UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

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Interpretive Educational Scheme (iED)
Clinical Scenario 1/2021 – Renal Transplantation

Dispatched on 1st June 2021

Summary of Results

A total of 47 responses were received, 18 from UK & Ireland (UK&I) based laboratories and 29 from Rest of the World (RoW) based laboratories.

Background:
A 16 year old patient, ABO blood group A Rh+, was referred to the laboratory in November 2020 for re-listing following failure of his first kidney transplant. The patient has received a number of transfusions.

The patient's HLA type is:
HLA-A3, A11; B52, B57; Cw6, Cw12; DR7, DR15; DQ6, DQ9; DQA1*01:03, DQA1*02:01; DPB1*03:01, DPB1*09:01

The patient's first transplant was a living donor from his mother in 2008.
The mother's HLA type is:
HLA-A3, A11; B35, B57; Cw4, Cw6; DR7, DR8; DQ4, DQ9; DPB1*03:01, DPB1*04:02

The patient has the following HLA specific antibodies well defined by One Lambda Single Antigen kits (the patient has no Class I antibodies). The average MFI range of these specificities over the last 3 years is provided:

Antibody Specificity	MFI Range
DR4	1,000-2,000
DR12	1,000-2,000
DQ2	>10,000
DQ4	>10,000
DQ7	>10,000
DQ8	>10,000
DQA1*03	>10,000
DQA1*04	>10,000
DQA1*05	>10,000
DQA1*06	>10,000

Question 1

The patient's family launch a social media campaign to find a living donor for their son. The clinical team receive more than 20 enquires.

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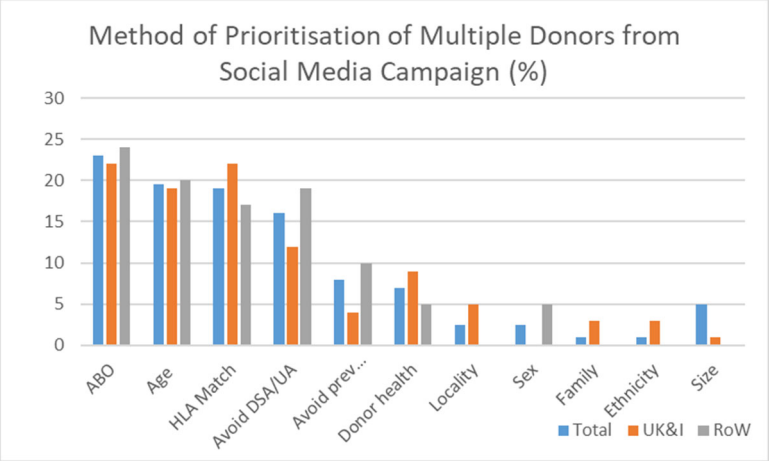
1.1 What advice would you give to the clinical team in terms of prioritising the potential donors?

Prioritise based on:	Total (%)	UK&I (%)	RoW (%)
ABO compatibility	**36 (23%)**	16 (22%)	20 (24%)
Age	**31 (19.5%)**	14 (19%)	17 (20%)
HLA Match	**30 (19%)**	16 (22%)	14 (17%)
Avoid DSA/UA	**25 (16%)**	9 (12%)	16 (19%)
Avoid previous mismatches	**12 (8%)**	3 (4%)	9 (10%)
Donor health	**11 (7%)**	7 (9%)	4 (5%)
Locality	**4 (2.5%)**	4 (5%)	0 (0%)
Sex	**4 (2.5%)**	0 (0%)	4 (5%)
Family	**2 (1%)**	2 (3%)	0 (0%)
Ethnicity	**2 (1%)**	2 (3%)	0 (0%)
Size	**1 (0.5%)**	1 (1%)	0 (0%)



Question 2

In April 2021 the clinical team receive an end of chain donor offer for the patient from Donor X who is a 25 year old male. The offer included the following HLA typing information for the donor.
Donor X's HLA Type: HLA-A1, A33; B52, B58; Cw10, Cw12; DR15, DR14; DQ5, DQ6; DPB1*04:01, DPB1*14:01
ABO: O Rh+

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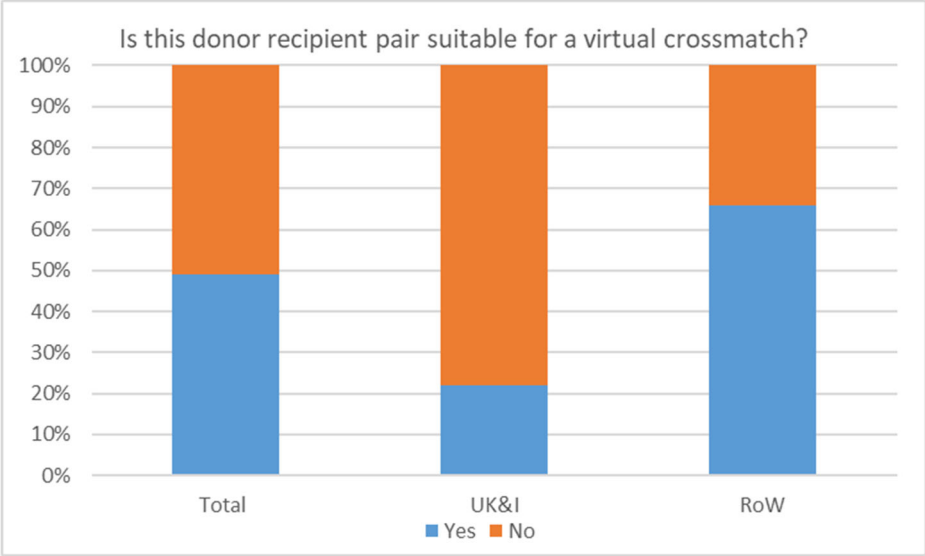
2.1 Is this donor recipient pair suitable for a virtual crossmatch?

