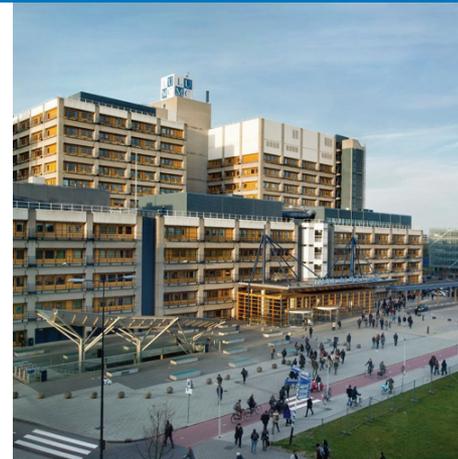


Patient based cases EPT - 2018

Sebastiaan Heidt

Eurotransplant Reference Laboratory

ET EXTRAMURAL MEETING 2019 - GRONINGEN





Patient case 2018-01: recipient information

Recipient age: 60

Recipient gender: male

Recipient blood group: B⁺

Recipient HLA type : A1, A19, A29, B8, B40, B60, Bw6, Cw3, Cw10, Cw7, DR3, DR17, DR52, DQ2

Immunizing events: transplant

Previous transplant: A1, A2, B7, B8, Bw6, Cw7, DR2, DR15, DR3, DR17, DR51, DR52, DQ1, DQ6, DQ2

Chance on a donor (AM program): 0.160

vPRA: 99%



Patient case 2018-01: antibody information

CDC antibody specificities: A2 A28

Luminex antibodies: HLA class I (baseline MFI)

Specificity	A2	A68	A69	A23	A24	B57	B58	A11	B73	B7
Highest MFI	24505	23618	22947	22467	21954	18698	15868	13185	9500	9093

HLA class II (baseline MFI)

Specificity	DQ5	DQ6	DP1	DP3	DP6	DP9	DR16	DP5	DP14	DR15	DP17	DR51	DP19	DP13
Highest MFI	25125	24905	23772	23741	23123	22850	22754	22631	22557	22502	22164	22130	22126	22117

Specificity	DR103	DP11	DP10	DP18	DP3	DR13	DQ7	DR12	DQ4	DQ9	DP20	DR11	DQ8	DP15
Highest MFI	22110	22109	21637	21039	20905	20451	20271	20098	19747	19341	19118	18300	17638	17070

Specificity	DR8	DR4	DR1	DP28	DP4	DR9	DR14	DP23	DR7	DR10
Highest MFI	16778	15303	13810	13423	12874	10166	8833	8654	8430	6059



Patient case 2018-01 : donor information

Donor type: DCD (AM offer)

Donor blood group: B⁺

Donor HLA type: A1, A33, A19, B8, B50, B21, Bw6, Cw6, Cw7, DR17, DR3, DR52, DQ2

CDC crossmatch results PBL:

(at donor center)

Unseparated		T cells		B cells		Final results	
(-) DTT	(+) DTT	(-) DTT	(+) DTT	(-) DTT	(+) DTT	(-) DTT	(+) DTT
Neg	Neg	-	-	-	-	Neg	Neg

CDC crossmatch results spleen:

(at recipient center)

Unseparated		T cells		B cells		Final results	
(-) DTT	(+) DTT	(-) DTT	(+) DTT	(-) DTT	(+) DTT	(-) DTT	(+) DTT
Pos	Pos	-	-	-	-	Pos	Pos

Autologous state: Dneg



Patient case 2018-01: Histocompatibility

Patient:

A1, A19, A29, B8, B40, B60, Bw6, Cw3, Cw10, Cw7, DR3, DR17, DR52, DQ2

Acceptable antigens:

A74, B50, B61, B41, B48, B72

Donor:

A1, A33, A19, B8, B50, B21, Bw6, Cw6, Cw7, DR3, DR17, DR52, DQ2

Green: match

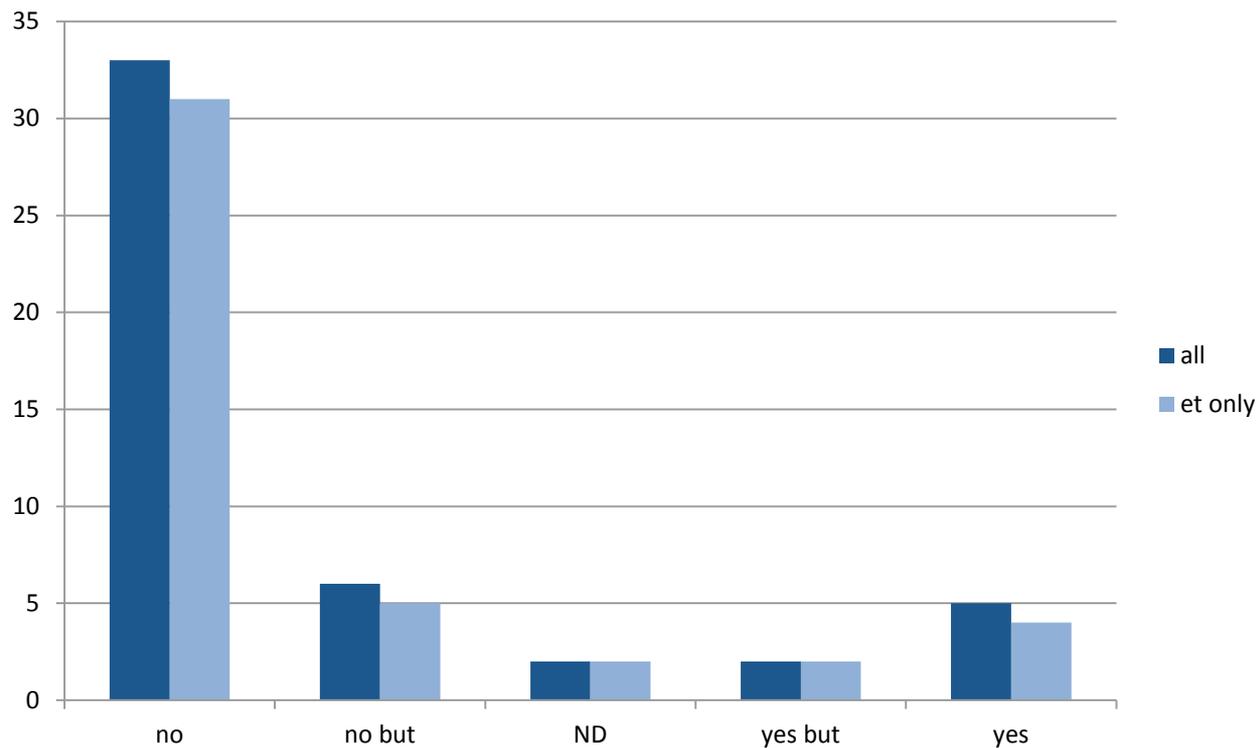
Blue: mismatch without proven antibodies

Black: mismatch with specificity CDC proven

Orange: mismatch with specificity Luminex proven

Underlined: acceptable mismatch

Patient case 2018-01: Results



Patient case 2018-01: discussion

- Donor turned out to be HLA-DP3 (MFI: 23741)
- How clinically relevant are antibodies against HLA-DP?

Impact of preformed and de novo anti-HLA DP antibodies in renal allograft survival



Dolores Redondo-Pachón^a, Julio Pascual^{a,*}, María J. Pérez-Sáez^a, Carmen García^b, Juan José Hernández^b, Javier Gimeno^c, Marisa Mir^a, Marta Crespo^a

^a Department of Nephrology, Institute Mar for Medical Research, Hospital del Mar, Barcelona, Spain

^b Laboratori de Referència de Catalunya, Barcelona, Spain

^c Department of Pathology, Hospital del Mar, Barcelona, Spain

ARTICLE INFO

Article history:

Received 1 September 2015
Received in revised form 12 November 2015
Accepted 17 November 2015
Available online 18 November 2015

Keywords:

Renal transplant
HLA-DP antibodies
Graft survival

ABSTRACT

The influence of antibodies against HLA-DP antigens detected with solid-phase assays on graft survival after kidney transplantation (KT) is uncertain. We evaluated with Luminex® the prevalence of pre- and posttransplant DP antibodies in 440 KT patients and their impact on graft survival. For 291 patients with available pretransplant samples, DP antibodies were present in 39.7% KT with pretransplant HLA antibodies and 47.7% with DSA. Graft survival of KT with pretransplant class-II DSA was worse than with non-DSA ($p = 0.01$). DP antibodies did not influence graft survival. Of 346 patients monitored post-KT, 17.1% had HLA class-II antibodies, 56% with DP antibodies. Class-II DSA was detected in 39%, 60.9% of them had DP antibodies. Graft survival was worse in patients with class-II DSA ($p = 0.022$). DP antibodies did not change these results. The presence of isolated DP antibodies was a rare event both pre- and posttransplantation (1.03 and 0.86%). The presence of pretransplant and posttransplant DSA is associated with a negative impact on graft survival. However, the presence of DP antibodies does not modify this impact significantly.

