

Virtual Cross Matching in Practice

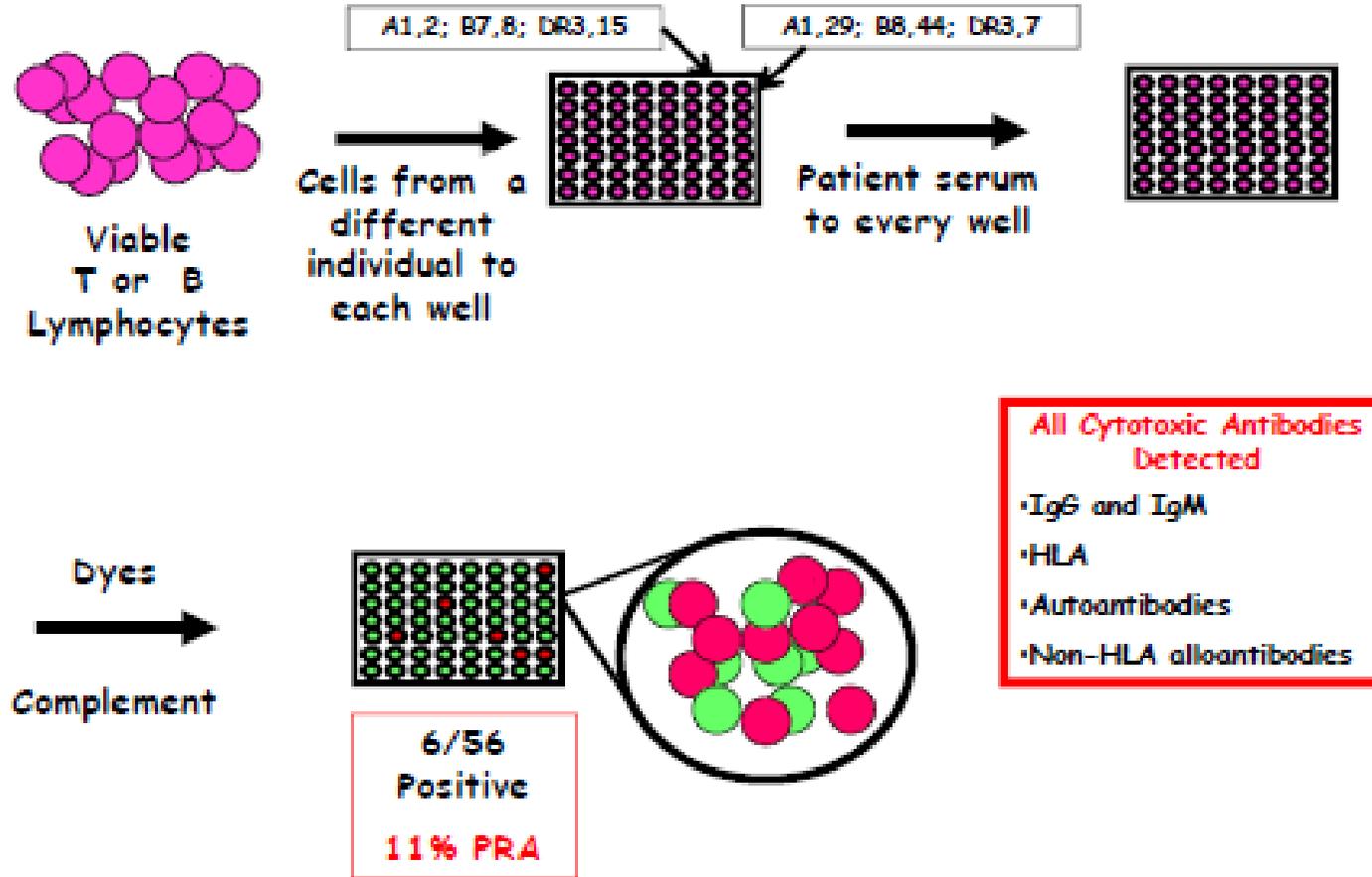
By Yadollah Shakiba
MD, PhD

How we detect of Anti HLA antibodies in IRAN

- Live cell: Cytotoxicity Assay (CDC)
- Solid phase assay: screening or single antigen assay
 - ✓ Elisa
 - ✓ Flow cytometry
 - ✓ Luminex

CDC screening (PRA)

Cytotoxicity Assays for Antibodies

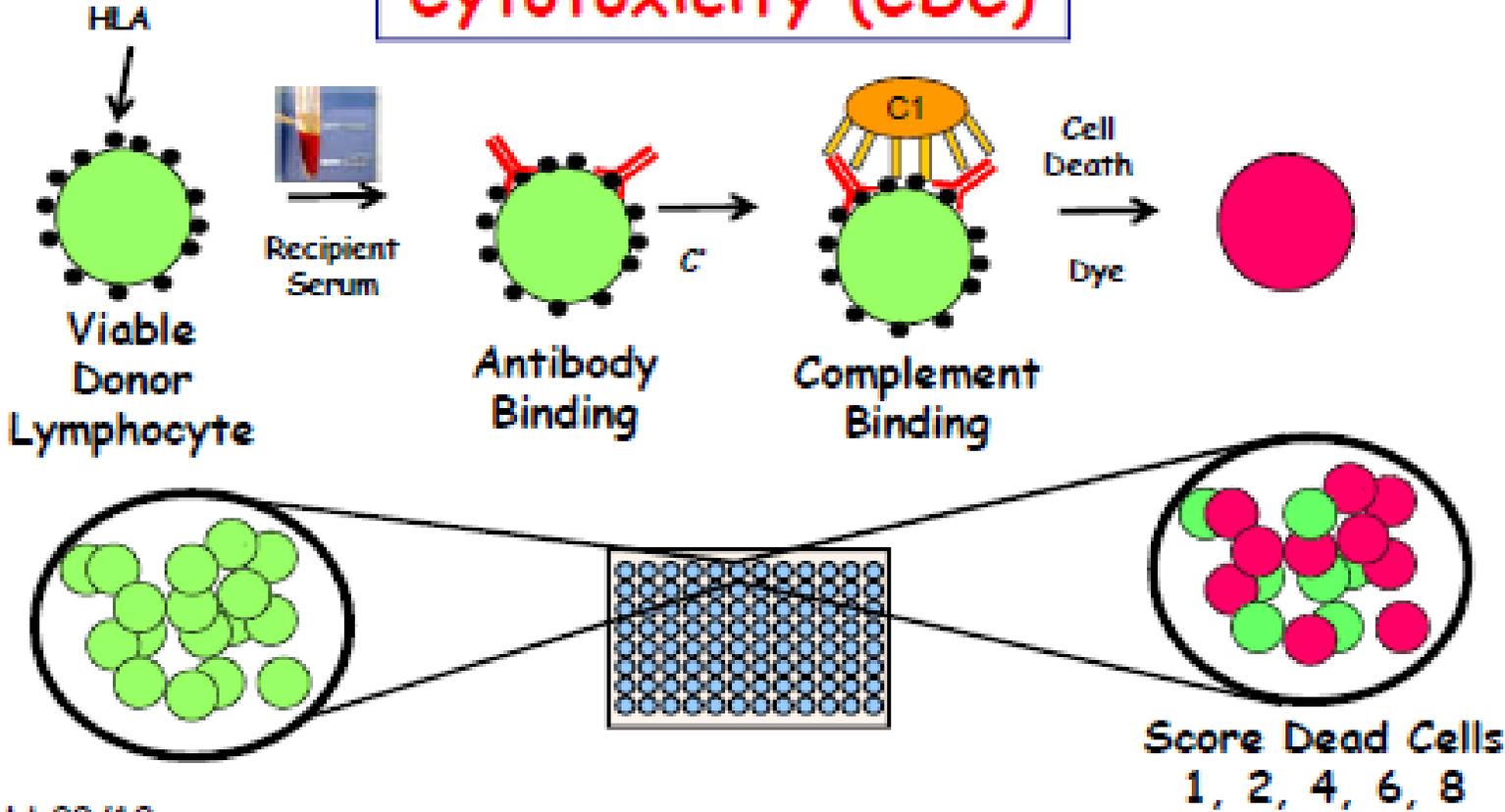


Mostly Class I HLA

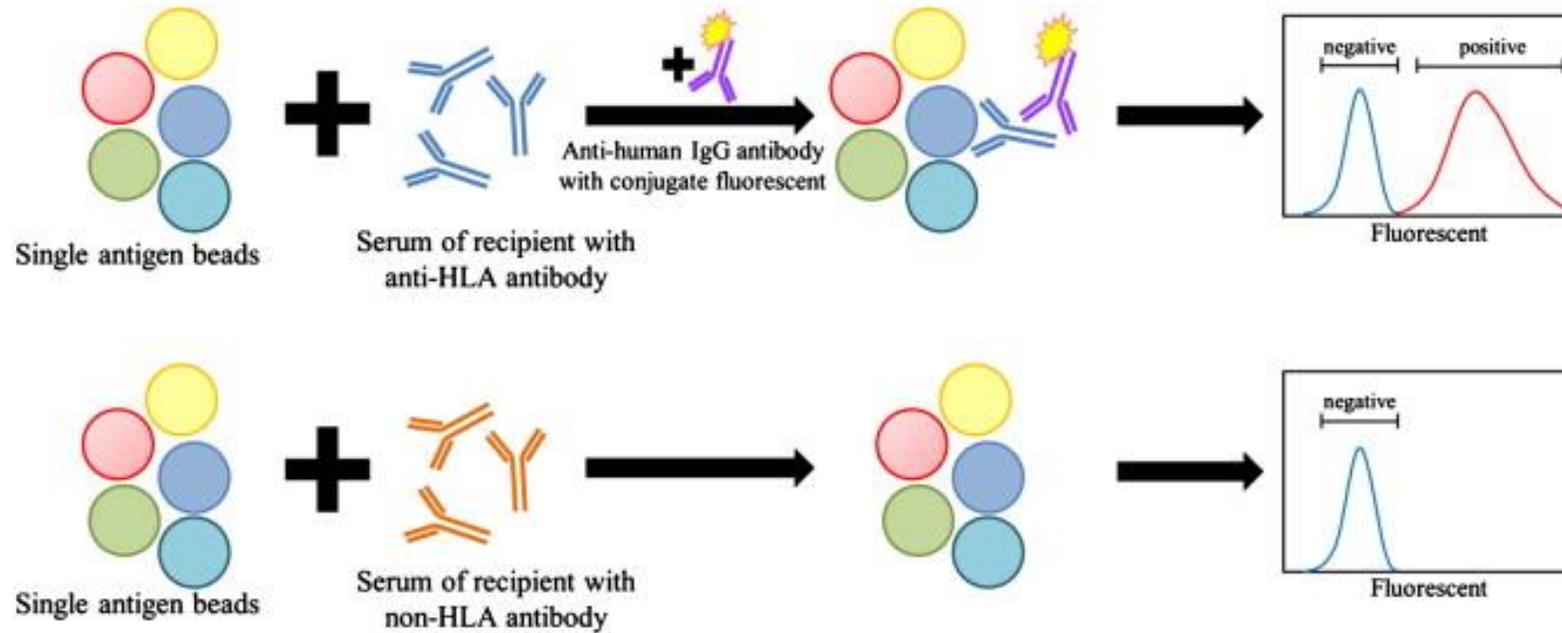
CDC Cross match

Crossmatch Detects HLA Antibodies Donor Cells and Recipient Serum

Cytotoxicity (CDC)