Response	Total (n=47)	UK&I (n=18)	RoW (n=29)	Selected Comments
Yes	**23 (49%)**	4 (22%)	19 (66%)	• Used at referral for compatibility assessment • Perform wet XM if donor proceeding to transplant • No apparent DSAs • ABO compatible • No repeat mismatches with previous transplant
No	**24 (51%)**	14 (78%)	10 (34%)	• Unknown donor HLA-DQA1 genotype and patient has DQA antibodies • Unclear how current antibody screening results are • High-level HLA Class II sensitisation • Previous transplant mismatches • Unclear when transfusion were given • A wet-XM is always used for live donors • Always perform a wet-XM on re-transplant patients



2.2 What further laboratory tests would you perform at this time?

Further Testing Identified	Total (%)	UK&I (%)	RoW (%)
Donor Genotyping (DQA)	**35 (28%)**	17 (32%)	18 (25%)
Flow Cytometry Crossmatch	**31 (25%)**	11 (21%)	20 (28%)
Single Antigen Testing	**24 (19%)**	12 (23%)	12 (17%)
Cytotoxicity Crossmatch	**23 (19%)**	4 (8%)	19 (27%)
Autologous Crossmatch	**4 (3%)**	3 (6%)	1 (1%)
Patient Genotyping	**2 (2%)**	2 (4%)	0 (0%)
Previous Graft Genotyping	**1 (1%)**	1 (2%)	0 (0%)
ABO Group	**1 (1%)**	1 (2%)	0 (0%)
Eplet Analysis	**1 (1%)**	0 (0%)	1 (2%)
Confirm Transfusion Dates	**1 (1%)**	1 (2%)	0 (0%)

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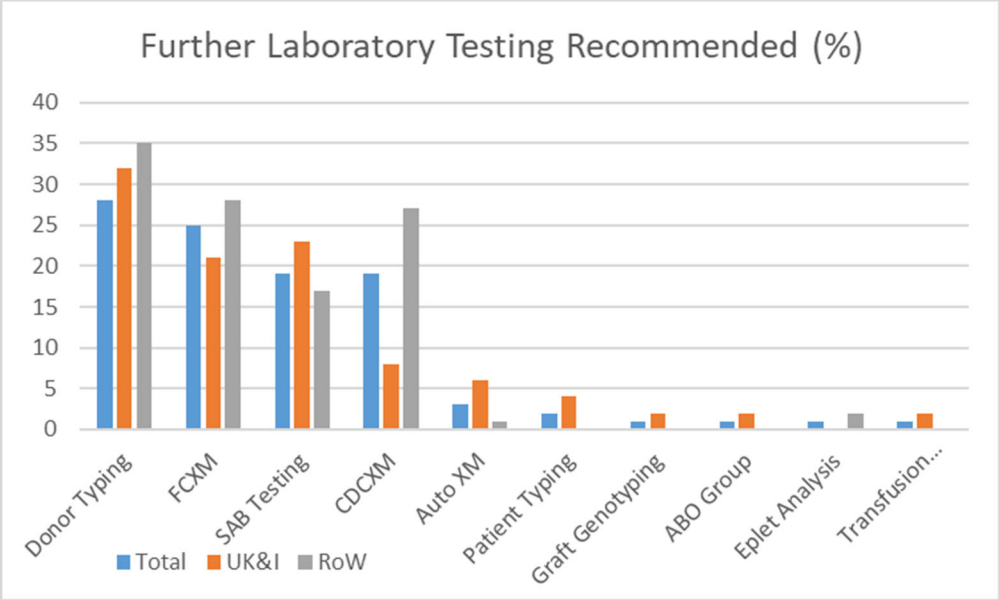

Question 3

A CDC and flow cytometry crossmatch were carried out. The results, which have been verified as technically valid, are shown below:

	Cytotoxic Crossmatch				Flow Crossmatch	
Serum Date	T cells	T cells with DTT	B cells	B cells with DTT	T cells Linear Channel Shift (LCS)	B cells Linear Channel Shift (LCS)
03/04/2008	1	1	1	1	2	0
11/04/2008	1	1	1	1	4	6
08/09/2014	1	1	6	1	0	0
01/08/2016	1	1	1	1	0	0
01/10/2018	1	1	1	1	0	0
12/08/2019	1	1	1	1	0	0
11/05/2020	1	1	1	1	0	0
14/04/2021	1	1	1	1	0	94

LCS Thresholds:

	Neg	Equivocal	Positive
T cells	<46		>=46
B cells	<35	>=35<63	>=63

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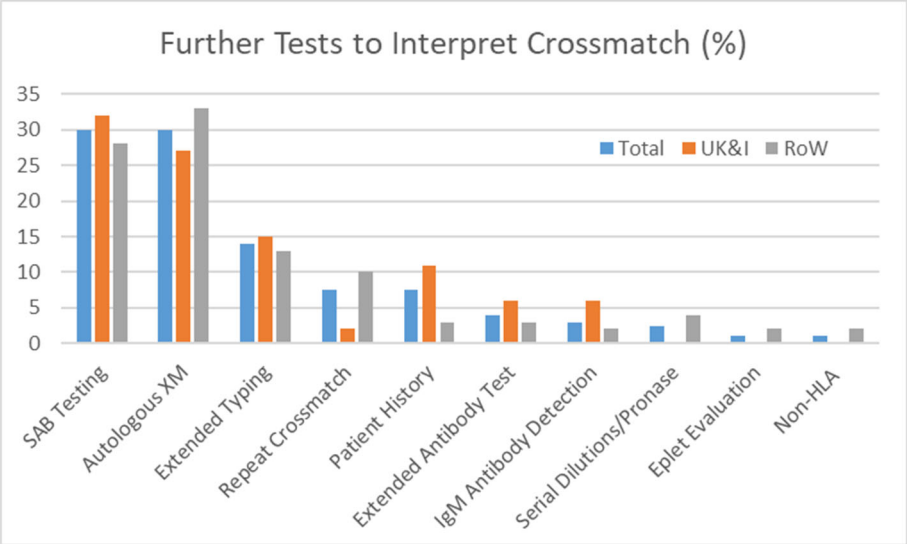
3.1 What further tests would be required to interpret the crossmatch results?

Further Testing	Total (%)	UK&I (%)	Row (%)
Single Antigen Antibody Testing	**36 (30%)**	17 (32%)	19 (28%)
Autologous Crossmatch	**36 (30%)**	14 (27%)	22 (33%)
Extended Genotyping e.g. DQA, DRB3/4/5	**17 (14%)**	8 (15%)	9 (13%)
Repeat Crossmatch	**8 (7.5%)**	1 (2%)	7 (10%)
Patient Sensitisation History	**8 (7.5%)**	6 (11%)	2 (3%)
Extended Antibody Panel/Second Kit	**5 (4%)**	3 (6%)	2 (3%)
IgM Antibody Detection	**4 (3%)**	3 (6%)	1 (2%)
Serial Dilutions/Pronase Crossmatch	**3 (2.5%)**	0	3 (4%)
Eplet Evaluation	**1 (1%)**	0	1 (2%)
Non-HLA Antibody Dectection	**1 (1%)**	0	1 (2%)



Question 4

The donor HLA type was verified using an Alpha Biotech QTYPE HLA Typing kit:

Locus	Genotype		Serological Equivalent
HLA-A	A*01	A*33:03	A1 A33
HLA-B	B*52	B*58	B52 B58 Bw4
HLA-C	C*03	C*12:02	Cw10 Undefined
HLA-DRB1	DRB1*14	DRB1*15:02	DR1404 DR15
HLA-DRB3/4/5	DRB3*02	DRB5*01	DR52 DR51
HLA-DQA1	DQA1*01:03	DQA1*01:04	
HLA-DQB1	DQB1*05:03	DQB1*06:01	DQ5 DQ6
HLA-DPA1	DPA1*02:01	DPA1*02:07	
HLA-DPB1	DPB1*04:01	DPB1*14:01	

The sample received 08/09/2014 was tested using a modified LABScreen Mixed assay and was negative for the presence of IgM alloantibody.

