

HLA-EMMA – Epitope Mismatch Algorithm version 1.06

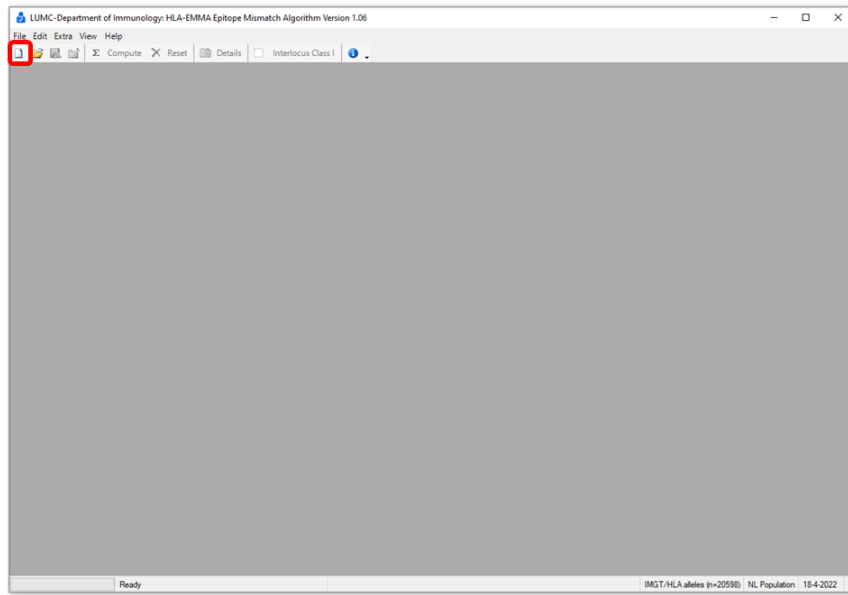


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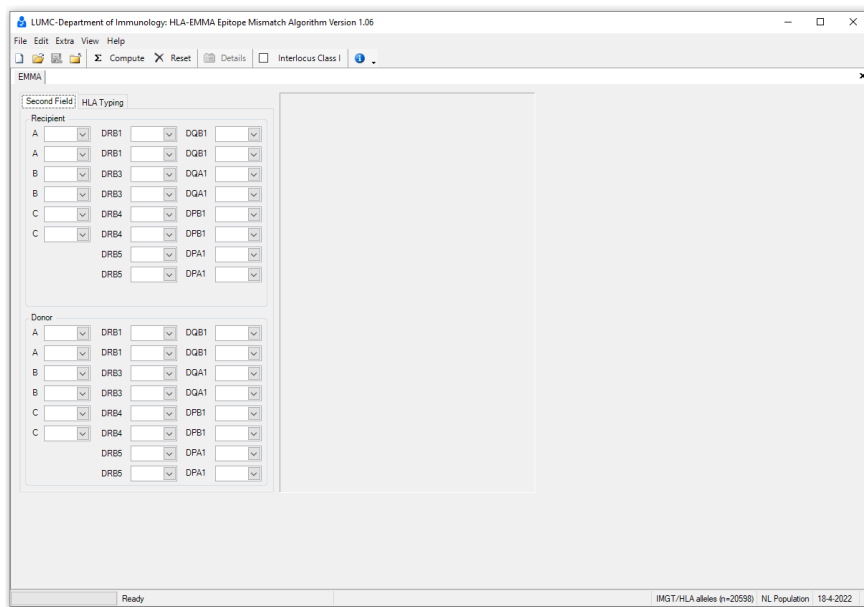
HLA-EMMA v1.06 user manual

Compatibility analysis of a single recipient-donor pair

1. Open HLA-EMMA.
2. Go to File and then new or click on the white paper icon (red square).



3. The interface will open.





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4. Insert the HLA typing of the recipient and donor.
 - a. Use **Second field** tab to type the HLA typing in the box or use dropdown option.