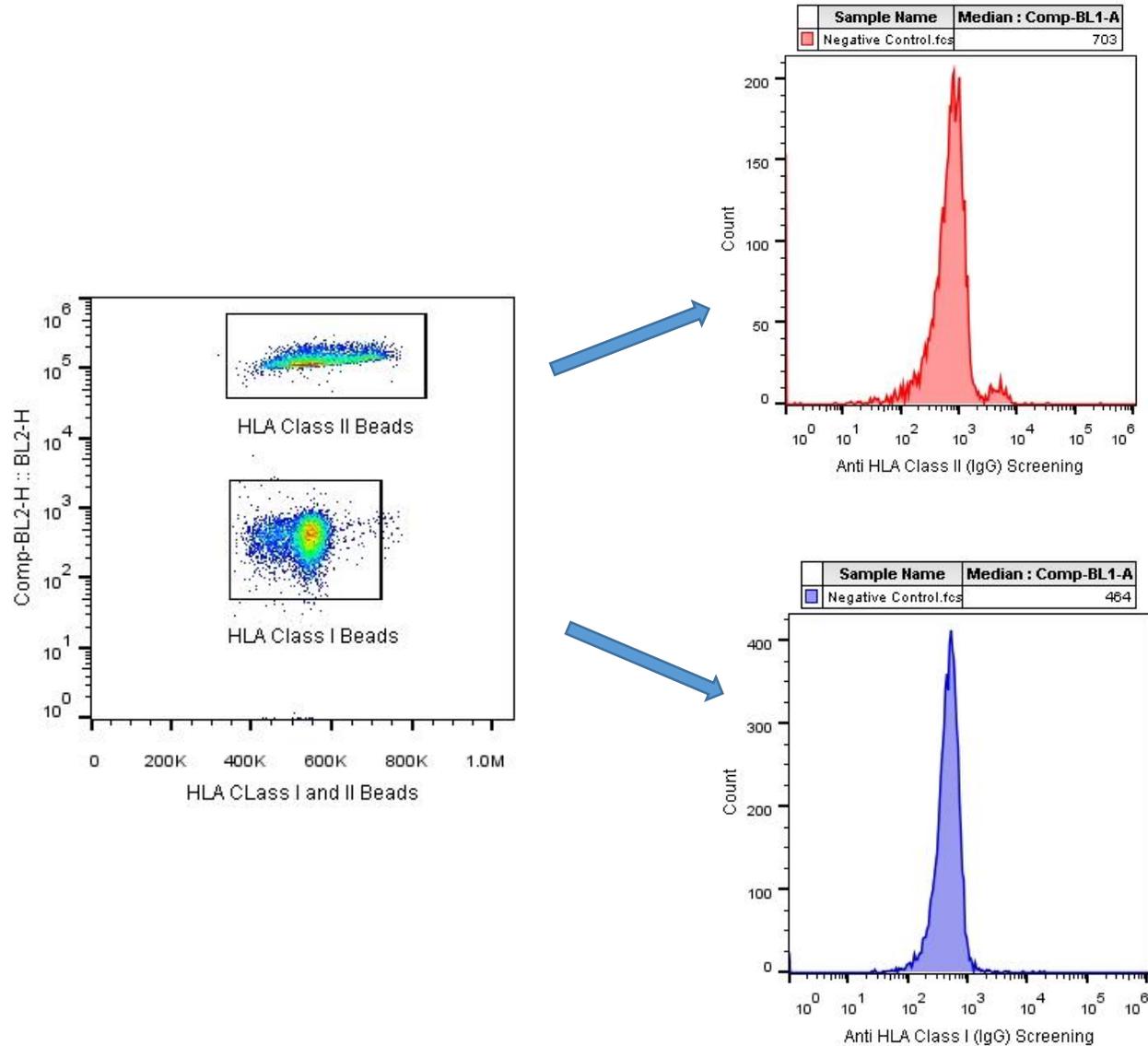


Flow Cytometric PRA

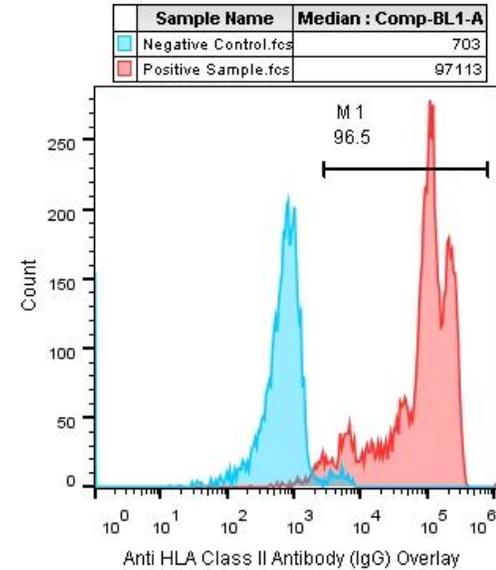
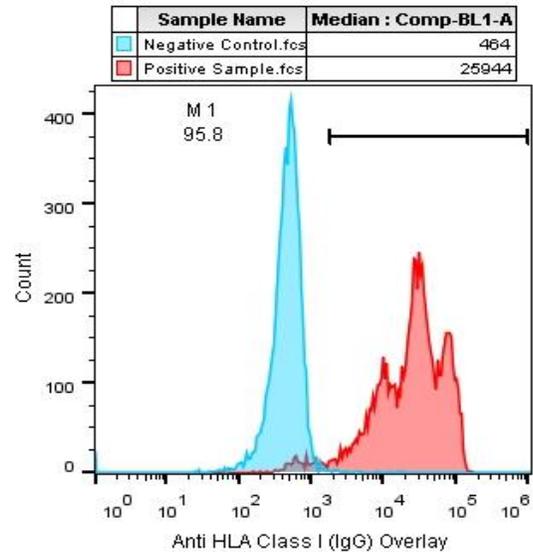
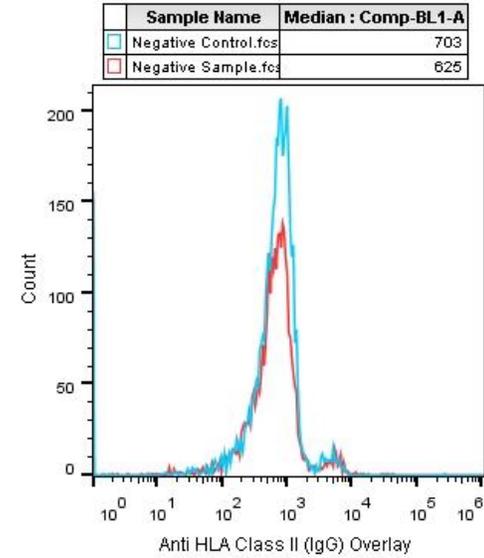
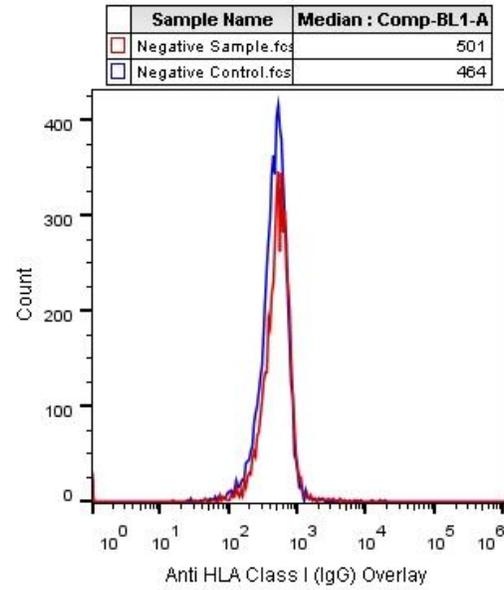


HLA class I	HLA class II
<p>A*01-A*02-A*03-A*11-A*23-A*24-A*25-A*26-A*29-A*30-A*31-A*32-A*33-A*34-A*36-A*66-A*68-A*69-A*74-A*80</p> <p>B*07- B*08- B*13-B*18- B*27-B*35-B*37-B*38-B*39-B*41-B*42-B*44-B*45-B*46-B*47-B*48-B*49- B*50-B*51-B*52-B*53 -B*54-B*55-B*56-B*57-B*58-B*59-B*60-B*61-B*62- B*63-B*64-B*65-B*67-B*71-B*72-B*73-B*75- B*78-B*81-BW4-BW6</p> <p>Cw1-Cw2-Cw4-Cw5-Cw6-Cw7-Cw8-Cw9-Cw10-Cw12-Cw14-Cw15-Cw16-Cw17-Cw18</p>	<p>DRB1*01-DRB1*01:03-DRB1*03:01-DRB1*03:02-DRB1*04-DRB1*07-DRB1*08-DRB1*09-DRB1*10-DRB1*11-DRB1*12-DRB1*13-DRB1*14- DRB1*15-DRB1*16-DRB3-DRB4-DRB5</p> <p>DQB1*02-DQB1*04-DQB1*05-DQB1*06-DQB1*03:01-DQB1*03:02-DQB1*03:03</p> <p>DPB1*01-DPB1*02-DPB1*03-DPB1*04-DPB1*05-DPB1*08-DPB1*09-DPB1*10-DPB1*11-DPB1*13-DPB1*14-DPB1*17-DPB1*18-DPB1*19-DPB1*105-DPB1*20-DPB1*21-DPB1*40</p>