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The sample received 14/04/2021 was tested using LABScreen Single Antigen – the class I test was negative, the results of the class II test are shown below (all controls were within acceptable ranges). Donor X alleles have been highlighted yellow=negative, red=positive:

Raw Data	Allele(s)	Raw Data	Allele(s)	Raw Data	Allele(s)
30265.77	DQA1*06:01 DQB1*03:01	712.09	DRB1*13:01	316.08	DRB5*02:02
29894.81	DQA1*05:01 DQB1*02:01	659.66	DQA1*01:02 DQB1*06:04	314.19	DRB1*09:01
28795.77	DQA1*05:03 DQB1*03:01	636.46	DRB4*01:01	307.41	DRB1*04:05
28305.3	DQA1*05:05 DQB1*03:19	629.56	DPA1*01:03 DPB1*01:01	304.81	DPA1*02:02 DPB1*11:01
24945.02	DQA1*03:02 DQB1*03:02	592.12	DPA1*01:03 DPB1*04:02	300.6	DRB1*01:01
24465.81	DQA1*03:03 DQB1*04:01	557.26	DQA1*01:01 DQB1*06:02	289.08	DRB1*16:01
23974.56	DQA1*03:02 DQB1*03:03	556.3	DRB3*02:02	286.73	DPA1*01:03 DPB1*11:01
22725.78	DQA1*04:01 DQB1*02:01	539.26	DRB5*01:01	276.56	DPA1*02:01 DPB1*03:01
20698.42	DQA1*04:01 DQB1*04:02	526.99	DRB1*03:01	274.79	DPA1*02:02 DPB1*13:01
20229.49	DQA1*03:01 DQB1*03:02	513.62	DPA1*03:01 DPB1*13:01	274.71	DPA1*01:03 DPB1*06:01
18862.42	DQA1*03:01 DQB1*02:01	500.84	DPA1*01:03 DPB1*19:01	273.11	DPA1*02:01 DPB1*18:01
13397.75	DQA1*03:01 DQB1*03:03	484.94	DPA1*01:05 DPB1*28:01	265.71	DPA1*01:05 DPB1*18:01
12047.53	DQA1*03:01 DQB1*03:01	481.38	DQA1*02:01 DQB1*03:01	264.92	DRB1*10:01
6867.1	DRB1*08:01	465.13	DPA1*01:04 DPB1*18:01	262.84	DPA1*01:03 DPB1*28:01
6863.74	DQA1*02:01 DQB1*04:02	462.1	DPA1*01:03 DPB1*03:01	262.38	DPA1*02:01 DPB1*06:01
6367.26	DQA1*02:01 DQB1*04:01	453.92	DPA1*01:03 DPB1*02:01	236.2	DPA1*03:01 DPB1*20:01
2911.29	DRB1*12:02	448.61	DRB1*14:02	233.38	DQA1*02:01 DQB1*03:03
2690.99	DRB1*12:01	439.02	DRB1*07:01	233.08	DPA1*02:01 DPB1*17:01
2637.88	DQA1*01:02 DQB1*05:02	435.46	DPA1*02:02 DPB1*10:01	232.58	DPA1*02:01 DPB1*09:01
2102.18	DPA1*01:03 DPB1*04:01	432.3	DPA1*02:01 DPB1*13:01	205.92	DPA1*02:01 DPB1*05:01
1525.07	DRB1*14:01	427.01	DQA1*02:01 DQB1*02:02	202.28	DRB1*09:02

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1518.53	DPA1*02:01 DPB1*01:01	413.26	DPA1*02:01 DPB1*15:01	201.1	DQA1*01:02 DQB1*06:02
1215.34	DPA1*01:03 DPB1*23:01	394.79	DRB1*15:01	195.58	DQA1*01:03 DQB1*06:03
1107.44	DRB1*13:03	383.56	DRB3*03:01	191.05	DPA1*02:01 DPB1*14:01
1095.38	DRB1*11:01	370.08	DRB1*01:03	190.55	DRB1*01:02
986.9	DRB1*14:54	368.08	DRB1*04:04	190.15	DPA1*01:05 DPB1*03:01
974.71	DQA1*01:02 DQB1*06:09	353.76	DRB4*01:03	180.47	DRB1*16:02
904.95	DPA1*02:02 DPB1*05:01	346.45	DRB1*04:01	161.41	DRB1*15:02
868.27	DRB1*11:04	330.89	DRB1*04:02	140.09	DQA1*01:01 DQB1*05:01
864.86	DPA1*04:01 DPB1*28:01	321.93	DRB3*01:01	133.89	DQA1*01:03 DQB1*06:01
792.85	DRB1*03:02	321.53	DQA1*02:01 DQB1*03:02	132.26	DRB1*15:03
722	DQA1*02:01 DQB1*02:01	319.91	DRB1*04:03		

LABScreen Single Antigen Class II MFI results for sample date 14/04/2021

An autologous flow crossmatch was performed using serum samples dated 11/05/2020 and 14/04/2021 and was negative. Using this information and the Single Antigen bead and donor verification type provided.

4.1/4.2 How would you report the allogeneic crossmatch to a Clinician? Give your reasons.

Response	Total (%)	UK&I (%)	RoW (%)	Reasons
Positive	**35 (74%)**	16 (89%)	19 (66%)	• B cell positive, T cell negative crossmatch. • Autologous crossmatch negative. • DR14 bead weak positive. • DPB1*04:01 bead positive. • Potential reactivity against DQ5. • Some donor alleles not present on antibody panel. • DRB1*14:04 shares a potential epitope with previous transplant mismatch DR8 (Y@aa16). DR8 positive in 14/04/21. • Cumulative DSA of 6,132 MFI.
Equivocal	**8 (17%)**	1 (5.5%)	7 (24%)	• Positive flow cytometric crossmatch in the absence of detectable HLA antibody. • CDC crossmatch negative but DSA to DPB1*04:01. • Some donor alleles not present on antibody panel. • DRB1*14:01, DPB1*04:01, DPA1*02:01 and DRB3*02 beads positive.
Negative	**4 (9%)**	1 (5.5%)	3 (10%)	• CDC crossmatch negative. • Not a DSA from previous transplant. • Potential DSAs but low MFI level would not suggest a positive FCXM. • Not all positive B cell XM are clinically relevant. • Unspecific reaction.

