGAGTG	HLA-DRB1*0301-2, *1101-4, *1106, *1301-5 *1401-3, *1405-8
DRB 1005	AGAAATAACACTCACCCG	HLA-DRB1*1201-2, *1404, *0801-4
DRB 2813	GTTCCTGGACAGATACTT	HLA-DRB1*1201, *1202, *1404, *0801-4
DRB 3703N	ACCAGGAGGAGAACGTGC	HLA-DRB1*0301-2, *1301-2, *1305, *1402-3
DRB 5701	GCCTGATGCCGAGTACTG	HLA-DRB1*0301-2, *1301-2, *1305, *1402-3 *0802, *0804
DRB 5702N	GCCTAGCGCCGAGTACTG	HLA-DRB1*1303-4, *0801, *08031-2
DRB 5703	GCCTGATGAGGAGTACTG	HLA-DRB1*1101-4
DRB 5704N	GCCTGCTGCGGAGCACTG	HLA-DRB1*1401, *1404
DRB 5705	GCCTGTCGCCGAGTCCTG	HLA-DRB1*1201-2
DRB 5708	GCCTGATGCTGAGTACTG	HLA-DRB1*1405
DRB 7002N	GACTTCCTGGAAGACAGG	HLA-DRB1*1101, *1104, *1106, *1202, *1305 *0801-2, *0804
DRB 7003N	GACCTCCTGGAAGACAGG	HLA-DRB1*1403
DRB 7004	GGCCGGGTGGACAACTAC	HLA-DRB1*0301-2
DRB 7007N	ACATCCTGGAAGACGAGC	HLA-DRB1*1103, *1301-2, *1304
DRB 7010	GGACATCCTGGAAGACAG	HLA-DRB1*1201, *08032, *0804 (*1103, *1301-4)
DRB 8601	AACTACGGGGTTGGTGAG	HLA-DRB1*0302, *1101, *1302-3, *1305 *1402-3, *1407, *0801-3
DRB 8602	AACTACGGGGCTGTGGAG	HLA-DRB1*1106, *1201-2
DRB 8603	AACTACGGGGTTGTGGAG	HLA-DRB1*0301, *1102-4, *1301, *1304 *1401, *1404-6, *1408, *0804

Table 9 Sequences and specificities of DRB SSO probes for HLA-DR2 typing

HLA-DR2 SSO probe	Sequence 5'-->3'	Specificity
DRB 1002N	CCTAAGAGGGAGTGTCAT	HLA-DRB1*1501, *1502, *1503, *1504 *1601, *1602
DRB 2813	GTTCCTGGACAGATACTT	HLA-DRB1*1501, *1502, *1503, *1504 *1601, *1602
DRB 7002	GACTTCCTGGAAGACAGG	HLA-DRB1*1601
DRB 7003	GACCTCCTGGAAGACAGG	HLA-DRB1*1602
DRB 7011	GACATCCTGGAGCAGGCG	HLA-DRB1*1501, *1502, *1503
DRB 8601	AACTACGGGGTTGGTGAG	HLA-DRB1*1502, *1601, *1602
DRB 8603	AACTACGGGGTTGTGGAG	HLA-DRB1*1501, *1503, *1504

Table 10 Hybridization pattern of DQA SSO probes for HLA-DQA1 typing

DQA SSO	DQA1* allele									
	0101	0102	0103	0201	0301 11	0301 12	0401	0501	0502	0601
2502	-	-	+	+	-	-	-	-	-	+
3401	+	-	-	-	-	-	-	-	-	-
3402	-	+	+	-	-	-	-	+	+	-
3403	-	-	-	-	-	-	+	-	-	+
4102	-	-	+	-	-	-	-	-	-	-
5501N	+	+	+	-	-	-	-	-	-	-
5502N	-	-	-	+	-	-	-	-	-	-
5503	-	-	-	-	+	+	-	-	-	-
5504	-	-	-	-	-	-	+	+	+	+
6903	-	-	-	-	-	+	-	+	+	-
0501	-	-	-	+	+	+	+	+	-	+
0502	-	-	-	-	-	-	-	-	+	-

Table 11 Hybridization pattern of DQB SSO probes for the HLA-DQB1 typing

DQB SSO	DQB1 allele																
	0501	0502	05031	05032	0504	0601	0602	0603	0604	0605	0201	0301	0302	03031	0303 2	0401	0402
2301	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2302N	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
2601	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2603	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-	-
2604	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
2606	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
3702N	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
4501N	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
4901	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5701	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
5702	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
5703	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
5704	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-
5705	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
5706	-	-	-	-	-	-	-	-	-	-	-	+	c	+	+	-	-
5707	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
0504	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+

Table 12 Hybridization pattern of DRB SSO probes for the HLA-DRB1 typing

DRB SSO	DRB*1 allele																																							
	0101	0102	0103	1501	1502	1503	1504	1601	1602	0301	0302	04	1101	1102	1103	1104	1106	1201	1202	1301	1302	1303	1304	1305	1401	1402	1403	1404	1405	1406	1407	1408	07	0801	0802	08031	08032	0804	09011	09012	1001
1001	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1002N	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1003	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-
1004	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1005	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+	-	-	-
1006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
1007	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
1008N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Table 13 Hybridization pattern of DRB SSO probes for the HLA-DR 52 associated DRB1 typing

DRB SSO	DRB1* allele																										
	0301	0302	1101	1102	1103	1104	1106	1201	1202	1301	1302	1303	1304	1305	1401	1402	1403	1404	1405	1406	1407	1408	0801	0802	08031	08032	0804
1003	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-
1005	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+
2813	-	-	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+
3703	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-
5701	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-	-	+
5702	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-
5703	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5704	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
5705	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5708	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
7002	-	-	+	-	-	+	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+
7003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
7004	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7007	-	-	-	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7010	-	-	-	-	c	-	-	+	-	c	c	c	c	-	-	-	-	-	-	-	-	-	-	-	-	+	+
8601	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	+	+	-	-	-	+	-	+	+	+	+	-
8602	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8603	+	-	-	+	+	+	-	-	-	+	-	-	+	-	+	-	-	+	+	+	-	+	-	-	-	-	+

Table 14 Hybridization pattern of DRB SSO probes for the HLA-DR2 group typing

DRB SSO	DRB1* allele					
	1501	1502	1503	1504	1601	1602
1002N	+	+	+	+	+	+
2813	+	+	-	+	+	+
7002N	-	-	-	-	+	-
7003N	-	-	-	-	-	+
7011	+	+	+	-	-	-
8601	-	+	-	-	+	+
8603	+	-	+	+	-	-