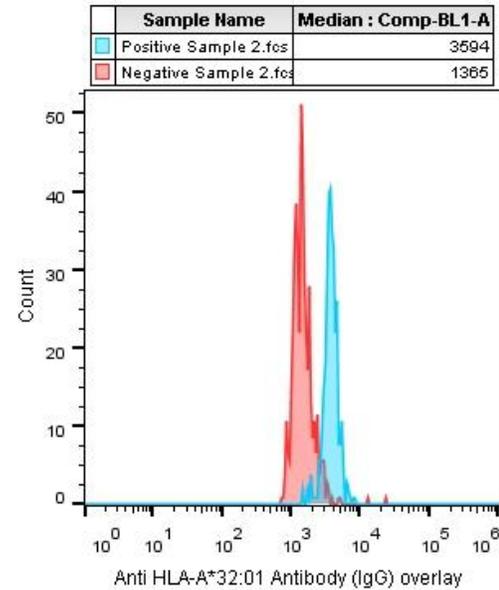
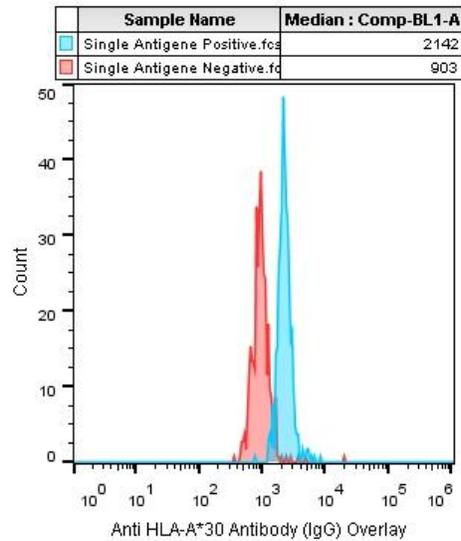
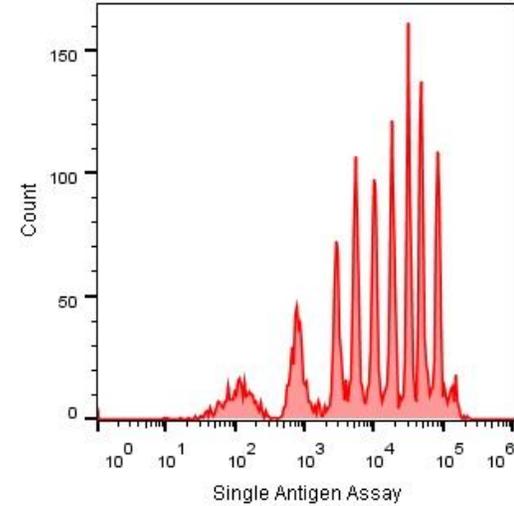
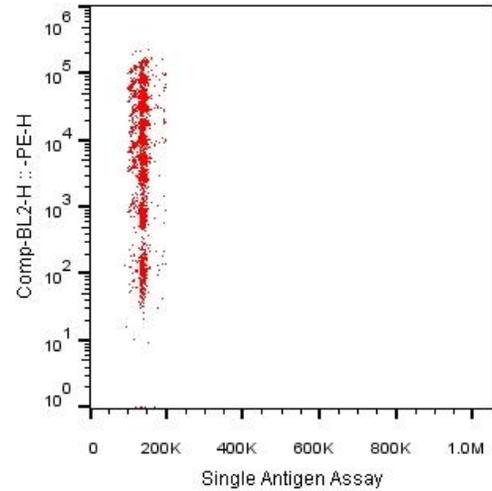
Flow PRA



Results of negative and positive samples



Flow Cytometric Single Antigen Assay



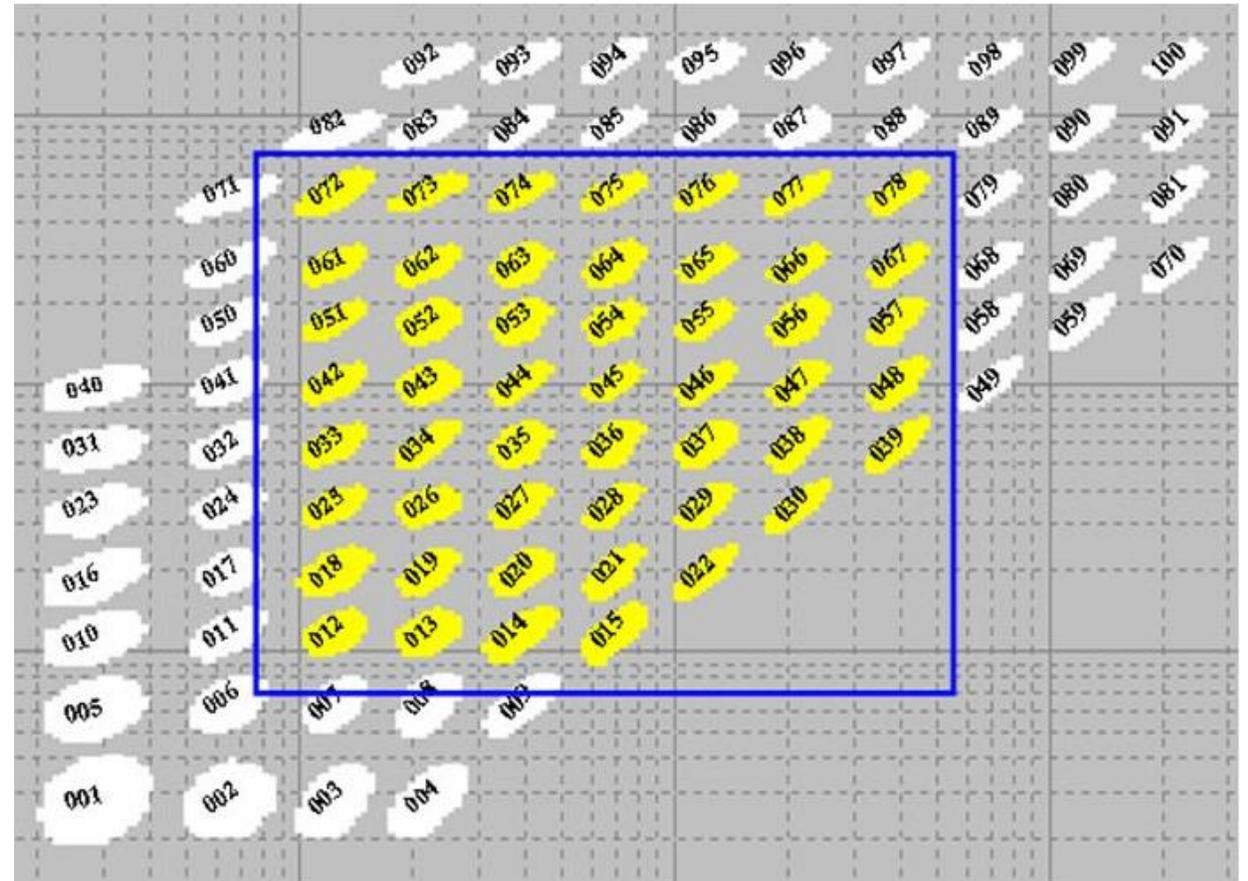
when we use luminex assay

Higher cost than the others

1- HLA antigen is not include in flow beads

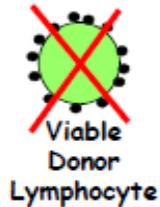
2- DSA is negative but flow XM is positive

Looking for antibody against C or other loci



What we can do with Single antigen data?

Virtual Crossmatch HLA Antibody Data Used to Predict Crossmatch Result



Recipient
HLA Antibodies
A2, A68, A69

Donor 1
A1,2;B8,44;DR2,17

Positive

Donor 2
A1,3;B8,44;DR2,17

Negative

labl 09/10

Calculated PRA (cPRA)

% deceased donors with unacceptable antigens

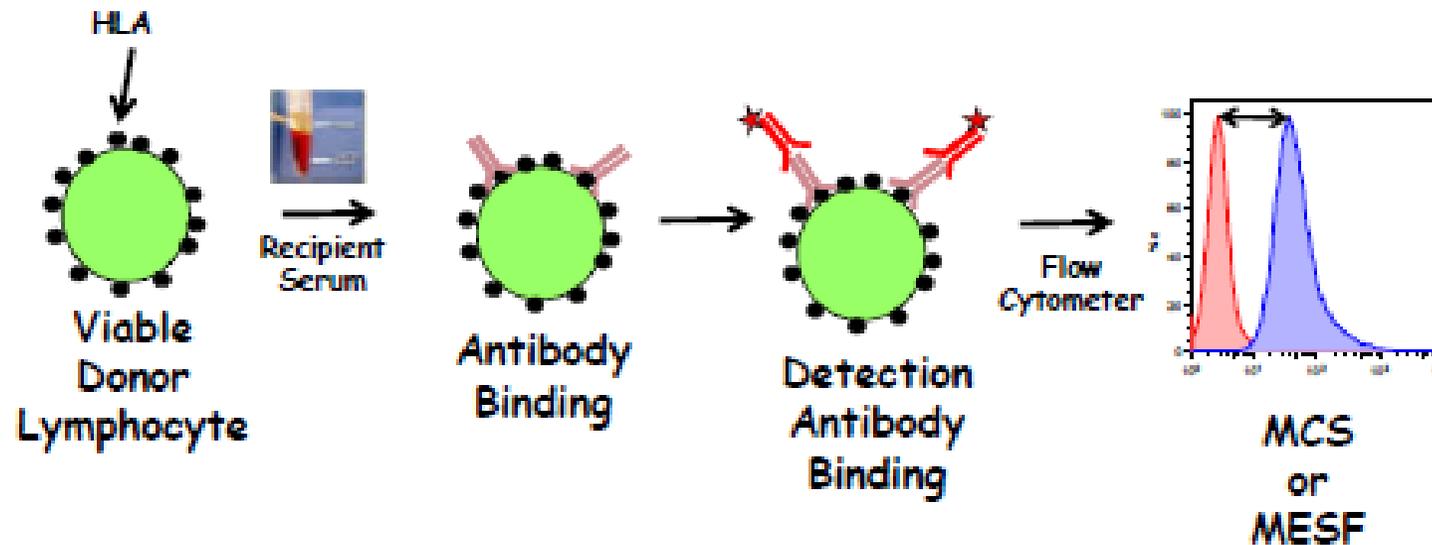
<u>Unacceptable Antigens</u>	<u>cPRA</u>
A2	47%
A2 A68 A69	53%
A80	0%
DR4	29%
A2 DR4	60%
Bw6	80%