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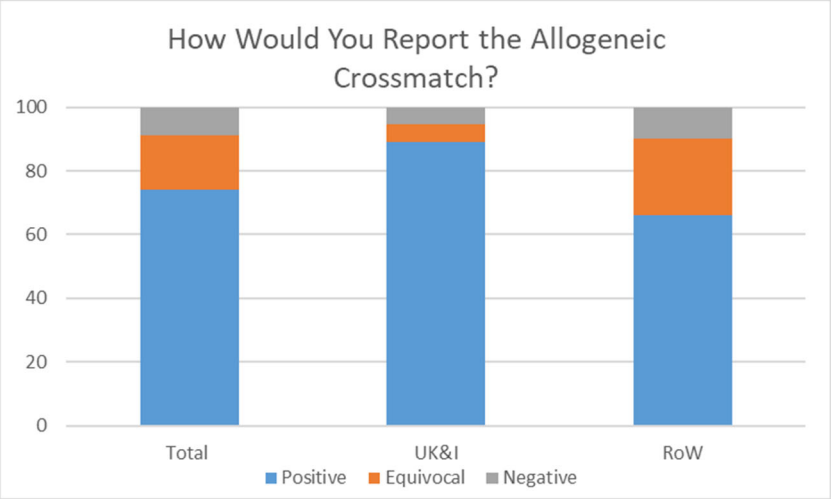
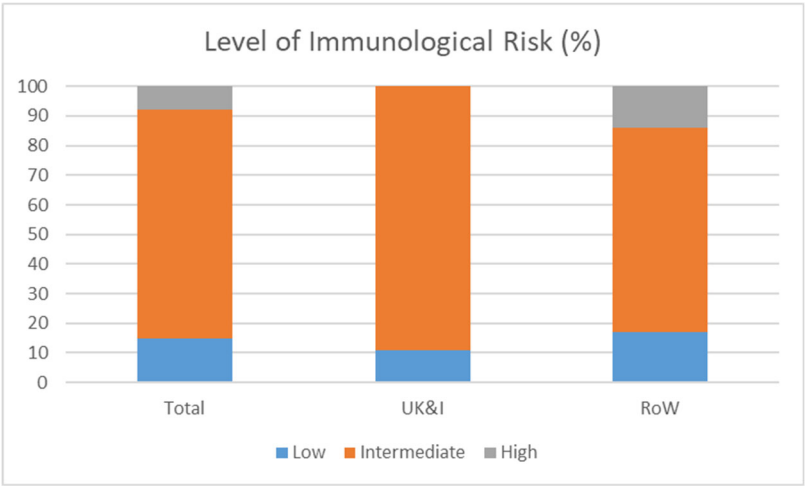


4.3/4.4 What level of immunological risk would you assign to this transplant? Give your reasons.

Response	Total (%)	UK&I (%)	RoW (%)	Reasons
Low/Standard	**7 (15%)**	2 (11%)	5 (17%)	• No DSA. • T and B cell negative crossmatch, DSA positive. • Low MFI <5000.
Intermediate	**36 (77%)**	16 (89%)	20 (69%)	• CDC crossmatch negative. • Flow cytometry crossmatch B cell positive (latest sample tested). • Potential for IgG Class II donor-specific antibody. • DRB1*14:04 not represented on standard One Lambda Single Antigen panel. • Unexplained positive crossmatch. • Shared eplets with previous transplant. • Second transplant. • Risk of antibody mediated rejection post-transplant.
High	**4 (8%)**	0	4 (14%)	• Immunised patient. • Previous transplant and transfusions. • Positive crossmatch.



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Question 5

As per laboratory policy the latest sample was also tested using Lifecodes Single Antigen kit. Results are shown below (all controls were within acceptable ranges). Donor X alleles have been highlighted, yellow=negative, red=positive:

Raw Value	Antigen	Raw Value	Antigen	Raw Value	Antigen
359	B35	192	A29(19)	151	B56(22)
348	B35	191	Cw1	150	B52(5)
315	B53	191	B65(14)	150	B61(40)
273	Cw4	190	A68(28)	149	A203
257	B54(22)	189	A68(28)	148	B41
245	Cw8	187	B2708	148	B72(70)
243	B75(15)	187	Cw10(w3)	148	B44(12)
238	A24(9)	186	Cw2	147	A11
220	Cw8	184	B82	146	B62(15)
218	Cw17	181	A43	145	A32(19)
217	Cw7	180	Cw18	144	A74(19)
217	B77(15)	180	Cw12	144	A34(10)
216	A1	179	Cw15	144	A66(10)
215	B71(70)	178	Cw16	143	Cw9(w3)
212	A2403	175	A31(19)	142	B47
208	B7	175	A33(19)	139	B59
206	Cw5	174	Cw14	139	B44(12)
205	Cw7	174	B63(15)	139	A29(19)
205	B46	173	B55(22)	138	B67
203	B27	173	B48	138	B45(12)
203	A2	167	A36	136	B51(5)
200	B42	166	B8	135	A26(10)
200	A2	163	Cw4	133	B81
200	A30(19)	162	B73	132	B50(21)
199	B13	162	B37	125	A25(10)
198	B64(14)	162	A66(10)	123	B60(40)
197	A69(28)	161	A23(9)	122	B58(17)
196	B3901	159	A33(19)	121	A3
196	B76(15)	158	B27	121	A11
195	Cw6	156	B38(16)	118	A80
193	B18	154	B78	114	B57(17)
192	B703	152	A2	113	B49(21)