Deleterious Impact of Donor-Specific Anti-HLA Antibodies Toward HLA-Cw and HLA-DP in Kidney Transplantation

Thomas Bachelet, MD, PhD,^{1,2} Charlie Martinez, MD,¹ Arnaud Del Bello, MD,³ Lionel Couzi, MD, PhD,^{1,2} Salima Keiji, MD,³ Gwendaline Guidicelli, PharmD, PhD,^{2,4} Sébastien Lepreux, MD, PhD,⁵ Jonathan Visentin, PharmD,^{2,4} Nicolas Congy-Jolivet, PharmD, PhD,⁶ Lionel Rostaing, MD, PhD,^{3,7} Jean-Luc Taupin, PharmD, PhD,^{2,4} Nassim Kamar, MD, PhD,^{3,7} and Pierre Merville, MD, PhD^{1,2}

Background. It is widely accepted that HLA donor-specific antibodies (DSA) are associated with antibody-mediated rejection and graft loss. However, in many transplant programs, preformed anti-HLA-Cw and anti-HLA-DP DSA are not considered in organ allocation policies because their clinical relevance is still uncertain. **Methods.** We analyzed the clinical impact of Cw/DP DSA through a retrospective study, comparing 48 patients transplanted with isolated preformed Cw/DP DSA (Cw/DP DSA group) with (i) 104 matched HLA-sensitized kidney transplant recipients with No DSA at DO (No DSA group) and (ii) 47 kidney transplant recipients with preformed A₂-B₂-DR₂-DQ DSA (A/B/DR/DQ DSA group). **Results.** A positive flow cytometry crossmatch in the Cw/DP DSA group was more frequent than in the No DSA group and as frequent as in the A/B/DR/DQ DSA group. Two years after transplantation, the biopsy-proven acute rejection-free survival was worse in the Cw/DP and A/B/DR/DQ DSA groups than in the No DSA group (65%, 84%, 93%, $P = 0.001$ and $P = 0.05$, respectively). Accordingly, graft survival was lower in the Cw/DP and the A/B/DR/DQ DSA groups than in the No DSA group (87%, 89%, 95%, $P = 0.02$ and $P = 0.1$, respectively). **Conclusions.** These results suggest that preformed anti-HLA-Cw and anti-HLA-DP DSA are as deleterious as anti-HLA A/B/DR/DQ DSA. It justifies their inclusion in kidney allocation programs and in immunological risk stratification algorithms.

(*Transplantation* 2016;100: 159–166)



Patient case 2018-02A: recipient information

Recipient age: 58

Recipient gender: female

Recipient blood group: A

Recipient HLA type : A1, A2, B8, B27, Bw4, Bw6, Cw2, Cw7, DR3, DR17, DR6, DR13, DR52, DQ1, DQ6, DQ2

Immunizing events: 2 prior transplants

Previous transplant 1: A1, A10, A66, B8, B41, Bw6, Cw7, Cw17, DR3, DR17, DR6, DR13, DR52, DQ2, DQ3, DQ7

Previous transplant 2: A2, B7, B17, B57, Bw4, Bw6, Cw7, DR6, DR14, DR9, DR52, DR53, DQ1, DQ5, DQ3, DQ9



Patient case 2018-02A: antibody information

Luminex antibodies: HLA class I (baseline MFI)

	Cw17	A66	Cw18	A25	A34	Cw6	A26	A68	Cw5	A69	B41	A43	Cw15	B49
Highest MFI	15368	13972	12038	11745	9237	8754	8541	6721	6485	5800	5567	4442	4370	4144

Specificity	A33	B72	B62	A11	B50	B71	B76	B39	Cw1	B45	B13	B42	A30	B75
Highest MFI	4036	3926	3693	3049	2822	2813	2696	2600	2310	2019	1974	1823	1808	1778