labl 09/10

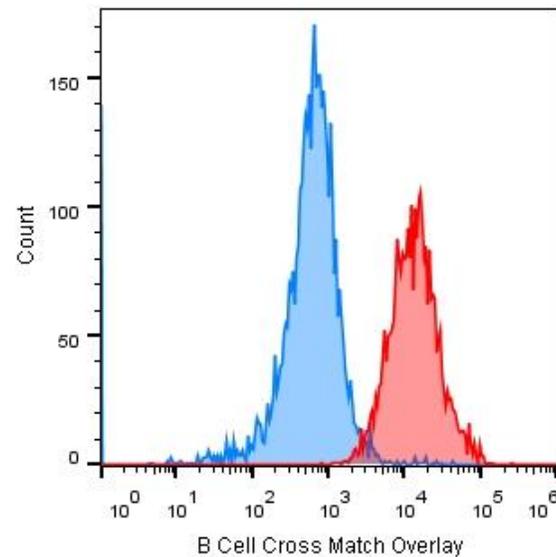
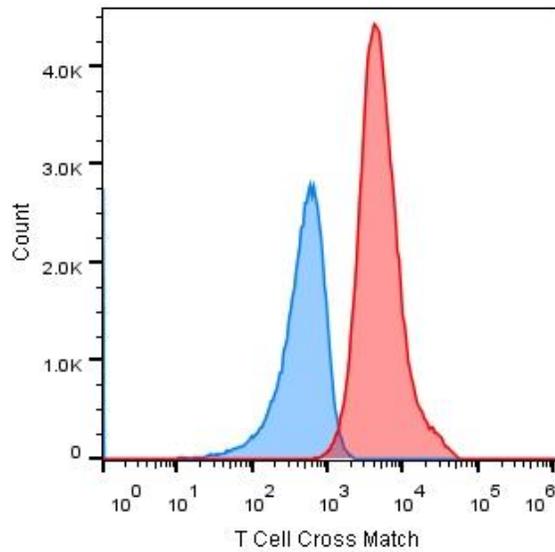
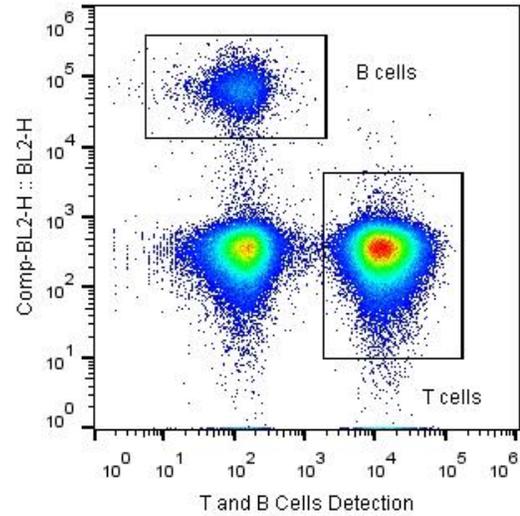
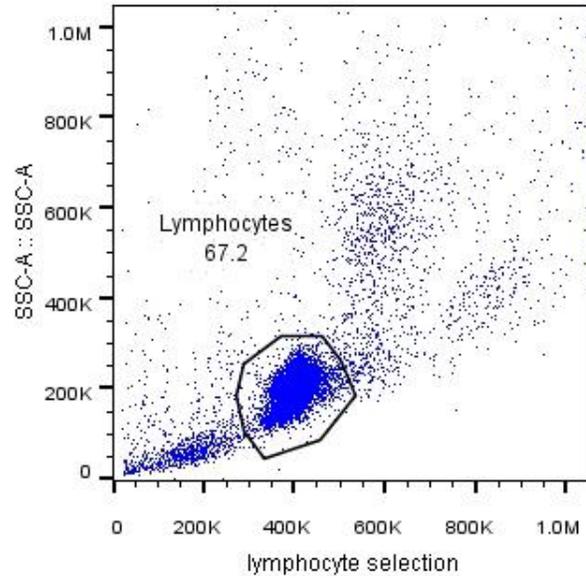
Flow Cytometry cross match

Crossmatch Detects HLA Antibodies Donor Cells and Recipient Serum

Flow Cytometry Crossmatch

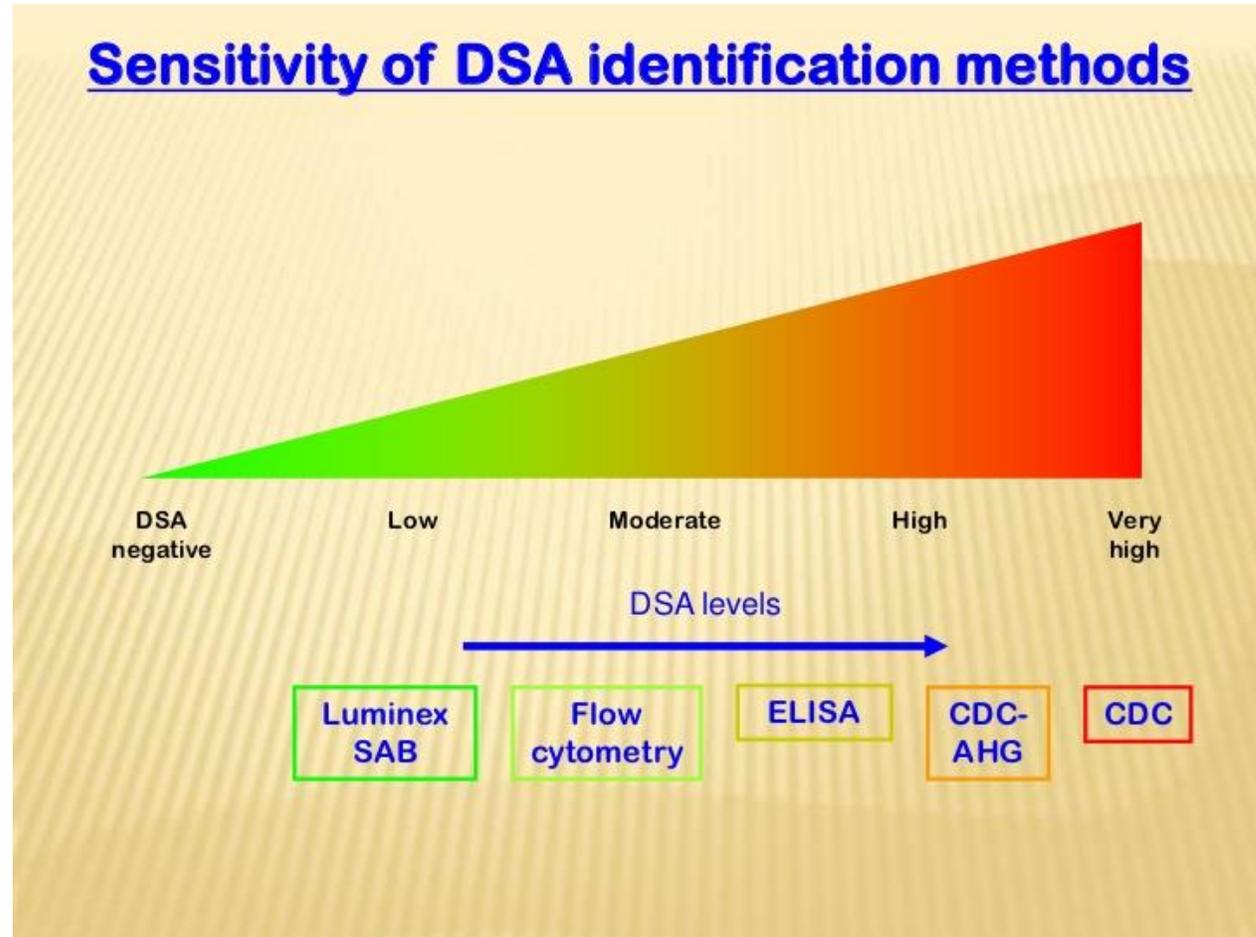


Flow Cytometric cross match



Difference between Anti-HLA antibody detection methods

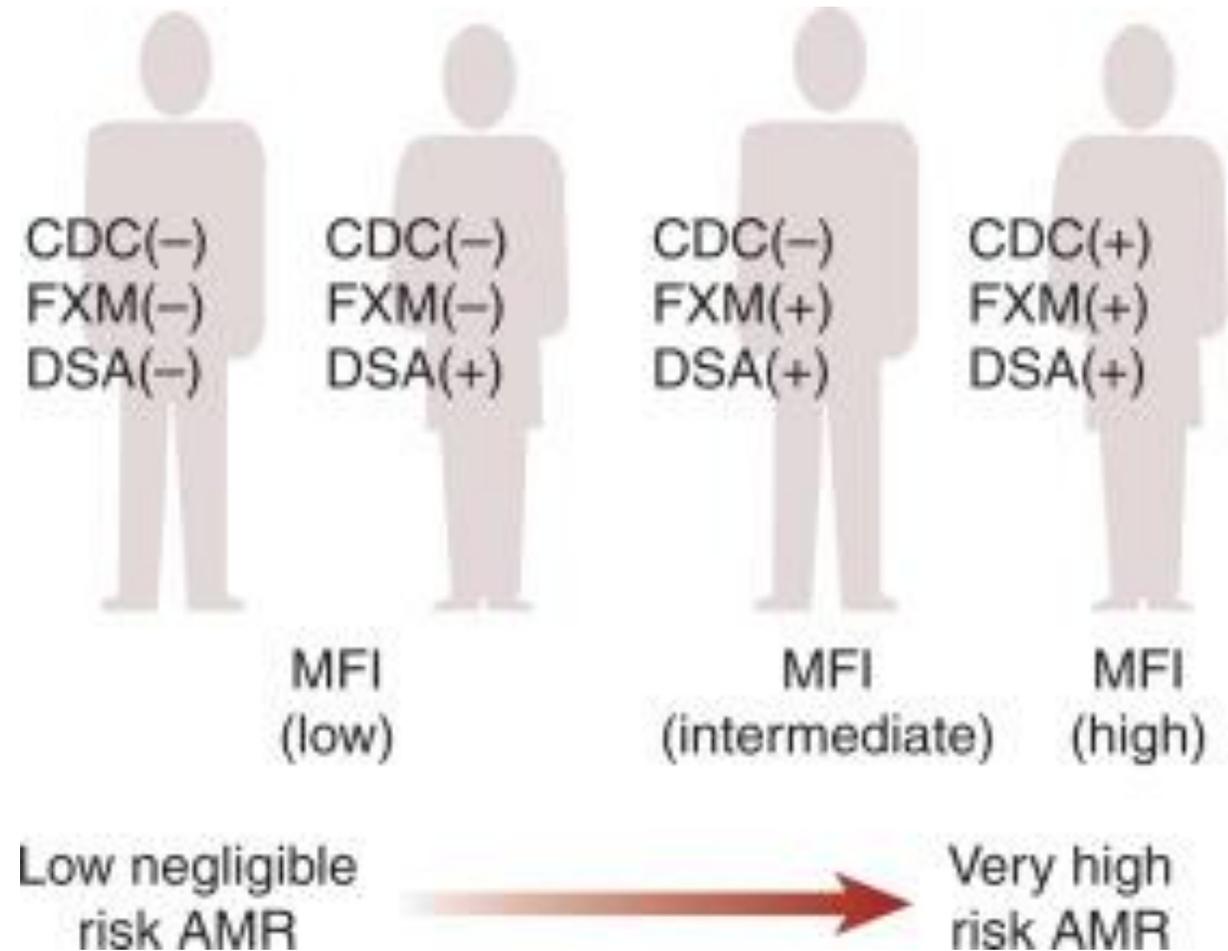
- Live cell: Cytotoxicity Assay (CDC)
- Flow cytometry
- Solid phase assay:
 - screening or single antigen assay (VXM)



Patient Classification based on cross match results

Which category should we select?