Lifecodes Class I Single Antigen bead data for the sample date 14/04/2021

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Raw Value	Allele(s)		Raw Value	Allele(s)		Raw Value	Allele(s)	
24523	DQA1*05:01	DQB1*03:01	482	DRB3*02:02		190	DPA1*02:02	DPB1*01:01
24293	DQA1*06:01	DQB1*03:03	464	DRB1*14:03		189	DQA1*02:01	DQB1*02:02
23330	DQA1*04:01	DQB1*03:03	448	DRB1*03:02		189	DQA1*01:02	DQB1*05:01
23238	DQA1*06:01	DQB1*04:02	415	DRB3*01:01		185	DQA1*01:04	DQB1*05:03
22608	DQA1*06:01	DQB1*03:01	379	DQA1*02:01	DQB1*03:02	185	DPA1*02:01	DPB1*01:01
22573	DQA1*04:01	DQB1*04:02	322	DQA1*01:02	DQB1*06:04	181	DRB1*07:01	
22390	DQA1*05:01	DQB1*04:01	314	DRB3*03:01		180	DPA1*02:01	DPB1*13:01
22367	DQA1*03:01	DQB1*03:02	303	DRB1*01:03		178	DRB1*16:01	
22206	DQA1*03:02	DQB1*03:03	289	DQA1*01:01	DQB1*05:01	177	DRB1*01:01	
21288	DQA1*03:01	DQB1*04:02	273	DQA1*01:02	DQB1*05:02	177	DPA1*03:01	DPB1*04:02
21220	DQA1*04:01	DQB1*04:01	261	DRB5*02:02		174	DRB1*16:02	
20704	DQA1*03:01	DQB1*03:01	261	DPA1*02:02	DPB1*28:01	171	DRB1*01:02	
19935	DQA1*03:02	DQB1*03:02	258	DPA1*03:01	DPB1*01:01	168	DPA1*01:03	DPB1*04:02
19293	DQA1*05:01	DQB1*02:02	257	DRB1*15:01		168	DPA1*03:01	DPB1*04:01
18846	DQA1*03:02	DQB1*03:01	256	DPA1*02:02	DPB1*05:01	163	DQA1*02:01	DQB1*02:01
18088	DQA1*05:01	DQB1*02:01	250	DRB1*10:01		162	DQA1*01:02	DQB1*06:02
11813	DQA1*03:02	DQB1*02:02	248	DRB1*04:05		160	DQA1*01:03	DQB1*06:03
3692	DRB1*08:02		246	DPA1*01:03	DPB1*01:01	159	DPA1*01:03	DPB1*04:01
3610	DRB1*14:04		245	DRB4*01:01		156	DPA1*02:02	DPB1*04:01
3351	DRB1*08:01		234	DRB1*04:04		151	DPA1*03:01	DPB1*05:01
3141	DQA1*02:01	DQB1*04:01	228	DPA1*02:01	DPB1*14:01	150	DPA1*01:03	DPB1*03:01
1088	DRB1*12:02		225	DRB1*04:03		146	DPA1*02:01	DPB1*04:01
1033	DRB1*12:01		224	DQA1*02:01	DQB1*06:01	144	DQA1*01:04	DQB1*06:01
726	DRB1*11:04		220	DPA1*02:01	DPB1*05:01	142	DPA1*01:03	DPB1*02:01
672	DRB1*13:05		219	DPA1*02:01	DPB1*15:01	138	DPA1*02:01	DPB1*17:01
666	DRB1*11:01		218	DRB5*01:01		132	DPA1*01:03	DPB1*18:01
653	DRB1*13:03		217	DRB1*04:02		121	DPA1*02:01	DPB1*11:01
581	DRB1*14:01		205	DRB1*04:01		120	DPA1*04:01	DPB1*13:01
561	DRB1*13:01		204	DRB1*15:02		116	DPA1*02:01	DPB1*09:01
528	DRB1*11:03		198	DRB1*09:01		110	DQA1*01:03	DQB1*06:01
515	DRB1*03:03		193	DPA1*04:01	DPB1*04:01	104	DPA1*02:01	DPB1*19:01
510	DRB1*03:01		191	DRB1*15:03		87	DPA1*01:03	DPB1*06:01

Lifecodes Class II Single Antigen bead data for the sample date 14/04/2021

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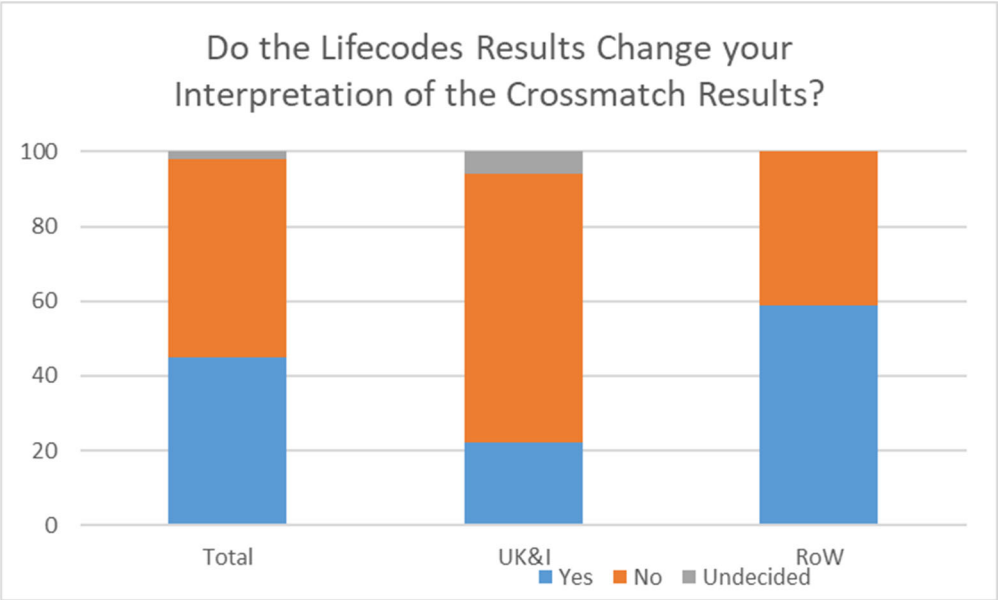
5.1/5.2 Do the Lifecodes results provided change the interpretation of the crossmatch test? Give your reasons.

Response	Total (%)	UK&I (%)	RoW (%)	Reasons
Yes	**21 (45%)**	4 (22%)	17 (59%)	• HLA-DRB1*14:04 DSA at MFI of 3610 explains positive B cell crossmatch. • HLA incompatible. • HLA-DPB1*04:01 negative on Lifecodes, potential false positive on One Lambda. • As DPB1*04:01 negative, crossmatch positivity possibly due to non-HLA.
No	**25 (53%)**	13 (72%)	12 (41%)	• Results are supportive of a positive crossmatch. • The already suspected presence of a DRB1*14:04 donor specific antibody has been confirmed. • DRB1*14:04, DPA1*02:07 and DQB1*05:03 donor alleles are not present on the One Lambda panel but are present in the Immucor kit. • Lifecodes results suggest antibody against epitope 16Y.
Undecided	**1 (2%)**	1 (6%)	0	• Further investigation required to determine whether the DRB1*14:04 antibody is only present in the sera causing the positive crossmatch and repeat crossmatch.