Specificity	B67	B38	Cw4	B61	B55	B60
Highest MFI	1605	1523	1492	1451	1297	1058

HLA class II (baseline MFI)

Specificity	DQ7	DR53	DQ9	DQ8
Highest MFI	23713	9729	4634	4114



Patient case 2018-02A: results

Luminex antibodies: HLA class I (baseline MFI)

	<u>Cw17</u>	<u>A66</u>	Cw18	A25	A34	Cw6	A26	A68	Cw5	A69	<u>B41</u>	A43	Cw15	B49
Highest MFI	15368	13972	12038	11745	9237	8754	8541	6721	6485	5800	5567	4442	4370	4144

Specificity	A33	B72	B62	A11	B50	B71	B76	B39	Cw1	B45	B13	B42	A30	B75
Highest MFI	4036	3926	3693	3049	2822	2813	2696	2600	2310	2019	1974	1823	1808	1778

Specificity	B67	B38	Cw4	B61	B55	B60
Highest MFI	1605	1523	1492	1451	1297	1058

HLA class II (baseline MFI)

Specificity	<u>DQ7</u>	<u>DR53</u>	<u>DQ9</u>	<u>DQ8</u>
Highest MFI	23713	9729	4634	4114

≥75% consensus

≥50% consensus



Patient case 2018-02B: recipient information



Recipient age: 46

Recipient gender: male

Recipient blood group: O

Recipient HLA type : A1, A2, B8, B16, B39, Bw6, Cw7, Cw12, DR3, DR17, DR4, DR52, DR53, DQ2, DQ3, DQ8

Immunizing events: unknown

Patient case 2018-02B: antibody information



Luminex antibodies:

HLA class II (baseline MFI)

	DQ5	DQ6	DP14	DP11	DP10
Highest MFI	7966	6675	1919	1476	1114

Patient case 2018-02B: results



Luminex antibodies:

HLA class II (baseline MFI)

	DQ5	DQ6	DP14	DP11	DP10
Highest MFI	7966	6675	1919	1476	1114

≥75% consensus

≥50% consensus



Patient case 2018-02C: recipient information

Recipient age: 57

Recipient gender: male

Recipient blood group: O

Recipient HLA type : A2, B7, B8, Bw6, Cw7, DR4, DR6, DR13, DR52, DR53, DQ3, DQ7

Immunizing events: prior transplant

Previous transplant: A1, A2, B5, B51, B15, B62, Bw4, Bw6, Cw3, Cw9, Cw4, DR1, DR6, DR13, DR52, DQ1, DQ5, DQ1, DQ6

Patient case 2018-02C: antibody information



Luminex antibodies:

HLA class I (baseline MFI)

	B63	B62	B75	B51	B49	B52	B77	B53	B35	B56	B57	B72	B76	B50
Highest MFI	4046	2806	2643	2452	2336	2314	2292	2028	1744	1694	1629	1597	1579	1366

Specificity	B71	B78	A24	A23	A1
Highest MFI	1354	1254	1219	1210	1129



Patient case 2018-02C: results

Luminex antibodies:

HLA class I (baseline MFI)

	<i>B63</i>	<u><i>B62</i></u>	<i>B75</i>	<u><i>B51</i></u>	<i>B49</i>	<i>B52</i>	<i>B77</i>	<i>B53</i>	<i>B35</i>	<i>B56</i>	<i>B57</i>	<i>B72</i>	<i>B76*</i>	<i>B50</i>
Highest MFI	4046	2806	2643	2452	2336	2314	2292	2028	1744	1694	1629	1597	1579	1366

Specificity	<i>B71</i>	<i>B78</i>	<i>A24</i>	<i>A23</i>	<i>A1</i>
Highest MFI	1354	1254	1219	1210	1129

Table 2: Frequency (%) and HLA specificity of antibodies detected by single antigen bead (SAB) assay in waiting list patients without a history of an immunizing event

HLA specificities	Median MFI (range)	Prevalence of SAB reactivity in waiting list patients (%)	Frequency of allele in Caucasian population (%) ¹
Class I			
A*24:02	4193 (1178–12 197)	8.8	8.7
A*31:01	3377 (1036–8710)	7.8	2.4
A*24:03	4487 (1010–11 025)	6.9	0.1
A*43:01	2019 (1557–3738)	6.9	0.0
A*23:01	4974 (1141–7108)	6.9	1.7
A*25:01	2426 (1235–5004)	6.9	1.9
A*66:01	2501 (1259–5226)	6.9	0.3
A*80:01	3416 (2312–8012)	5.9	0.0
A*30:01	2386 (1253–5497)	5.9	1.3
A*30:02	2547 (1183–4959)	4.9	0.9
A*11:02	2562 (1007–7126)	3.9	0.0
B*15:12 (B76)	2289 (1001–6022)	21.6	0.0