- Age of the patient
- cPRA of the patient
- Emergency of the transplant
- Donor limitations
- Desensitized patients



Virtual cross matching in IRAN

- Living related donor
- Living unrelated donor (unique)
- Cadaveric donor

Case 1- Virtual XM in familial transplant

- **32 years old woman**
- **Second time candidate for kidney transplant**
- **Dialysis Status: Positive**
- **Pregnancy: 0**
- **Blood transfusion: Positive**
- **CDC PRA: 55%**

Flow cytometric evaluation

Repeatedly positive flow cross match with living donors

Results:

Anti HLA class I antibody:	Anti HLA class II antibody
94%	77%

Single bead assay and cPRA

A*01:01	0	Low Risk Antigen
A*02:01	20800	High Risk Antigen
A*03:01	32000	High Risk Antigen
B*49:01	17600	High Risk Antigen
A*25:01	275200	High Risk Antigen
A*29:02	20800	High Risk Antigen
A*30:01	36800	High Risk Antigen
A*26:01	73600	High Risk Antigen
A*68:01	102400	High Risk Antigen
A*11:01	64000	High Risk Antigen
A*34:01	89600	High Risk Antigen
A*24:02	200	Low Risk Antigen
A*32:01	38400	High Risk Antigen
A*33:01	30400	High Risk Antigen
A*31:01	44800	High Risk Antigen
A*23:01	3200	High Risk Antigen
B*51:01	100	Low Risk Antigen
B*13:01	400	Low Risk Antigen
B*18:01	19200	High Risk Antigen
B*35:01	38400	High Risk Antigen
B*15:01	128000	High Risk Antigen
B*45:01	6400	High Risk Antigen
B:40:01	100	Low Risk Antigen
B*44:02	0	Low Risk Antigen
B*38:01	400	High Risk Antigen
B*57:01	300	High Risk Antigen
B*07:02	9600	High Risk Antigen
B*52:01	14400	High Risk Antigen
B*27:05	9600	High Risk Antigen
B*08:01	90	High Risk Antigen
B*14:02	0	Low Risk Antigen
B*55:01	0	Low Risk Antigen

DRB1*01:01	100	Low Risk Antigen
DRB1*01:03	100	Low Risk Antigen
DRB1*04:01	100	Low Risk Antigen
DRB1*07:01	0	Low Risk Antigen
DRB1*08:01	100	Low Risk Antigen
DRB1*04:05	50	Low Risk Antigen
DRB1*10:01	100	Low Risk Antigen
DRB1*11:01	0	Low Risk Antigen
DRB1*12:01	0	Low Risk Antigen
DRB1*13:01	0	Low Risk Antigen
DRB1*13:03	200	Low Risk Antigen
DRB1*14:01	50	Low Risk Antigen
DRB1*15:01	40000	High Risk Antigen
DRB1*16:01	52000	High Risk Antigen
DRB1*03:01	200	Low Risk Antigen
DRB1*03:02	0	Low Risk Antigen
DRB5*01:01	9000	High Risk Antigen
DRB3*02:02	0	Low Risk Antigen
DRB4*01:03	100	Low Risk Antigen
DRB1*01:02	0	Low Risk Antigen
DRB1*04:04	100	Low Risk Antigen
DRB1*09:01	0	Low Risk Antigen
DRB1*12:02	100	Low Risk Antigen
NA	-	
DRB1*15:02	0	Low Risk Antigen
DQB1*02:01	12000	High Risk Antigen
DQB1*04:02	0	Low Risk Antigen
DQB1*05:01	100	Low Risk Antigen
DQB1*06:02	150	Low Risk Antigen
DQB1*03:01	200	Low Risk Antigen
DQB1*03:02	10000	High Risk Antigen
DQB1*03:03	250	Low Risk Antigen

cPRA 99%

Table 4. Estimated number of match runs needed to have a 95% probability of finding an acceptable donor based on candidate cPRA	
cPRA, %	Theoretical number of match runs to have a 95% chance of finding an acceptable donor
10	2
20	2
30	3
40	4
50	5
60	6
70	9
80	14
85	19
90	29
95	59
99	300
99.5	600
99.9	3000
99.99	30,000
99.999	300,000

cPRA, calculated panel-reactive antibody.

Searching for familial donor

A*01:01	0	Low Risk Antigen
A*02:01	20800	High Risk Antigen
A*03:01	32000	High Risk Antigen
B*49:01	17600	High Risk Antigen
A*25:01	275200	High Risk Antigen
A*29:02	20800	High Risk Antigen
A*30:01	36800	High Risk Antigen
A*26:01	73600	High Risk Antigen
A*68:01	102400	High Risk Antigen
A*11:01	64000	High Risk Antigen
A*34:01	89600	High Risk Antigen
A*24:02	200	Low Risk Antigen
A*32:01	38400	High Risk Antigen
A*33:01	30400	High Risk Antigen
A*31:01	44800	High Risk Antigen
A*23:01	3200	High Risk Antigen
B*51:01	100	Low Risk Antigen
B*13:01	400	Low Risk Antigen
B*18:01	19200	High Risk Antigen
B*35:01	38400	High Risk Antigen
B*15:01	128000	High Risk Antigen
B*45:01	6400	High Risk Antigen
B*40:01	100	Low Risk Antigen
B*44:02	0	Low Risk Antigen
B*38:01	400	High Risk Antigen
B*57:01	300	High Risk Antigen
B*07:02	9600	High Risk Antigen
B*52:01	14400	High Risk Antigen
B*27:05	9600	High Risk Antigen
B*08:01	90	High Risk Antigen
B*14:02	0	Low Risk Antigen
B*55:01	0	Low Risk Antigen

DRB1*01:01	100	Low Risk Antigen
DRB1*01:03	100	Low Risk Antigen
DRB1*04:01	100	Low Risk Antigen
DRB1*07:01	0	Low Risk Antigen
DRB1*08:01	100	Low Risk Antigen
DRB1*04:05	50	Low Risk Antigen
DRB1*10:01	100	Low Risk Antigen
DRB1*11:01	0	Low Risk Antigen
DRB1*12:01	0	Low Risk Antigen
DRB1*13:01	0	Low Risk Antigen
DRB1*13:03	200	Low Risk Antigen
DRB1*14:01	50	Low Risk Antigen
DRB1*15:01	40000	High Risk Antigen
DRB1*16:01	52000	High Risk Antigen
DRB1*03:01	200	Low Risk Antigen
DRB1*03:02	0	Low Risk Antigen
DRB5*01:01	9000	High Risk Antigen
DRB3*02:02	0	Low Risk Antigen
DRB4*01:03	100	Low Risk Antigen
DRB1*01:02	0	Low Risk Antigen
DRB1*04:04	100	Low Risk Antigen
DRB1*09:01	0	Low Risk Antigen
DRB1*12:02	100	Low Risk Antigen
NA	-	
DRB1*15:02	0	Low Risk Antigen
DQB1*02:01	12000	High Risk Antigen
DQB1*04:02	0	Low Risk Antigen
DQB1*05:01	100	Low Risk Antigen
DQB1*06:02	150	Low Risk Antigen
DQB1*03:01	200	Low Risk Antigen
DQB1*03:02	10000	High Risk Antigen
DQB1*03:03	250	Low Risk Antigen

Rel.	Class I PCR	Class II PCR
Mother	A*11-A*24 B*35-B*51 C*04-C*14	DQB1*03- DQB1*05 DRB1*01-DRB1*11 DRB3
Sibling 1	A*01-A*11 B*14-B*35 C*04-C*08	DQB1*05 DRB1*01
Sibling 2	A*24 B*35-B*51 C*12-C*14	DQB1*03- DQB1*05 DRB1*11-DRB1*14 DRB3
Sibling 3	A*01-A*24 B*14-B*51 C*08-C*14	DQB1*03- DQB1*05 DRB1*01-DRB1*11 DRB3
Patient	A*01-A*24 B*14-B*51 C*08-C*14	DQB1*03- DQB1*05 DRB1*01-DRB1*11 DRB3

Negative cross match with full HLA match sibling

Physician: Dr. Shakiba	Sample ID: 12-1931	Test date: 96/12/20
Recipient Name: Zahra Delfani	Donor name: Amin Delfani	Relationship: related
Recipient gender/age: F/32	Donor gender/age: M/23	Recipient blood group: O+ Donor blood group: O+
Previous transplant: Positive	Blood transfusion: Positive	Number of pregnancy:

Method: **Flow Cytometry**

Donor cells were incubated with recipient serum. Donor B and T cells were separated using specific fluorescent monoclonal antibodies. The presence of recipient IgG antibodies on donors B and T cells evaluated using monoclonal anti- IgG Fc antibody. Shift in median channel fluorescence compared to negative control calculated and reported.

Results:

T cell Median Channel Shift: 10 channels

B cell Median Channel Shift: 16 channels

Negative T and B cell cross match.

Case 2- Finding a DSA negative Donor with VXM between living unrelated donors

- Registering recipients and Donors with previous positive XM (CDC or Flow cytometry) in recent days
- Performing manual virtual cross match
- Finding an ABO compatible with acceptable antigen for Recipient
- Informing patients nephrologist.
- Communication with responsible NGO for Allocation
- Flow cytometric cross match
- Transplant

Virtual cross matching with living unrelated donors

Physician: Dr. Einollahi	Sample ID: 03-1745	Test date: 97/03/23
Name: Sobhan	Sex/age: M/36	Dialysis Status: Positive
Previous transplant: Positive	Blood transfusion: Positive	Number of pregnancy: - Blood Group: B+

Anti HLA class I antibody:	Anti HLA class II antibody
5%	80%

Negative CDC PRA

Sensitized patient.

Recommendations:

- **Anti HLA class II antibody single antigen assay for determination of donor specific antibodies.**
- **Flow Cytometric cross match before transplant.**
- **HLA typing of donor before transplant.**

Antibody identification

Reactive Antigens	
High Risk Antigens (MFI >1000)	DRB1*09:01- DQB1*02:01- DQB1*04:02- DQB1*05:01- QB1*06:02
Moderate Risk Antigens (MFI 500-1000)	
Calculated PRA: 85%. High chance to find a suitable donor. HLA typing of potential donors and virtual cross matching is recommended.	