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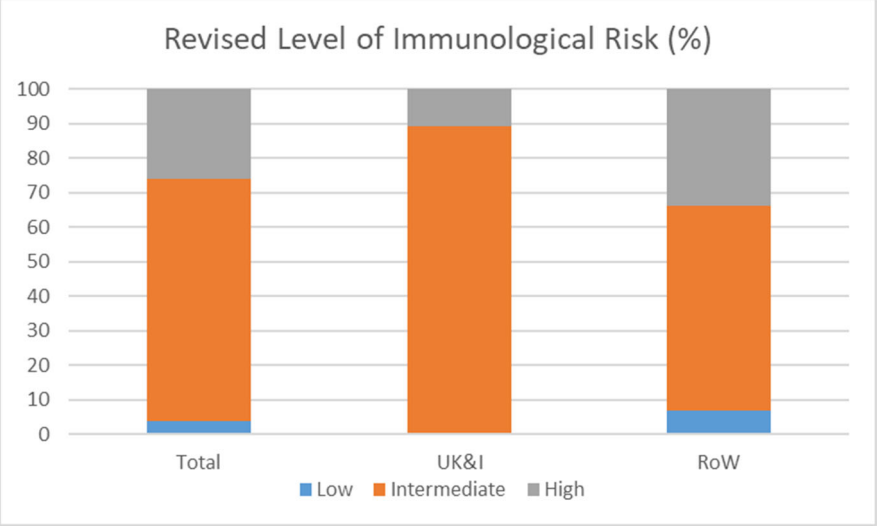
5.3/5.4 What level of immunological risk would you now assign?

Response	Total (%)	UK&I (%)	RoW (%)	Reasons
Low	**2 (4%)**	0	2 (7%)	• MFI <5,000. • No DSA detected, only potential weak DPA1 antibodies.
Intermediate	**33 (70%)**	16 (89%)	17 (59%)	• HLA-DR donor specific antibody. • CDCXM negative. • FCXMT cell negative, B cell positive. • DSA (CII IgG) present at time of transplant. • DSA has low MFI. • Risk of antibody mediated rejection post-transplant. • Oversensitivity not noted in Lifecodes test makes antibodies detected by this kit more clinically relevant. • Non-HLA antibodies.
High	**12 (26%)**	2 (11%)	10 (34%)	• Highly immunised patient. • Re-transplant. • Positive B cell FCXM with circulating DSA. • Donor DPA1*02:07 not represented on single antigen panels.



5.5 What would suggest, if anything, to the clinical team to increase the chances of a successful transplant?

Further Testing Identified	Total (%)	UK&I (%)	RoW (%)
Enhanced immunosuppression	**26 (30%)**	17 (36%)	9 (23%)
Regular post-transplant monitoring	**24 (28%)**	15 (32%)	9 (23%)
Desensitisation	**22 (26%)**	8 (17%)	14 (36%)
Source alternate donor	**14 (16%)**	7 (15%)	7 (18%)

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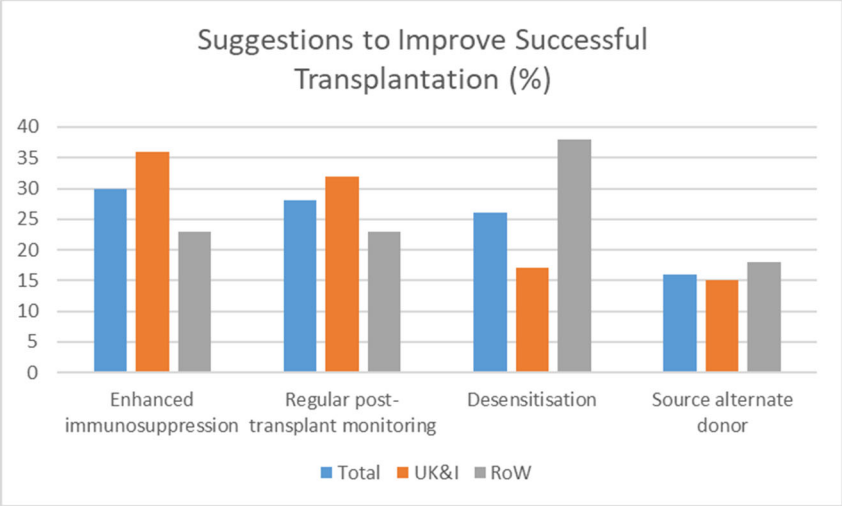

Question 6

A potential live donor, Donor Y, was identified from the social media campaign. Donor Y, a 24 year old male, was deemed medically fit to donate.
Donor Y's HLA Type:
HLA-A2, A29; B44, B57; Cw6, Cw16; DR7, DR17; DQ2, DQ9; DQA1*02, DQA1*05; DPB1*04:01, DPB1*20:01
ABO O Rh+
A cytotoxic and flow cytometry crossmatch against the patient was negative.

6.1/6.2 What level of immunological risk would you assign to this transplant? Give your reasons.

Risk Level	Total (%)	UK&I (%)	RoW (%)	Reasons
Low/Standard	**10 (21%)**	9 (50%)	1 (3%)	• FCXM negative. • Potential DSA. • DQA antibodies (allele type of donor not known). • DQA1*05 shares epitope with DQA1*04 (mismatch from previous transplant). • Clinical relevance of DQA DSA questionable.
Intermediate	**7 (15%)**	1 (6%)	6 (21%)	• Negative crossmatch. • Presence of DSA (DQ2 and DQA1*05). • Potential for antibody mediated rejection.
High	**30 (64%)**	8 (44%)	22 (76%)	• Crossmatch negative but potential prozone effect. • DQ, DQA and DPB1*04:01 DSA with high MFI. • Poor local experience of transplant across DQ antibodies. • Patient sensitisation history. • Risk of antibody mediated rejection.