Recipient	
A	01:01 DRB1 11:01 DQB1 03:01
A	32:01 DRB1 13:01 DQB1 06:03
B	08:01 DRB3 02:01 DQA1 01:03
B	40:02 DRB3 05:05
C	02:02 DRB4 DPB1 04:01
C	07:01 DRB4 DPB1 105:01
	DRB5 DPA1 01:03
	DRB5 DPA1

- b. Use **HLA typing** tab to type or copy the HLA typing.

Recipient Typing: A*01:01, A*32:01, B*08:01, B*40:02, C*02:02, C*07:01, DRB1*11:01, DRB1*13:01, DRB3*02:01, DQB1*03:01, DQB1*06:03, DQA1*01:03, DQA1*05:05, DPB1*04:01, DPB1*105:01, DPA1*01:03

Donor Typing: A*02:01, B*07:02, B*35:03, C*04:01, C*07:02, DRB1*04:01, DRB1*11:04, DRB4*01:03N, DQB1*03:01, DQB1*03:02, DQA1*03:01, DQA1*05:05, DPB1*05:01, DPB1*05:01, DPA1*01:03

5. Click **compute** to start analysis.

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File Edit Extra View Help

Compute Reset Details Interlocus Class I

EMMA

Second Field HLA Typing

Recipient

A	01:01	DRB1	11:01	DQB1	03:01
A	32:01	DRB1	13:01	DQB1	06:03
B	08:01	DRB3	02:01	DQA1	01:03
B	40:02	DRB3		DQA1	05:05
C	02:02	DRB4		DPB1	04:01
C	07:01	DRB4		DPB1	105:01
		DRB5		DPA1	01:03
		DRB5		DPA1	

Donor

A	02:01	DRB1	04:01	DQB1	03:01
A		DRB1	11:04	DQB1	03:02
B	07:02	DRB3		DQA1	03:01
B	35:03	DRB3		DQA1	05:05
C	04:01	DRB4	01:03N	DPB1	05:01
C	07:02	DRB4		DPB1	
		DRB5		DPA1	01:03
		DRB5		DPA1	

Ready

IMGT/HLA alleles (v=20598) NL Population 18-4-2022

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6. Results are shown for each donor HLA allele.
 - a. Per **donor HLA allele** the following results are shown:
 - The total amino acid mismatches (Total AA mismatches)
 - The solvent accessible amino acid mismatches (Solvent accessible AA mismatches)
 - Allele mismatch number
 - b. For each locus a subtotal of the number of mismatches is provided (yellow).
 - c. Dark green indicates donor alleles that either are matched or mismatched donor alleles with zero total and solvent accessible amino acid mismatches.
 - d. Light green indicates donor alleles that are mismatched and have total amino acid mismatches but no solvent accessible amino acid mismatches.

The screenshot shows the HLA-EMMA software interface. On the left, there are dropdown menus for selecting HLA alleles for Recipient and Donor across various loci (A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPB1, DPA1). The main area displays a table with the following columns: HLA Allele, Total AA Mismatches, Solvent Accessible AA Mismatches, and Allele Mismatches. The table lists various HLA alleles and their corresponding mismatch counts. Subtotals for each locus are highlighted in yellow. The status bar at the bottom indicates 'Ready' and 'IMGT/HLA alleles (n=20598) NL Population 18-4-2022'.

HLA Allele	Total AA Mismatches	Solvent Accessible AA Mismatches	Allele Mismatches
A*02:01	14	12	1
Subtotal HLA-A	14	12	1
B*07:02	9	6	1
B*35:03	12	6	1
Subtotal HLA-B	19	11	2
C*04:01	11	5	1
C*07:02	1	0	1
Subtotal HLA-C	12	5	2
DRB1*04:01	8	7	1
DRB1*11:04	0	0	1
DRB4*01:03N	0	0	1
Subtotal HLA-DR	8	7	3
DQB1*03:01	0	0	0
DQB1*03:02	2	2	1
Subtotal HLA-DQB1	2	2	1
DQA1*03:01	17	13	1
DQA1*05:05	0	0	0
Subtotal HLA-DQA1	17	13	1
DPB1*05:01	9	6	1
Subtotal HLA-DPB1	9	6	1
DPA1*01:03	0	0	0
Subtotal HLA-DPA1	0	0	0

7. Double click on donor HLA allele or **details** button to open details view.

The screenshot shows the HLA-EMMA software interface with the 'Details' button highlighted in red. The main table is the same as in the previous screenshot, but the 'A*02:01' row is highlighted with a red border, indicating it is the selected allele for the details view. The status bar at the bottom indicates 'Ready' and 'IMGT/HLA alleles (n=20598) NL Population 18-4-2022'.