DRB1*09:01	X	1000	High Risk Antigen
DQB1*02:01	X	3500	High Risk Antigen
DQB1*04:02	X	10100	High Risk Antigen
DQB1*05:01	X	32600	High Risk Antigen
DQB1*06:02	X	16950	High Risk Antigen

Name	Rel.	Class I PCR	Class II PCR
سبحان	Recipient	A*24-A*32	DQB1*03
		B*27-B*51	DRB1*04
		C*02- C*14	DRB4

Table1. HLA class I allele frequencies in Iranian Cord Blood Donors

Allele	N	Frequency (%)	Allele	N	Frequency (%)
A*01	111	9.25	B*07	57	4.75
A*02	218	18.16	B*08	51	4.25
A*03	145	12.08	B*13	47	3.91
A*11	125	10.41	B*14	36	3
A*23	27	2.25	B*15	38	3.16
A*24	197	16.41	B*18	52	4.33
A*26	82	6.83	B*27	31	2.58
A*29	30	2.5	B*35	260	21.66
A*30	56	4.66	B*37	12	1
A*31	74	6.16	B*38	52	4.33
A*32	68	5.66	B*39	11	0.91
A*33	44	3.66	B*40	43	3.58
A*43	2	0.16	B*41	33	2.75
A*66	3	0.25	B*42	2	0.165
A*68	50	4.16	B*44	50	4.165
A*69	2	0.16	B*45	2	0.165
A*74	5	0.41	B*47	2	0.165
A*80	2	0.16	B*48	6	0.50
			B*49	30	2.50
			B*50	43	3.58
			B*51	160	13.33
			B*52	42	3.50
			B*53	3	0.25
			B*54	2	0.165
			B*55	42	3.50
			B*56	6	0.50
			B*57	12	1.00
			B*58	23	1.915

How many match runs do we need?

Table 4.

Estimated number of match runs needed to have a 95% probability of finding an acceptable donor based on candidate cPRA

cPRA, %	Theoretical number of match runs to have a 95% chance of finding an acceptable donor
10	2
20	2
30	3
40	4
50	5
60	6
70	9
80	14
85	19
90	29
95	59
99	300
99.5	600
99.9	3000
99.99	30,000
99.999	300,000

Calculated PRA 85%

[Clin J Am Soc Nephrol](#). 2016 Apr 7; 11(4): 684–693.

living unrelated donor VXM

Reactive Antigens	
High Risk Antigens (MFI >1000)	DRB1*09:01- DQB1*02:01- DQB1*04:02- DQB1*05:01- DQB1*06:02

DRB1*09:01	1000	High Risk Antigen
DQB1*02:01	3500	High Risk Antigen
DQB1*04:02	10100	High Risk Antigen
DQB1*05:01	32600	High Risk Antigen
DQB1*06:02	16950	High Risk Antigen

Name	Rel.	Class I PCR	Class II PCR
صديقه	Donor	A*02-A*26 B*08-B*40 C*03- C*07	DQB1*03- DQB1*05 DRB1*09-DRB1*10 DRB4

T cell Median Channel Shift: 13 channels

B cell Median Channel Shift: 200 channels

Negative T cell cross match. Positive B cell cross match.

The presence of class II DSA is likely. Transplant is not recommended

VXM between living unrelated donors

Reactive Antigens	
High Risk Antigens (MFI >1000)	DRB1*09:01- DQB1*02:01- DQB1*04:02- DQB1*05:01- DQB1*06:02

Name	Rel.	Class I PCR	Class II PCR
سبحان	Recipient	A*24-A*32 B*27-B*51 C*02- C*14	DQB1*03 DRB1*04 DRB4

Name	Rel.	Class I PCR	Class II PCR
سارا	Donor	A*03-A*32 B*18-B*35 C*04- C*12	DPB1*02- DPB1*04 DQB1*03 DRB1*07-DRB1*11 DRB3- DRB4

T cell Median Channel Shift: 8 channels

B cell Median Channel Shift: 256 channels

Negative T cell cross match. Positive B cell cross match. Because of previous history of Rituximab infusion, B cell cross match result is not reliable. For more clarification single antigen assay of recipient, HLA typing of donor and virtual cross matching is recommended.

Case 3- Virtual cross match with living unrelated donor

Physician: Dr.	Sample ID: 03-585	Test date: 97/03/7
Name: Noori	Sex/age: M/28	Dialysis Status: Positive
Previous transplant: positive	Blood transfusion: Negative	Number of pregnancy: - Abortion:-

CDC PRA: 10%

Multiple Flow XM with donors

Anti HLA class I antibody:	Anti HLA class II antibody
34%	33%

Sensitized patient.

Recommendations:

- 1. Anti HLA class I and II antibody single antigen assay for determination of donor specific antibodies.**
- 2. Flow Cytometric cross match before transplant.**

HLA typing of donor before transplant

Single antigen assay

Reactive Antigens	
High Risk Antigens (MFI >1000)	A*03:01- A*11:01- A*30:01- A*31:01- A*32:01- B*44:02- B*45:01
High Risk Antigens (MFI >1000)	DQB1*03:02
Moderate Risk Antigens (MFI 500-1000)	DRB1*10:01

A*03:01	2600	High Risk Antigen
A*30:01	1370	High Risk Antigen
A*11:01	1700	High Risk Antigen
A*32:01	5950	High Risk Antigen
A*31:01	3050	High Risk Antigen
B*45:01	4900	High Risk Antigen
B*44:02	1950	High Risk Antigen
DRB1*10:01	400	Moderate Risk Antigen
DQB1*03:01	280	Low Risk Antigen
DQB1*03:02	750	Moderate Risk Antigen
DQB1*03:03	220	Low Risk Antigen
DPB1*01:01,02:01,03:01,04:01,05:01,11:01,13:01,17:01	2000	Low Risk Antigen

Table 4.

Estimated number of match runs needed to have a 95% probability of finding an acceptable donor based on candidate cPRA

cPRA, %	Theoretical number of match runs to have a 95% chance of finding an acceptable donor
10	2
20	2
30	3
40	4
50	5
60	6
70	9
80	14
85	19
90	29
95	59
99	300
99.5	600
99.9	3000
99.99	30,000
99.999	300,000

Calculated class I and II PRA: 84%. Moderate chance to find a suitable donor. In order to increase transplantability, HLA typing of potential donors and virtual cross matching is recommended.

DSA negative donor

Reactive Antigens	
High Risk Antigens (MFI >1000)	A*03:01- A*11:01- A*30:01- A*31:01- A*32:01- B*44:02- B*45:01
High Risk Antigens (MFI >1000)	DQB1*03:02
Moderate Risk Antigens (MFI 500-1000)	DRB1*10:01

CREG	Antigens included
A1C	A1, 3, 11 , 19 (29, 30, 31), 36, 80
A2	A2, 9 (23, 24), 28 (68, 69), B17 (57, 58)
A10C	A10 (25, 26, 34, 66), 32, 33, 43, 74
BW4	A9 (23, 24), 25, 32 , B13, 27, 37, 38, 44 , 47, 49, 51 , 52, 53, 57, 58, 59, 63, 77
B5C	B5 (51, 52.) 18, 35, 53
B5C2	B5 (51, 52), 15 (62, 63, 71, 72, 75, 76, 77), 17 (57, 58), 21 (49, 50), 35, 53, 73, 78
BW6	B7, 8, 14, 18, 35, 39, 40 (60, 61), 41, 42, 45 , 46, 48, 50 , 54, 55, 56, 62, 64, 65, 67, 71, 72, 73, 75, 76
B7C	B7, 8, 13, 27, 41, 42, 47, 48, 54, 55, 56, 60, 61, 81
B8C	B8, 18, 38, 39, 64, 65
B12C	B12 (44, 45), 13, 37, 41, 47, 21 (49, 50), 40 60, 61)

Name	Rel.	Class I PCR	Class II PCR
نوری	Recipient	A*24-A*26	DPB1*02- DPB1*04
		B*35-B*39	DQB1*03- DQB1*05
		C*04- C*12	DRB1*11-DRB1*16
پیمان	Donor	A*24-A*26	DPB1*04- DPB1*105
		B*50-B*51	DQB1*02- DQB1*03
		C*06- C*15	DRB1*07-DRB1*11
			DRB3- DRB4

Is DQB1*03 a DSA or not?

High resolution typing of DQB1

Reactive Antigens	
High Risk Antigens (MFI >1000)	A*03:01- A*11:01- A*30:01- A*31:01- A*32:01- B*44:02- B*45:01
High Risk Antigens (MFI >1000)	DQB1*03:02
Moderate Risk Antigens (MFI 500-1000)	DRB1*10:01

Name	Rel.	Class I PCR	Class II PCR
نوری	Recipient		DQB1*03:01- DQB1*05:02
پیمان	Donor		DQB1*02:02- DQB1*03:01

T cell Median Channel Shift: 20 channels

B cell Median Channel Shift: 32 channels

Negative T and B cell cross match.

Case 4- Virtual cross match with cadaveric donor

- **23 years old woman**
- **Third time candidate for kidney transplant**
- **Dialysis Status: Positive**
- **Pregnancy: 0**
- **Blood transfusion: Positive**
- **CDC PRA: 25%**

Flow cytometric evaluation

Results:

Anti HLA class I antibody:	Anti HLA class II antibody
45%	2%

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Reactive Antigens	
High Risk Antigens (MFI >1000)	A*02:01- A*23:01- A*24:02- A*68:01- B*57:01
Moderate Risk Antigens (MFI 500-1000)	

]

CREG	Antigens included
A1C	A1, 3, 11, 19 (29, 30, 31), 36, 80
A2	A2, 9 (23, 24), 28 (68, 69), B17 (57, 58)
A10C	A10 (25, 26, 34, 66), 32, 33, 43, 74
BW4	A9 (23, 24), 25, 32, B13, 27, 37, 38, 44, 47, 49, 51, 52, 53, 57, 58, 59, 63, 77
B5C	B5 (51, 52.) 18, 35, 53
B5C2	B5 (51, 52), 15 (62, 63, 71, 72, 75, 76, 77), 17 (57, 58), 21 (49, 50), 35, 53, 73,78
BW6	B7, 8, 14, 18, 35, 39, 40 (60, 61), 41, 42, 45, 46, 48, 50, 54, 55, 56, 62, 64, 65, 67, 71, 72, 73, 75, 76
B7C	B7, 8, 13, 27, 41, 42, 47, 48, 54, 55, 56, 60, 61, 81
B8C	B8, 18, 38, 39, 64, 65
B12C	B12 (44, 45), 13, 37, 41, 47, 21 (49, 50), 40 60, 61)

Calculated PRA

Calculated PRA: 73%

Table 4. Estimated number of match runs needed to have a 95% probability of finding an acceptable donor based on candidate cPRA

cPRA, %	Theoretical number of match runs to have a 95% chance of finding an acceptable donor
10	2
20	2
30	3
40	4
50	5
60	6
70	9
80	14
85	19
90	29
95	59
99	300
99.5	600
99.9	3000
99.99	30,000
99.999	300,000

cPRA, calculated panel-reactive antibody.