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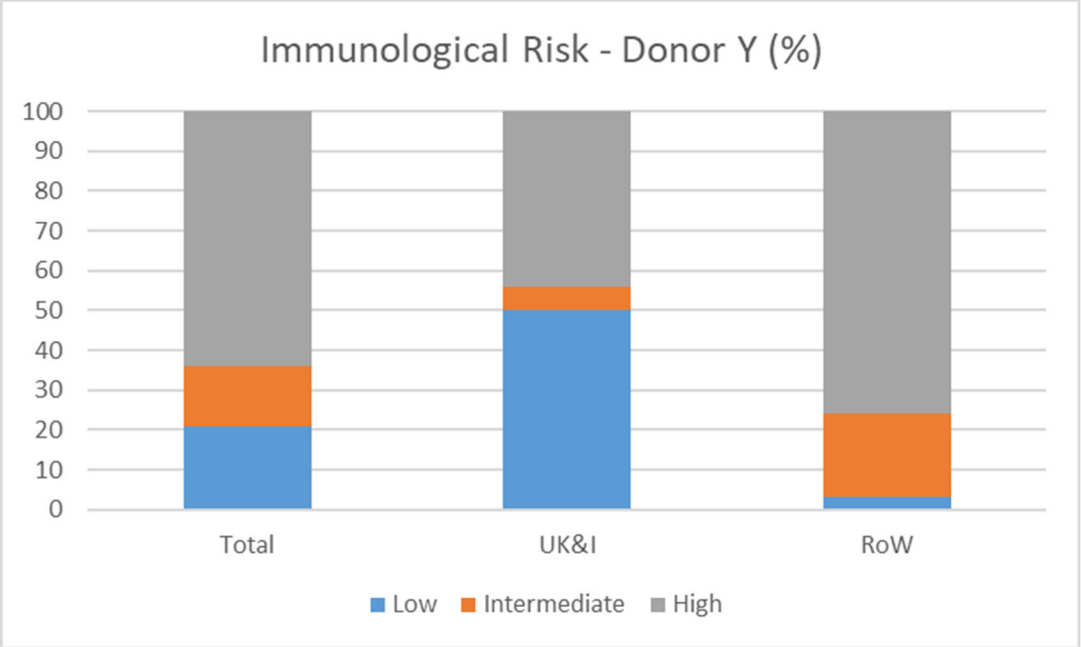

6.3/6.4 Which donor would you preferentially select for this patient? Give your reasons.

Response	Total (%)	UK&I (%)	RoW (%)	Reasons
Donor X	**28 (60%)**	9 (50%)	19 (65%)	• DSA present but <5,000 MFI. • No DSA. • Better HLA compatibility - less impact on finding future donors for paediatric patient (Donor Y A2 mismatch). • CDC crossmatch negative. • Can utilise pre-transplant desensitisation. • Questionable validity of crossmatch results for Donor Y.
Donor Y	**15 (32%)**	9 (50%)	6 (21%)	• Negative crossmatch. • No donor chain. • DQA DSA not clinically significant. • Detected antibodies may not be complement binding. • Crossmatch more indicative of donor antigen expression than single antigen testing.
No response	**4 (8%)**	0	4 (14%)	• Neither donor suitable. • DSA to both donors. • Need further testing to assess suitability.

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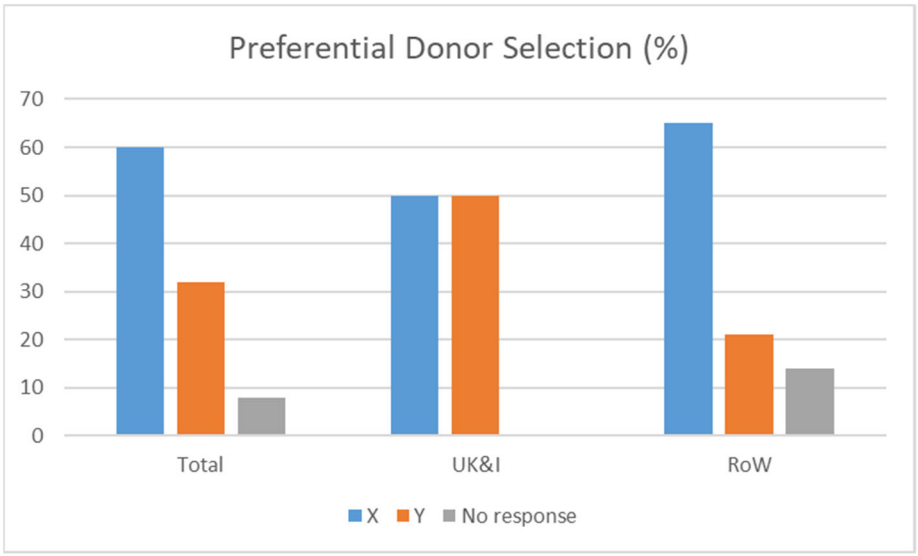
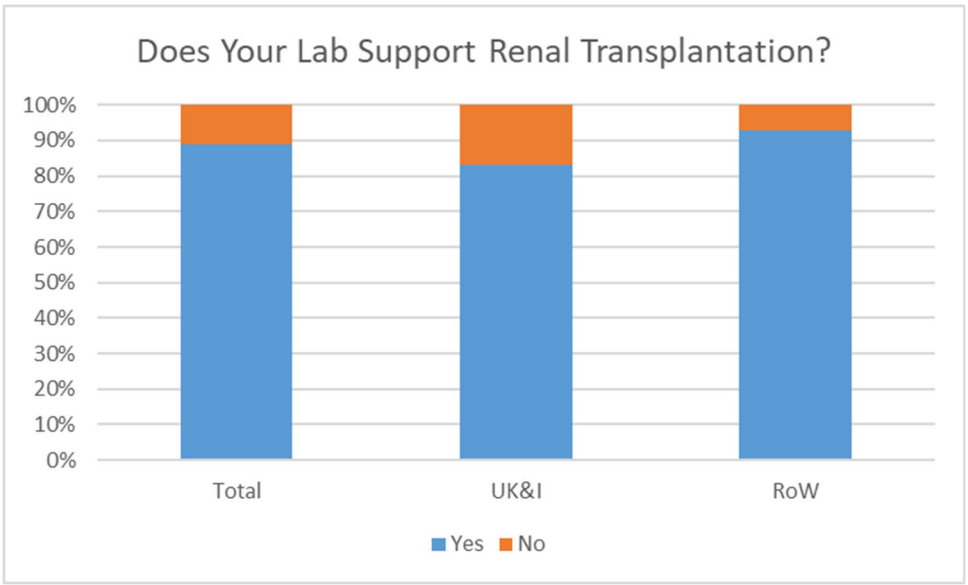


7 Does your centre provide services to support renal transplantation?

Response	Total (%)	UK&I (%)	RoW (%)
Yes	**42 (89%)**	15 (83%)	27 (93%)
No	**5 (11%)**	3 (17%)	2 (7%)