HLA Allele	Total AA Mismatches	Solvent Accessible AA Mismatches	Allele Mismatches
A*02:01	14	12	1
Subtotal HLA-A	14	12	1
B*07:02	9	6	1
B*35:03	12	6	1
Subtotal HLA-B	19	11	2
C*04:01	11	5	1
C*07:02	1	0	1
Subtotal HLA-C	12	5	2
DRB1*04:01	8	7	1
DRB1*11:04	0	0	1
DRB4*01:03N	0	0	1
Subtotal HLA-DR	8	7	3
DQB1*03:01	0	0	0
DQB1*03:02	2	2	1
Subtotal HLA-DQB1	2	2	1
DQA1*03:01	17	13	1
DQA1*05:05	0	0	0
Subtotal HLA-DQA1	17	13	1
DPB1*05:01	9	6	1
Subtotal HLA-DPB1	9	6	1
DPA1*01:03	0	0	0
Subtotal HLA-DPA1	0	0	0



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8. Details view shows the donor HLA allele and the recipient HLA alleles to which the donor HLA allele was compared to, and the total and solvent accessible amino acid mismatches.
 - a. With **compact**, only the positions and type of amino acid mismatches are shown.
 - b. With **full**, the whole amino acid sequences can be consulted.
 - c. By clicking on a specific amino acid residue, the **properties of the residue** are shown at the top.

Sequence Overview		Residue Properties												
<input type="radio"/> Full <input checked="" type="radio"/> Compact		Position: 66 Solvent Accessibility: Exposed Amino Acid Group: Positive												
Info	HLA Allele	62	66	74	76	77	95	97	105	107	114	116	127	142
Recipient	A*01:01	G	N	D	A	N	I	I	P	G	R	D	N	I
Recipient	A*32:01	G	N	D	E	S	I	M	P	G	Q	D	N	I
Donor	A*02:01	G	K	H	V	D	V	R	S	W	H	Y	K	T
Total AA Mismatches	14													
Solvent Accessible AA Mismatches	12													

Compatibility analysis of a multiple recipient-donor pairs

1. The batch template is provided to you via the github repository (under CaseStudies/SolidOrgan, the file is called **HLA-EMMA practice input file.xlsx**).
2. Prepare the HLA-EMMA template by filling in the HLA typing of the donor and recipient and save it as .xlsx.
 - a. Use unique code to indicate donor and recipient by using R for recipient and D for donor.
 - b. Batch analysis will still run if HLA typing of a locus is missing.
 - c. No output will be generated for a pair if the HLA typing of donor or recipient contains an incorrect HLA typing.