Virtual cross match with donor

Class I PCR	Class II PCR
A*30-A*31	DQB1*02- DQB1*03
B*44-B*53	DRB1*03-DRB1*04
C*04- C*07	DRB3- DRB4

CREG	Antigens included
A1C	A1, 3, 11, 19 (29, 30, 31), 36, 80
A2	A2, 9 (23, 24), 28 (68, 69), B17 (57, 58)
A10C	A10 (25, 26, 34, 66), 32, 33, 43, 74
BW4	A9 (23, 24), 25, 32, B13, 27, 37, 38, 44, 47, 49, 51, 52, 53, 57, 58, 59, 63, 77
B5C	B5 (51, 52.) 18, 35, 53
B5C2	B5 (51, 52), 15 (62, 63, 71, 72, 75, 76, 77), 17 (57, 58), 21 (49, 50), 35, 53, 73,78
BW6	B7, 8, 14, 18, 35, 39, 40 (60, 61), 41, 42, 45, 46, 48, 50, 54, 55, 56, 62, 64, 65, 67, 71, 72, 73, 75, 76
B7C	B7, 8, 13, 27, 41, 42, 47, 48, 54, 55, 56, 60, 61, 81
B8C	B8, 18, 38, 39, 64, 65
B12C	B12 (44, 45), 13, 37, 41, 47, 21 (49, 50), 40 60, 61)

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Reactive Antigens	
High Risk Antigens (MFI >1000)	A*02:01- A*23:01- A*24:02- A*68:01- B*57:01
Moderate Risk Antigens (MFI 500-1000)	

No DSA no CREG specific antibody

Cadaveric donor

Physician: Dr. Shakiba	Sample ID: 10-2247	Test date: 96/10/24
Recipient Name: Fateme Chob Bor	Donor name: cadaveric donor	Relationship: Unrelated
Recipient gender/age: F/23	Donor gender/age: ?	Recipient blood group: ? Donor blood group: ?
Previous transplant: Positive	Blood transfusion: Positive	Number of pregnancy: -

Results:

T cell Median Channel Shift: 20 channels

B cell Median Channel Shift: 90 channels

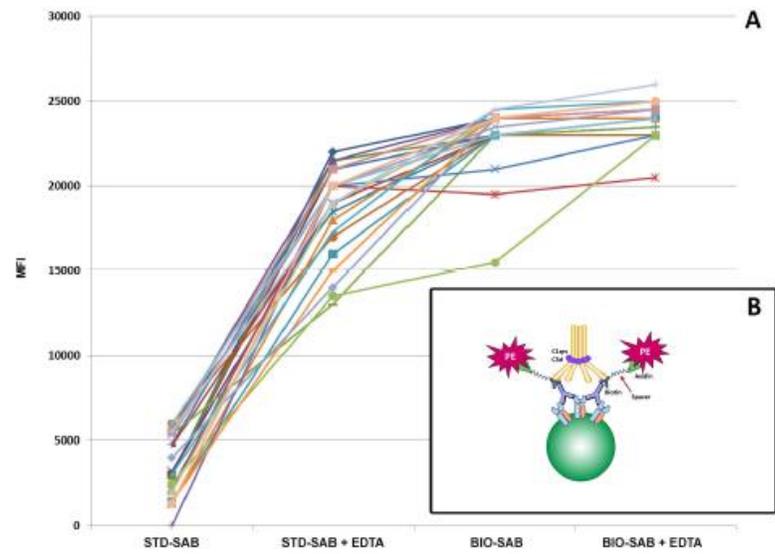
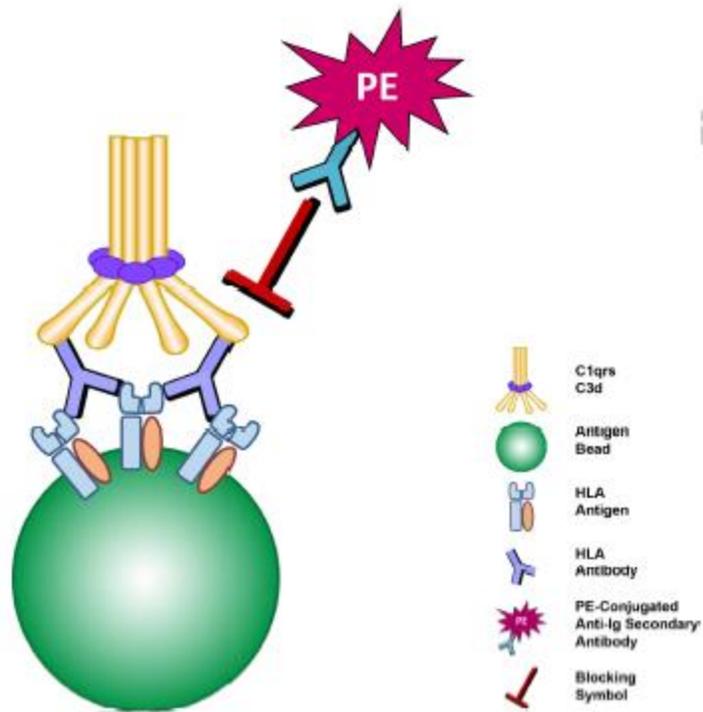
Negative T cell cross match. Positive B cell cross match.

Based on recipient single antigen assay and donor HLA typing, patient has no DSA. Positive B cell cross match could be due to previous Rituximab therapy in recipient.

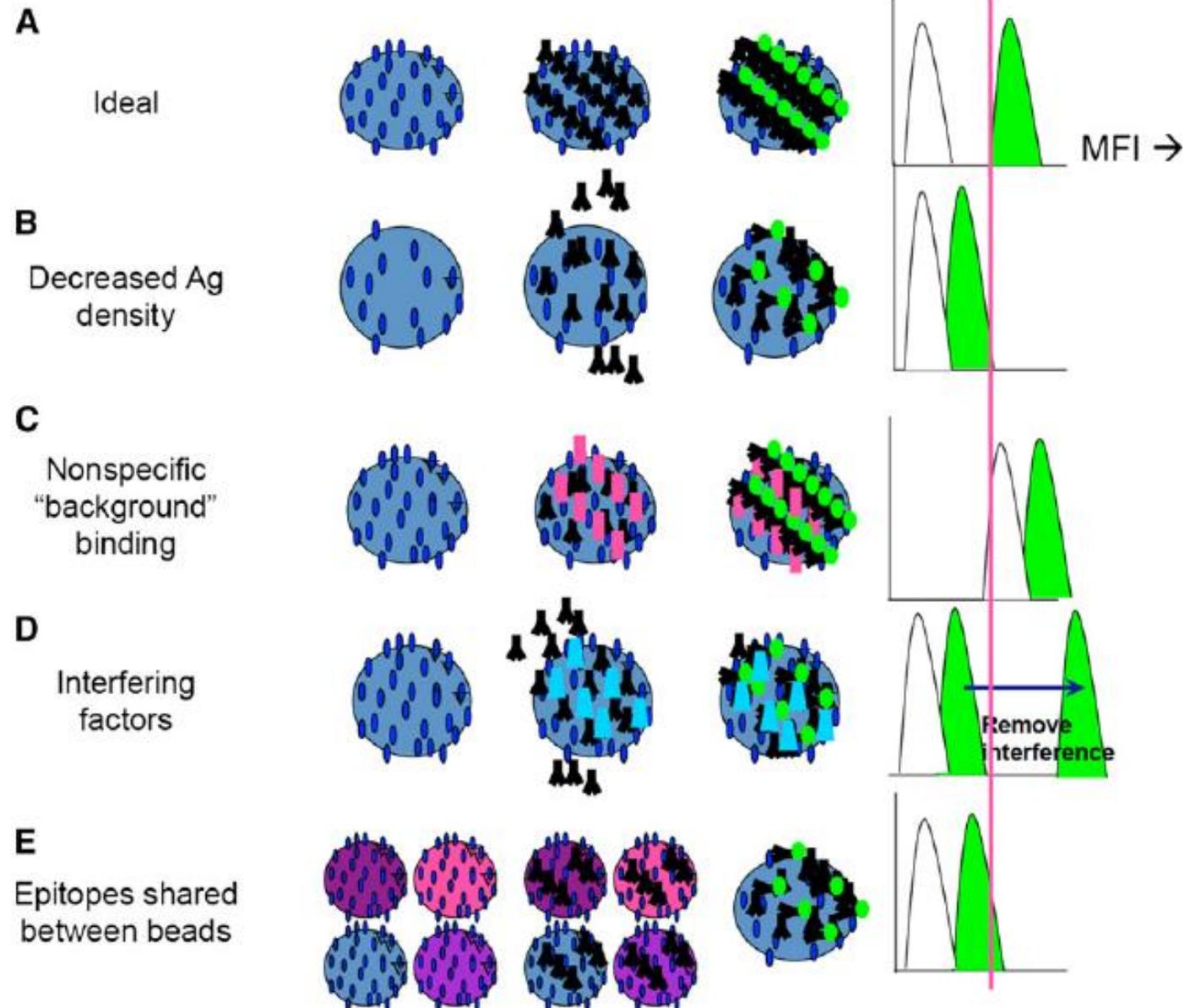
Prozone effect in Anti-HLA Antibody Assay

A case study

ACCEPTED



Solid phase assays limitations

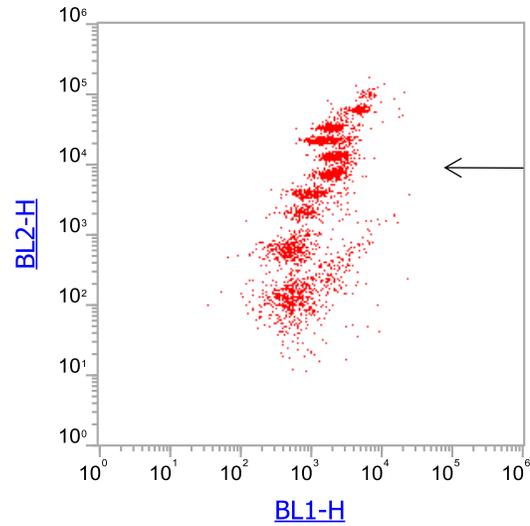


Why we encounter prozone effect?