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General Comments

- These transplant situations would require extensive discussions with the clinical team. The paediatric patient will require multiple future transplants and an effort ought to be made to minimise additional sensitisation. Although Donor Y is preferable considering risk level, consider also the MFI of the donor-specific antibodies, and that e.g. HLA-A2,B44 mismatches will likely limit future transplant opportunities.
- BSHI/BTS guidelines describe risk as 'standard, intermediate and high'. This NEQAS paper asks us to grade as 'low, intermediate and high'... this is not the same thing.
- We would recommend continued search for a live donor for this patient, either for direct transplantation or entry into the national sharing scheme. Explore further options before progressing with Donor Y. Information about the recipient's clinical situation would have been helpful in this scenario, e.g. whether they are stable on dialysis, and also compliance with medication as the latter has implications for DSA resurgence/development.
- Good case highlighting value of analysing epitopes, high resolution extended typing and use of multiple different commercial kits.
- Crossmatching of Donor Y would not have been performed by our centre due to the Single Antigen Class II assay results showing potential DSA to the HLA-DQA1*05:01 beads at such a strong level.
- Crossmatch with Donor Y negative, therefore lower immunological risk due to negative crossmatch despite strong DSA detected. However neither donor is ideal for a young recipient – suggest investigating KSS or other live donor options. Would also DQA1 type the mother that was the donor from the previous transplant to determine whether patient's DQA1 antibodies are donor-specific.
- Donor X is not standard risk but is not contraindicated. Donor Y has a Negative crossmatch which should make them standard risk but the negative crossmatch result needs to be explained possibly using high resolution HLA typing. The donor Y crossmatch needs to be repeated before the level of risk can be ascertained.
- Would have been good to know the following about the patient: diagnosis; cause of transplant failure; whether allograft has been removed; if patient remained on immunosuppression after graft failure (in relation to serum sample dates); what transfusions were given and when.
- Social media campaign to find a living donor is forbidden in our country.
- DSA anti HLA-DQA1*05:01 may not cytotoxic (based on negative Flow and CDC crossmatch) but we wouldn't risk such a transplant with Single Antigen based strong DSA in our Center.
- The results of the FXCM are very surprising in these two cases: A positive FXCM with very low titre donor-specific HLA antibodies could also imply the presence of non-HLA antibodies - a negative FXCM with DSA (MFI >25000) then seems quite implausible.
- In France we don't use living donors from media campaign, only from family or friends.
- We would have concerns about both of these donors and further sensitisation of the patient, especially given his age and the likely need for future transplants. Our laboratory does not use LifeCodes so we are unfamiliar with cut-offs and therefore interpreting results is difficult.

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Comments and suggested results from H&I experts providing scenario

Question 1

We would advise prioritising potential donors by initial health screening then selecting young ABO O and A donors. If the patient is based in the UK we would also recommend that, due to the breadth of sensitisation meaning most donors would be HLA incompatible, priority is given to the ABO blood group O donors to increase the likelihood of receiving a match in the UK Living Kidney Sharing Scheme (https://www.odt.nhs.uk/living-donation/uk-living-kidney-sharing-scheme/). The recipient is ABO A and UK data shows that recipient A with donor O has the greatest chance of a match within the Scheme.

Question 2

We do not feel a virtual crossmatch is appropriate as there is insufficient donor HLA typing information to make the assessment. The patient has DQA1* antibodies and the donor DQA1* type is not known.
Further tests could include:

- Cytotoxic and flow crossmatch.
- Donor verification HLA typing.
- Recipient antibody testing using single antigen kits.

Question 3

Further tests required to interpret the crossmatch results could include verification of the donor HLA type including DQA and DPA genotyping.
We would suggest that sera dated 08/09/2014 could to be investigated for the presence of IgM alloantibodies as the CDC positive reaction was reduced by DTT.
We would also suggest that sera dated 14/04/2021 could be investigated for IgG alloantibodies due to the positive FCXM result.

Question 4

We would classify this crossmatch as positive due to the positive flow cytometry B cell result in the current sample. Using the current BSHI/BTS guidelines (https://bshi.org.uk/wp-content/uploads/2019/05/BSHI_BTS_Ab_Guidelines_Revision_June_2014.pdf) this would be classified as Standard Risk transplant (a positive FCXM in current sample with negative Luminex single antigen bead result).

The allele level typing reveals the donor is DRB1*14:04 and there is no cognate bead on the LABScreen panel. The DQA typing has ruled out FCXM reactivity due to DQA antibodies that the patient was known to have.

There is also a potential DPB1*04:01 donor specific antibody.

Question 5

We concluded that the Lifecodes results do change the interpretation of the crossmatch as the presence of a DRB1*14:04 bead on the Lifecodes panel has enabled the detection of a donor directed antibody.

Local experience with the Lifecodes Single Antigen assay has shown that the MFI values are generally much lower than those in the One Lambda assay. From our testing the MFI level of the DRB1*14:04 bead could equate to a positive B cell FCXM. We therefore concluded that the FCXM was positive due to donor directed antibody.
Following the BSHI/BTS guidelines a positive flow cytometry crossmatch in a current sample due to IgG HLA class II antibody is classed as Intermediate Risk.

To increases the chances of successful transplant we suggest the follow:

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- Enhanced immunosuppression including induction therapy.
- Desensitisation.
- Post plasma exchange antibody screen to assess the effect of the plasma exchange on an antibody titre.
- Regular post-transplant DSA monitoring.

Question 6

This scenario presents a negative crossmatch with detectable donor specific antibodies to HLA-DQ2, DQA1*05 in both One Lambda and Lifecodes kits and a possible DPB1*04:01 antibody positive only in the One Lambda assay. There is also reactivity to DQ9 (a self-antigen) that is potentially due to a DQA antibody but requires further investigation. We would consider repeating the crossmatch to verify the negative result. Also it could be informative to perform third party testing against other cells expressing DQ2 and DQA1*05.

We would consider this Intermediate immunological risk. In our experience we have noted crossmatch negative results in cases where DQ antibodies with high MFI values have been present.

Follow up on the patient:

In the case that this scenario was based on Donor X was being assessed as the potential donor.

At the point where it was determined that the positive B cell crossmatch was positive due to the allele specific HLA-DRB1*14:04 donor directed antibody as defined by Lifecodes the case was discussed with the clinical team.

The clinical team felt the patient's chance of a transplant was severely limited by the breadth of his class II sensitisation (high titre antibodies to all DQ antigens except self) and therefore, following discussion with the family, they wanted to proceed with transplant.

The patient had two sessions of plasma exchange and IvIg the day before the transplant. The transplant went ahead and the patient is very well 3 months post-transplant. His current SCr is 72 and the MFI of the donor directed bead in the Lifecodes assay is currently below the test cut off at 424.