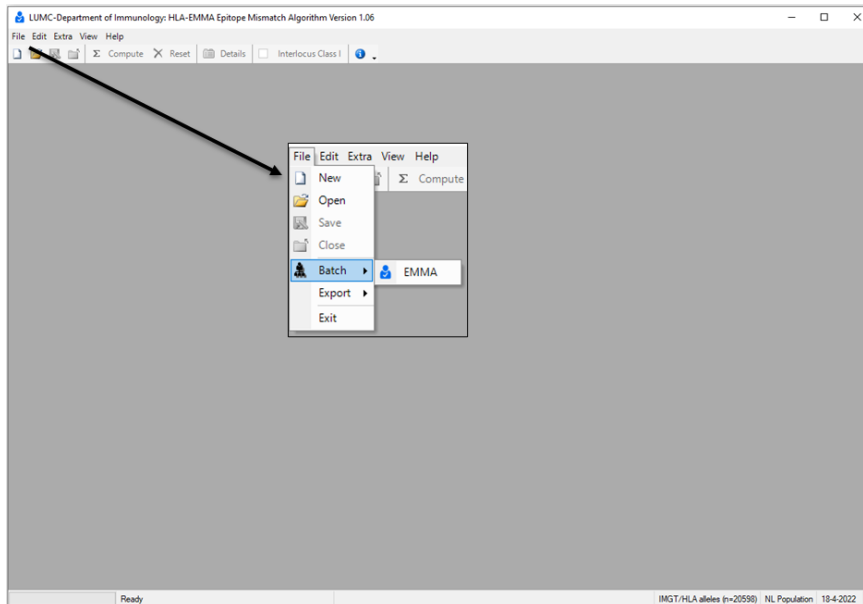
Code	A_1	A_2	B_1	B_2	C_1	C_2	DRB1_1	DRB1_2	DRB3/4/5_1	DRB3/4/5_2	DQB1_1	DQB1_2	DQA1_1	DQA1_2	DPB1_1	DPB1_2	DPA1_1	DPA1_2
R1	A*01:01	A*32:01	B*08:01	B*40:02	C*02:02	C*07:01	DRB1*11:01	DRB1*13:01	DRB3*02:01		DQB1*03:01	DQB1*06:03	DQA1*01:03	DQA1*05:05	DPB1*04:01	DPB1*105:01	DPA1*01:03	
D1	A*02:01		B*07:02	B*35:03	C*04:01	C*07:02	DRB1*04:04	DRB1*11:04	DRB4*01:03N		DQB1*03:19	DQB1*03:02	DQA1*03:01	DQA1*05:05	DPB1*05:01	DPB1*05:01	DPA1*01:03	
R2	A1	A2	B7	B37	Cw6	Cw7	DR8	DR15	DR51		DQ4	DQ6						
D2	A1	A68	B61	B51	Cw2		DR7	DR13	DR52	DR53	DQ9	DQ6						
R3	A*01:01	A*02:686	B*07:02	B*57:01	C*06:02	C*07:02	DRB1*03:01		DRB3*01:01		DQB1*02:01	DQB1*05:01	DQA1*01:01	DQA1*05:01	DPB1*04:01		DPA1*01:03	
D3	A*32:01	A*68:02	B*14:02	B*44:02	C*04:01	C*08:02	DRB1*13:03		DRB3*01:01	DRB4*01:03	DQB1*03:01		DQA1*03:03	DQA1*05:05	DPB1*04:01	DPB1*02:01	DPA1*01:03	

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3. In HLA-EMMA go to file, **batch**, and then click **EMMA**.
 - a. Select the High-Resolution Conversion of choice in case input HLA typing needs to be convert to second field based.



4. Save the batch as .xml file.
5. Go to excel and open your .xml file.
6. Batch output contains the following information:
 - a. Original input
 - b. Number, type, and position of amino acid mismatches
 - c. The type and positions of recipient amino acid of the mismatched positions
 - d. Information on the HLA-EMMA version used for analysis.
 - e. See for more detailed description of batch output at end of user manual.