≥75% consensus

≥50% consensus

Gombos et al., AJT 2013



Patient case 2018-02D: recipient information



Recipient age: 59

Recipient gender: female

Recipient blood group: O

Recipient HLA type : A3, A30, A19, B17, B57, B27, Bw4, Cw1, Cw18, DR1, DR6, DR13, DR52, DQ1, DQ5

Immunizing events: blood transfusion

Patient case 2018-02D: antibody information

Luminex antibodies:

HLA class I (baseline MFI)

	B8	B75	B71	B76	B62	B18	B50	B72	B39	B7	B67	B45	B60	B41
Highest MFI	9358	9226	8956	8910	8856	8787	8730	8657	8454	8239	8120	8107	8021	8006

Specificity	B78	B61	B64	B48	B81	B35	B27	B56	B42	B55	B54	B82	B65	B44
Highest MFI	7988	7966	7780	7727	7477	7413	7315	7303	7265	7059	6942	6580	6287	2155

Specificity	B49	B13	B37	B38	B51	B53	B77	B51
Highest MFI	1827	1529	1493	1459	1413	1207	1150	1035

HLA class II (baseline MFI)

Specificity	DR16	DR4	DQ8	DP13	DP1	DR10	DP19	DR52	DQ7	DQ2	DR1	DQ9	DR51	DP5
Highest MFI	4457	4094	3929	3056	2601	1906	1778	1644	1597	1512	1308	1256	1133	1041

Specificity	DP11
Highest MFI	1005



Patient case 2018-02D: results

Luminex antibodies:

HLA class I (baseline MFI)

	B8	B75	B71	B76	B62	B18	B50	B72	B39	B7	B67	B45	B60	B41
Highest MFI	9358	9226	8956	8910	8856	8787	8730	8657	8454	8239	8120	8107	8021	8006

Specificity	B78	B61	B64	B48	B81	B35	B27	B56	B42	B55	B54	B82	B65	B44
Highest MFI	7988	7966	7780	7727	7477	7413	7315	7303	7265	7059	6942	6580	6287	2155

Specificity	B49	B13	B37	B38	B51	B53	B77	B51
Highest MFI	1827	1529	1493	1459	1413	1207	1150	1035

HLA class II (baseline MFI)

Specificity	DR16	DR4	DQ8	DP13	DP1	DR10	DP19	DR52	DQ7	DQ2	DR1	DQ9	DR51	DP5
Highest MFI	4457	4094	3929	3056	2601	1906	1778	1644	1597	1512	1308	1256	1133	1041

Specificity	DP11
Highest MFI	1005

≥75% consensus

≥50% consensus



Patient case 2018-03: recipient information

Recipient age: 27

Recipient gender: female

Recipient blood group: A⁺

Recipient HLA type : A3, A11, B5, B51, B35, Bw4, Bw6, Cw4, Cw15, DR6, DR14, DR10, DR52, DQ1, DQ5

Immunizing events: transplant

Previous transplant: A3, A11, B5, B51, B18, Bw4, Bw6, Cw5, Cw15, DR4, DR6, DR14, DR52, DR53, DQ1, DQ5, DQ3, DQ8

Recent PRA: 46%

Highest PRA: 84%



Patient case 2018-03: antibody information

CDC antibody specificities: DR4, DR53, DQ3, DQ7, DQ9

Luminex antibodies: HLA class II (baseline MFI)

Specificity	DQ7	DR53	DQ9	DQ4	DQ8	DQ2	DQ6	DR13	DR103	DR4
Highest MFI	18327	17995	17932	16171	15715	13560	7667	4424	3066	2260



Patient case 2018-03 : donor information

Donor type: DCD

Donor blood group: A⁺

Donor HLA type: A2, A11, B12, B44, B35, Bw4, Bw6, Cw4, Cw5, DR1, DR2, DR15, DR51, DQ1, DQ5, DQ6

CDC crossmatch results PBL:

Unseparated		T cells		B cells		Final results	
(-) DTT	(+) DTT	(-) DTT	(+) DTT	(-) DTT	(+) DTT	(-) DTT	(+) DTT
Neg	Neg	-	-	-	-	Neg	Neg

Autologous state: Neg



Patient case 2018-03: Histocompatibility

Patient:

A3, A11, B5, B51, B35, Bw4, Bw6, Cw4, Cw15, DR6, DR14, DR10, DR52, DQ1, DQ5

Donor:

A2, A11, B12, B44, B35, Bw4, Bw6, Cw4, Cw5, DR1, DR2, DR15, DR51, DQ1, DQ5, DQ1, DQ6

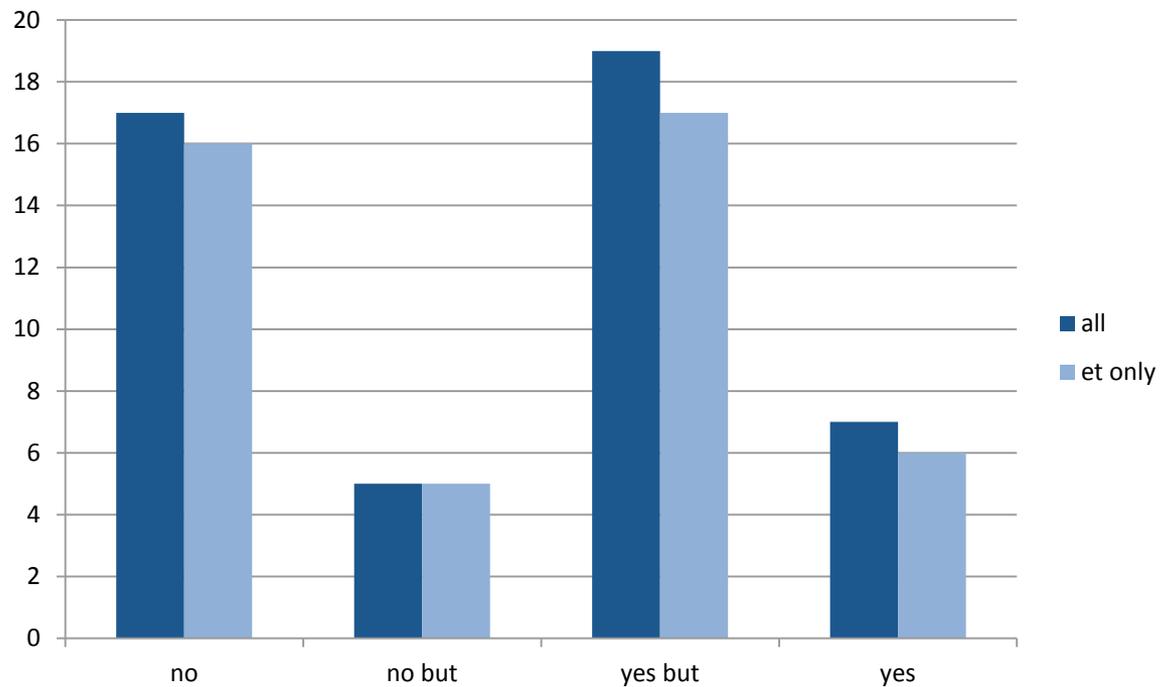
Green: match

Blue: mismatch without proven antibodies

Black: mismatch with specificity CDC proven

Orange: mismatch with specificity Luminex proven

Patient case 2018-03: Results





No:

- presence of DQ6 DSA (unacceptable antigen), epitopes (45GV/46VY₃/77T) shared with DQ8 (1st donor)
- 1-1-2 mismatch
- because of young age and future need of more organs be very careful in selecting donors
- Cw5 is repeat mismatch (unacceptable antigen/could be boosted after transplantation/no antibodies detectable)

Yes:

- CDC cross match negative
- with negative B cell cross match no contraindication
- chance of finding a homozygous DQ5 is low, and thus move on with (high risk) transplantation
- DQ6 antibodies found in luminex cannot be explained by immunization history and are not found in CDC

Thank you for participating in the patient based cases EPT

The ETRL team:

Marian Witvliet

Yvonne Zoet

Geert Haasnoot

Marissa Linden-van Oevelen

Petra van der Kroef

Anouk de Jong

Sebastiaan Heidt

Frans Claas