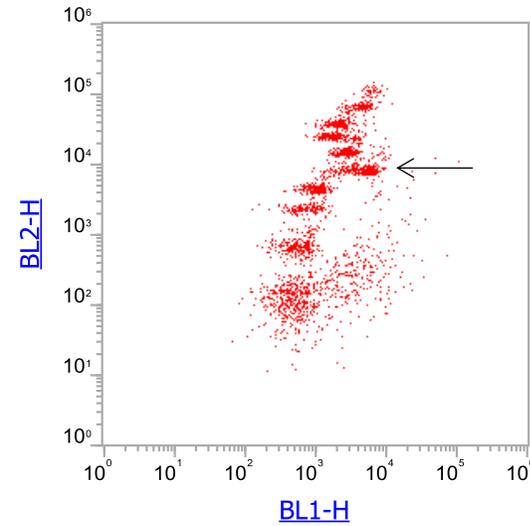
- High titer IgG interference attachment
- There is anti HLA IgM antibody in serum of highly sensitized patient.
- High titer Ab activates Complement (bad sign)

Diluted Single Antigen Class I

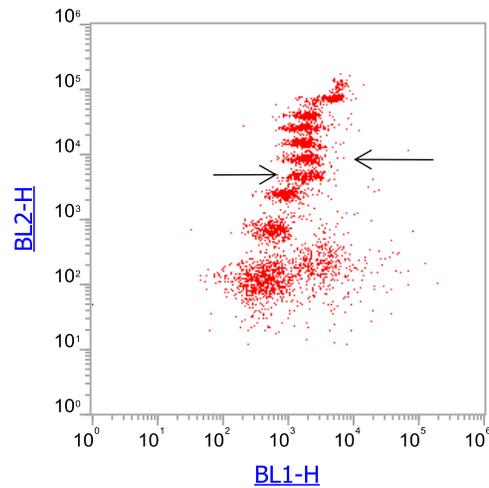
R2 - undil 1-3



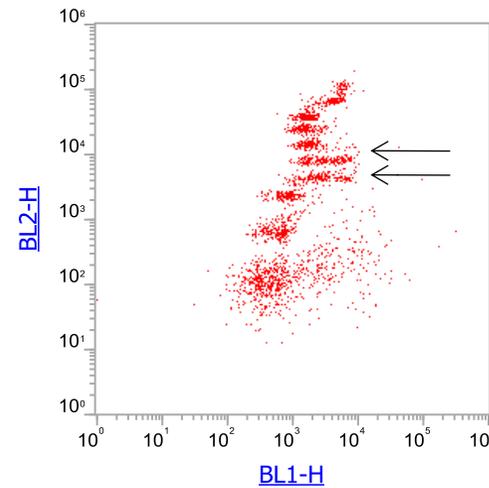
R2 - dil 1-3



R2 - undil 2-2



R2 - dil 2-2



Patient information?

- **Male**
- **57 y/o**
- **First transplant: living Related Donor- 4 years ago**
- **Blood transfusion: Negative**
- **Dialysis Status: Positive**
- **Candidate for second transplant**

Antibody screening

Anti HLA class I antibody:	Anti HLA class II antibody
82%	99%

Reactive Antigens

High Risk Antigens (MFI >1000)
 A*02:01- A*25:01- A*26:01- A*29:02- A*32:01-
 A*68:01- B*07:02- B*08:01- B*13:01- B*14:02-
 B*18:01- B*27:05- B*38:01- B:40:01- B*44:02-
 B*45:01- B*49:01- B*52:01- B*57:01-

Moderate Risk Antigens (MFI 500-1000)
 A*23:01- A*24:02- A*31:01- A*33:01- A*34:01-
 B*15:01- B*51:01

High Risk Antigens (MFI >1000)
 DRB1*01:03- DRB1*03:01- DRB1*03:02-
 DRB1*08:01- DRB1*09:01- DRB1*10:01-
 DRB1*11:01- DRB1*12:02- DRB1*13:01-
 DRB1*13:03- DRB1*14:01- DRB1*15:01-
 DRB1*15:02- DRB1*16:01- DRB3*02:02-
 DRB5*01:01- DQB1*03:01- DQB1*04:02-
 DQB1*05:01- DQB1*06:02

Moderate Risk Antigens (MFI 500-1000)
 DRB1*01:01- DRB1*12:01- DQB1*02:01-
 DQB1*03:02

**Previous transplant
 living Related Donor- Brother**

Calculated PRA: >99%

MFI after dilution

Class I before and after dilution

A*01:01	200	350
A*02:01	3900	16400
A*03:01	0	180
B*49:01	4000	13600
A*25:01	9950	57100
A*29:02	5200	12500
A*30:01	100	200
A*26:01	1700	5200
A*68:01	650	19200
A*11:01	100	700
A*34:01	700	19100
A*24:02	450	31600
A*32:01	1300	84550
A*33:01	300	10200
A*31:01	360	700
A*23:01	350	46500
B*51:01	350	7800
B*13:01	2300	23200
B*18:01	500	300
B*35:01	150	300
B*15:01	350	500
B*45:01	800	17000
B:40:01	1900	11200
B*44:02	880	15800
B*38:01	4650	16300
B*57:01	10250	32400
B*07:02	10400	40600
B*52:01	5250	23050
B*27:05	8800	44100
B*08:01	600	1450
B*14:02	350	1300
B*55:01	250	600

Class II before and after dilution

DRB1*01:01	500	2750
DRB1*01:03	1000	3900
DRB1*04:01	100	500
DRB1*07:01	0	500
DRB1*08:01	1800	6150
DRB1*04:05	140	1600
DRB1*10:01	750	2250
DRB1*11:01	4100	13300
DRB1*12:01	600	1200
DRB1*13:01	3600	11100
DRB1*13:03	2900	10100
DRB1*14:01	2400	7300
DRB1*15:01	2900	9100
DRB1*16:01	2500	4600
DRB1*03:01	2600	8900
DRB1*03:02	2400	8800
DRB5*01:01	4900	11200
DRB3*02:02	2200	2900
DRB4*01:03	0	700
DRB1*01:02	150	1050
DRB1*04:04	200	600
DRB1*09:01	1920	5750
DRB1*12:02	650	1260
DRB1*15:02	900	2600
DQB1*02:01	350	0
DQB1*04:02	6900	6200
DQB1*05:01	7700	10150
DQB1*06:02	6250	7750
DQB1*03:01	5200	15900
DQB1*03:02	380	200
DQB1*03:03	150	0
DPB1	0	0

Can we predict desensitization outcome?

Combining desensitization and VXM may help HSP

Discrimination between complement fixating and none complement fixating antibodies may increase chance to find a better donor

Plasma exchange lowers complement increases MFI

Rebound or prozone effect?

Case 6- Prozone effect and negative Flow XM

Physician: Dr. Miladi	Sample ID: 03-668	Test date: 97/03/8
Name:	Sex/age: M/79	Dialysis Status: Positive
Previous transplant: Positive	Blood transfusion: Positive	Number of pregnancy: - Abortion:-

Anti HLA class I antibody:	Anti HLA class II antibody
5%	63%

Third kidney transplant candidate
First transplant HLA matched sibling
Second transplant from living unrelated donor

Flow XM results

Physician: Dr.	Sample ID: 03-668	Test date: 97/03/8
Recipient Name:	Donor name:	Relationship: Unrelated
Recipient gender/age: M/79	Donor gender/age:M/33	Recipient blood group: O+ Donor blood group: O+
Previous transplant: Positive	Blood transfusion: Positive	Number of pregnancy: -

T cell Median Channel Shift: 19 channels

B cell Median Channel Shift: 49 channels

Negative T and B cell cross match. In order to rule out DSA, single antigen assay of recipient, HLA typing of donor and virtual cross matching is recommended.

Single antigen assay and cPRA

Reactive Antigens	
High Risk Antigens (MFI >1000)	DQB1*03:01- DQB1*03:02- DQB1*03:03- DQB1*04:02
Calculated class II PRA: 62%. High chance to find a DSA negative donor. HLA typing of potential donors and virtual cross matching is recommended.	

Name	Rel.	Class I PCR	Class II PCR
	Recipient	A*02	DPB1*02- DPB1*04
		B*55-B*57	DQB1*05- DQB1*06
		C*03- C*06	DRB1*14-DRB1*15
			DRB3- DRB5

Name	Rel.	Class I PCR	Class II PCR
	Donor	A*02-A*33	DPB1*04- DPB1*13
		B*14-B*51	DQB1*03- DQB1*05
		C*08- C*15	DRB1*01-DRB1*04
			DRB4

Table 4. Estimated number of match runs needed to have a 95% probability of finding an acceptable donor based on candidate cPRA

cPRA, %	Theoretical number of match runs to have a 95% chance of finding an acceptable donor
10	2
20	2
30	3
40	4
50	5
60	6
70	9
80	14
85	19
90	29
95	59
99	300
99.5	600
99.9	3000
99.99	30,000
99.999	300,000

cPRA, calculated panel-reactive antibody.

Prozone effect removal

Change in MFI after 1/50 dilution

DQB1*04:02	1500	17400	High Risk Antigen
DQB1*03:01	3450	57700	High Risk Antigen
DQB1*03:02	4800	73000	High Risk Antigen
DQB1*03:03	3000	52400	High Risk Antigen

T cell Median Channel Shift: 19 channels

B cell Median Channel Shift: 49 channels

Negative T and B cell cross match. In order to rule out DSA, single antigen assay of recipient, HLA typing of donor and virtual cross matching is recommended.

Negative CDC cross match. False negative B cell cross match.

What is your plan for transplant?

Which category should we select?

- Emergency of the transplant?
- cPRA of the patient?
- Age of the patient?

Table 1. Predictability of positive crossmatch results with solid phase assays shows excellent correlation with flow cytometric crossmatch tests. Results from a multi-center collaborative study (5).

Target Cells	Ab ¹ Detection Method	# Tests	% Correct Predictions
T Lymphocytes	ELISA ²	137	86.1
	SAB ³ by Flow Cytometry	580	93.8
B Lymphocytes	ELISA	145	91.0
	SAB by Flow Cytometry	698	97.9
	Multi-Ag ⁴ Beads - Flow or Luminex	16	93.8

¹ Ab = antibody

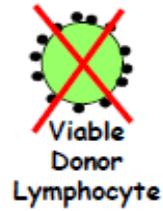
² ELISA = enzyme-linked immunosorbent assay

³ SAB = single antigen beads

⁴ Ag = antigen

What we can do in virtual cross match era?

Virtual Crossmatch HLA Antibody Data Used to Predict Crossmatch Result



Recipient
HLA Antibodies
A2, A68, A69

Donor 1
A1,2;B8,44;DR2,17

Positive

Donor 2
A1,3;B8,44;DR2,17

Negative

labl 09/10

Calculated PRA (cPRA)

% deceased donors with unacceptable antigens

<u>Unacceptable Antigens</u>	<u>cPRA</u>
A2	47%
A2 A68 A69	53%
A80	0%
DR4	29%
A2 DR4	60%
Bw6	80%

labl 09/10