Code_recipient	Locus_recipient	HLA_allele_recipient	Code_donor	Locus_donor	HLA_allele_donor	Allele_Mismatches	Total_AA_Mismatches	Solvent_Accessibility	Total_AA_Mismatches_Value	Solvent_Accessibility_Value	Profile_recipient	Info
R1	A_1	A*01:01	D1	A_1	A*02:01	1	14	12.626 66K74H 76V 77D 95V 97R 105S 107W 1 62G 66K76V 77D 97R 105S 107W 114H 11 62Q 66N 74D 75A 77N 95V 97R 105P	Version: (1.06), Date: 18-4-2022			
R1	A_2	A*32:01	D1	A_2								Version: (1.06), Date: 18-4-2022
			D1		Subtotal HLA-A:	1	14	12.626 66K74H 76V 77D 95V 97R 105S 107W 1 62G 66K76V 77D 97R 105S 107W 114H 11 62Q 66N 74D 75A 77N 95V 97R 105P	Version: (1.06), Date: 18-4-2022			
R1	B_1	B*08:01	D1	B_1	B*07:02	1	9	6.9V 67Y 68A 70Q 71A 114D 152E 156R 178K 68A 70Q 71A 114D 152E 178K	9D 67F 68T 70N 71T 114N 152V 156D 178T	Version: (1.06), Date: 18-4-2022		
R1	B_2	B*40:02	D1	B_2	B*38:03	1	12	6.9V 24A 48T 94Y 95Y 97R 103L 114D 116F 131S 45T 103L 114D 131S 163L 194V 116Y 131R 163T 194H	9D 24S 48E 94T 96L 97S 103V 114N 116Y 131R 163T 194H	Version: (1.06), Date: 18-4-2022		
R1	C_1	C*02:02	D1	C_1	C*04:01	1	11	11.9V 24A 48T 67Y 68A 70Q 71A 94Y 95Y 97R 102 45T 68A 70Q 71A 103L 114D 131S 152E 163L 178K 194V	1C 9V 11A 14R 49A 99Y 114D 116S 99Y	Version: (1.06), Date: 18-4-2022		
R1	C_2	C*07:01	D1	C_2	C*07:02	1	1	5 10 95 115 14V 49E 99F 114N 116F 156R 219 10 14V 49E 219V 275K	99Y	Version: (1.06), Date: 18-4-2022		
			D1		Subtotal HLA-C:	2	12	5 10 95 115 14V 49E 99F 99S 114N 116F 156R 10 14V 49E 219V 275K		Version: (1.06), Date: 18-4-2022		
R1	DRB1_1	DRB1*11:01	D1	DRB1_2	DRB1*11:04	1	0	0			Version: (1.06), Date: 18-4-2022	
	DRB1_2	DRB1*13:01	D1	DRB1_1	DRB1*04:01	1	8	7 10Q 11V 13H 33H 96Y 96E 120N 130L	10Q 11S 12T 13S 33N 47Y 58E 67F 70D 71R 96H 98K 104S 123S 146H	Version: (1.06), Date: 18-4-2022		
R1	DRB3/4/5_1	DRB3*02:01	D1	DRB3/4/5_1	DRB4*02:03N	1	0	0			Version: (1.06), Date: 18-4-2022	
R1	DRB3/4/5_2		D1	DRB3/4/5_2							Version: (1.06), Date: 18-4-2022	
			D1		Subtotal HLA-DR:	3	8	7 10Q 11V 13H 33H 96Y 96E 120N 130L	10Q 11V 13H 33H 96Y 96E 120N		Version: (1.06), Date: 18-4-2022	
R1	DQB1_1	DQB1*03:01	D1	DQB1_1	DQB1*03:01	0	0	0			57D 185T	Version: (1.06), Date: 18-4-2022
R1	DQB1_2	DQB1*06:03	D1	DQB1_2	DQB1*03:02	1	2	2 57A 185S	57A 185S		Version: (1.06), Date: 18-4-2022	
			D1		Subtotal HLA-DQB1:	1	2	2 57A 185S	57A 185S		Version: (1.06), Date: 18-4-2022	
R1	DQA1_1	DQA1*01:08	D1	DQA1_1	DQA1*03:01	1	17	13 26S 34E 47Q 50L 53R 56R 61F 64T 66 68L 76P 26S 47Q 50L 53R 56R 61F 64T 82S 130S 17 26T 34Q 47R 50E 53K 56G 61G 64R	Version: (1.06), Date: 18-4-2022			
R1	DQA1_2	DQA1*05:06	D1	DQA1_2	DQA1*05:05	0	0	0			Version: (1.06), Date: 18-4-2022	
			D1		Subtotal HLA-DQA1:	1	17	13 26S 34E 47Q 50L 53R 56R 61F 64T 66 68L 76P 26S 47Q 50L 53R 56R 61F 64T 82S 130S 17 26T 34Q 47R 50E 53K 56G 61G 64R	Version: (1.06), Date: 18-4-2022			
R1	DPB1_1	DPB1*04:01	D1	DPB1_1	DPB1*05:01	1	9	6 35L 55E 84D 85E 86A 87V 96K 17Q 205M	84D 85E 86A 87V 96K 205M	35F 55A 84G 85G 86P 87M 96R 17Q	Version: (1.06), Date: 18-4-2022	
R1	DPB1_2	DPB1*105:01	D1	DPB1_2		1					Version: (1.06), Date: 18-4-2022	
			D1		Subtotal HLA-DPB1:	1	9	6 35L 55E 84D 85E 86A 87V 96K 17Q 205M	84D 85E 86A 87V 96K 205M		Version: (1.06), Date: 18-4-2022	
R1	DPA1_1	DPA1*01:03	D1	DPA1_1	DPA1*01:03	0	0	0			Version: (1.06), Date: 18-4-2022	
R1	DPA1_2		D1	DPA1_2							Version: (1.06), Date: 18-4-2022	
			D1		Subtotal HLA-DPA1:	0	0	0			Version: (1.06), Date: 18-4-2022	

Input

Number of AA mismatches

Type and position of AA mismatches

Type and position of recipient AA

Info



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Column header	Description
Code_recipient	Recipient ID
Locus_recipient	ID of recipient allele, corresponds with input file
HLA_allele_recipient	HLA allele of recipient
Code_donor	Donor ID
Locus_donor	ID of donor allele corresponds with input file. Donor alleles are aligned with recipients' alleles. E.g., recipient DQB1_1 is DQB1*03:01 and donor DQB1_2 is DQB1*03:01 then these alleles will be aligned, so you first see DQB1_2 and then DQB1_1 in the output list
HLA_allele_donor	HLA allele of donor.
Allele_Mismatches	Indicates if the donor allele is mismatched with recipient 0 then allele is matched 1 then allele is mismatched
Total_AA_Mismatches	The number of total amino acids mismatches for the donor allele of that row with all recipient alleles of that locus.
Solvent_Accessibility	The number of solvent accessible amino acids mismatches for the donor allele of that row with all recipient alleles of that locus.
Total_AA_Mismatches_Value	The position and type of amino acid that are mismatched
Solvent_Accessibility_Value	The position and type of amino acid that are mismatched
Profile_recipient	The amino acids of the recipient's HLA allele on the mismatched positions, divided by to separate each HLA allele within the locus.
Info	The HLA-EMMA version used for batch analysis, the run date and additional information of batch settings.

7. The batch output can be used for further analysis.

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Example of batch output:

B*39:11 of donor is mismatched, and after comparison with recipients B*08:01 and B*55:01 8 total amino acid mismatches were defined and 1 solvent amino acid mismatch (158T)

The row shows results of this donor allele, the donor allele is compared with both recipient alleles of same locus

Indicates if donor allele is mismatched 1 = yes

Only 1 solvent accessibility amino acid mismatch between donor B*39:11 & recipient B*08:01 and B*55:01
Mismatch is on position 158 and amino acid is T (Threonine)

Both recipients B alleles have an alanine on position 158

Code_recipient	Locus_recipient	HLA_allele_recipient	Code_donor	Locus_donor	HLA_allele_donor	Allele_Mismatches	Total_AA_Mismatches	Solvent_Accessibility	Total_AA_Mismatches_Value	Solvent_Accessibility_Value	Profile_recipient	Info
R1	A_1	A*02:01	D1	A_1	A*02:01	0	0	0	0	0		Version: (1.04), Date:17-12-2020,
R1	A_2	A*24:02	D1	A_2	A*24:02	0	0	0	0	0		Version: (1.04), Date:17-12-2020,
R1	B_1	B*08:01	D1	B_1	B*39:11	1	8	1	11S 12V 67C 74Y 97R 116F 156R 1	158T	11A 12M 67F 74D 97S 116Y 156D 158A 67Y 97T 116L 156L	Version: (1.04), Date:17-12-2020,
R1	B_2	B*55:01	D1	B_2	B*40:08	1	9	4	9H 11S 12V 24T 32L 41T 45K 74Y 132L 41T 45K 163E		9D 11A 12M 24S 32Q 41A 45E 74D 163T	Version: (1.04), Date:17-12-2020,
R1	C_1	C*03:03	D1	C_1	C*03:04	1	0	0				Version: (1.04), Date:17-12-2020,
R1	C_2	C*07:01	D1	C_2	C*07:02	1	1	0.995			99Y	Version: (1.04), Date:17-12-2020,
R1			D1		Subtotal Class I:	4	15	5	9H 11S 12V 24T 32L 41T 45K 67C 732L 41T 45K 158T 163E			Version: (1.04), Date:17-12-2020,
R1	DRB1_1	DRB1*03:01	D1	DRB1_1	DRB1*04:03	1	12	10	10Q 11V 13H 32Y 33H 37Y 71R 74F 10Q 11V 13H 32Y 33H 71R 74E 9	149H 180V 71E 74A	10Y 11S 12T 13S 26Y 32H 33N 37N 47F 71K 73G 74R 77N 96H 98K 104S 120S	Version: (1.04), Date:17-12-2020,
R1	DRB1_2	DRB1*13:02	D1	DRB1_2	DRB1*14:02	1	1	1	71R	71R	26Y 28D 47F 71K 73G 74R 77N 86V 71E	Version: (1.04), Date:17-12-2020,
R1	DRB3/4/5_1	DRB3*01:01	D1	DRB3/4/5_1	DRB3*01:01	0	0	0				Version: (1.04), Date:17-12-2020,
R1	DRB3/4/5_2	DRB3*03:01	D1	DRB3/4/5_2	DRB4*01:03	1	27	19	4Q 10Q 11A 13C 18L 25W 26N 28I 4Q 10Q 11A 13C 18L 25W 31I 32Y 41N 44L 48Q 70R 71R 74E 81Y 96Q 120N			Version: (1.04), Date:17-12-2020,
R1	DQB1_1	DQB1*02:01	D1	DQB1_1	DQB1*03:01	1	5	3	13A 26Y 45E 57D 167H	45E 57D 167H	13G 26L 45G 57A 167R	Version: (1.04), Date:17-12-2020,
R1	DQB1_2	DQB1*03:02	D1	DQB1_2	DQB1*03:02	0	0	0				Version: (1.04), Date:17-12-2020,
R1	DQA1_1	DQA1*03:01	D1	DQA1_1	DQA1*03:01	0	0	0				Version: (1.04), Date:17-12-2020,
R1	DQA1_2	DQA1*05:01	D1	DQA1_2	DQA1*05:03	1	1	1	160S	160S	160A	Version: (1.04), Date:17-12-2020,
R1	DPB1_1	DPB1*01:01	D1	DPB1_1	DPB1*03:01	1	6	3	11L 36V 55D 56E 57D 65L	56E 57D 65L	11G 36A 55A 56A 57E 65I	Version: (1.04), Date:17-12-2020,
R1	DPB1_2	DPB1*04:01	D1	DPB1_2	DPB1*04:02	1	4	1	36V 55D 56E 178M	56E	36A 55A 56A 178L	Version: (1.04), Date:17-12-2020,
R1	DPA1_1	DPA1*01:03	D1	DPA1_1	DPA1*01:03	0	0	0				Version: (1.04), Date:17-12-2020,
R1	DPA1_2	DPA1*02:01	D1	DPA1_2								Version: (1.04), Date:17-12-2020,
R1			D1		Subtotal Class II:	7	45	30	4Q 10Q 11A 11L 11V 13A 13C 13H 4Q 10Q 11A 11V 13C 13H 18L 25W 31I 32Y 33H 41N 44L 45E 48Q 56E 57D			Version: (1.04), Date:17-12-2020,

It can occur that on allele level you have a mismatch but that all amino acids of that allele are present on one or both recipients' HLA allele than you have zero Total AA mismatches and zero Solvent Accessibility mismatches