

DIRECT DETECTION OF DONOR-DERIVED EXTRACELLULAR VESICLES IN KIDNEY TRANSPLANTATION: TOWARDS CLINICAL APPLICABILITY

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SUPPLEMENTARY FILES

Supplementary Data S1

LUMC

Class I Single Antigen Results

Batch ID: L1_LC_24-08-2021_I

Patient Name: A3-PE 150

Patient DOB:

Draw Date:

Donor Number:

Expiration Date:14-01-2022

Sample ID: A3-PE 150

Lot ID: 3010654 3010500-SA1

Donor Center # :

Accession # :

Patient HLA Type:

Report By: Lab Supervisor

Method Type: Standard Calculation

Positive CON MFI : 1

Negative CON MFI : 0

Analysis Mode: Manual

% PRA: 1

Bead Count	Antigen ID	Cut-off	Raw Value	MFI/LRA	Assignment	BG Adjusted	AD-MFI	AD-BG Adjusted	A	B	C	Bw	A Serology	B Serology	C Serology	Epitopes
72	109	3.49	8722	8722.00	Positive	8554	7565	7419	A*03:01				A3			161D
82	191	3.61	11	11.00	Negative	-276	20	-495.51			C*07:02				Cw7	
97	181	4.04	10	10.00	Negative	-187	9	-171.72		B*82:02		Bw6				
82	170	3.98	9	9.00	Negative	-201	9	-197.64		B*53:01		Bw4		B53		
78	190	3.65	8	7.50	Negative	-288	12	-472.09			C*07:01				Cw7	
80	180	3.73	7	7.00	Negative	-174	6	-160.81		B*81:01		Bw6		B81		
81	198	3.95	7	7.00	Negative	-350	12	-602.41			C*17:01					
76	199	3.45	7	7.00	Negative	-254	9	-329.44			C*18:01					
86	159	4.30	6	6.00	Negative	-234	6	-231.45		B*42:01		Bw6		B42		
104	169	4.34	6	6.00	Negative	-222	6	-212.85		B*52:01		Bw4		B52(5)		
96	179	3.90	5	5.00	Negative	-199	5	-193.02		B*78:01		Bw6		B78		
60	197	3.41	5	5.00	Negative	-245	7	-361.36			C*16:01					
80	149	3.83	4	4.00	Negative	-192	4	-172.20		B*27:05		Bw4		B27		
95	168	4.26	4	4.00	Negative	-210	4	-205.68		B*51:01		Bw4		B51(5)		
83	189	3.34	4	4.00	Negative	-268	4	-291.94			C*06:02				Cw6	
78	196	3.35	4	4.00	Negative	-242	5	-292.62			C*15:02					
83	148	3.84	3	3.00	Negative	-184	2	-152.82		B*27:03		Bw4		B27		
94	157	4.04	3	3.00	Negative	-204	3	-185.29		B*40:02		Bw6		B61(40)		
82	158	4.31	3	3.00	Negative	-231	3	-250.81		B*41:01		Bw6		B41		
83	167	3.99	3	3.00	Negative	-193	3	-166.24		B*50:01		Bw6		B50(21)		
98	177	3.40	3	3.00	Negative	-153	2	-123.19		B*67:01		Bw6		B67		
62	178	4.21	3	3.00	Negative	-209	3	-196.61		B*73:01				B73		
97	185	3.47	3	3.00	Negative	-260	3	-269.99			C*03:04				Cw10(w3)	
95	188	3.64	3	3.00	Negative	-289	5	-493.17			C*05:01				Cw5	
78	195	3.39	3	3.00	Negative	-252	3	-278.15			C*14:02					
96	176	3.73	3	2.50	Negative	-183	2	-165.76		B*59:01		Bw4		B59		
103	138	3.99	2	2.00	Negative	-187	2	-158.88		B*14:01		Bw6		B64(14)		
79	139	4.73	2	2.00	Negative	-262	2	-260.44		B*14:02		Bw6		B65(14)		
77	147	3.90	2	2.00	Negative	-195	2	-170.45		B*18:01		Bw6		B18		
84	156	3.69	2	2.00	Negative	-176	2	-150.04		B*40:01		Bw6		B60(40)		
93	161	4.27	2	2.00	Negative	-228	2	-193.71		B*44:03		Bw4		B44(12)		
81	164	3.93	2	2.00	Negative	-201	2	-185.08		B*47:01		Bw4		B47		
98	165	4.38	2	2.00	Negative	-270	3	-355.26		B*48:01		Bw6		B48		
104	166	3.80	2	2.00	Negative	-179	2	-148.55		B*49:01		Bw4		B49(21)		
106	173	4.00	2	2.00	Negative	-194	2	-181.99		B*56:01		Bw6		B56(22)		
94	174	3.76	2	2.00	Negative	-186	2	-160.62		B*57:01		Bw4		B57(17)		
81	175	4.06	2	2.00	Negative	-204	2	-189.59		B*58:01		Bw4		B58(17)		
76	182	3.41	2	2.00	Negative	-266	2	-301.59			C*01:02				Cw1	
83	184	3.44	2	2.00	Negative	-252	2	-290.32			C*03:03				Cw9(w3)	
102	186	3.86	2	2.00	Negative	-330	6	-988.02			C*04:01				Cw4	
70	187	3.42	2	2.00	Negative	-259	3	-331.63			C*04:03					
86	192	3.29	2	2.00	Negative	-236	2	-290.64			C*08:01				Cw8	
86	194	3.51	2	2.00	Negative	-252	2	-268.09			C*12:02					
100	117	3.94	2	1.50	Negative	-212	2	-241.16	A*29:01				A29(19)			
76	183	3.55	2	1.50	Negative	-269	3	-500.00			C*02:02				Cw2	
72	108	4.03	1	1.00	Negative	-215	1	-197.43	A*02:05				A2			
100	110	3.64	1	1.00	Negative	-169	1	-146.07	A*11:01				A11			
81	111	3.77	1	1.00	Negative	-195	1	-180.56	A*11:02				A11			
73	114	3.74	1	1.00	Negative	-190	1	-166.96	A*24:03			Bw4	A2403			
81	115	3.47	1	1.00	Negative	-172	1	-157.08	A*25:01			Bw4	A25(10)			
82	116	3.65	1	1.00	Negative	-187	1	-178.61	A*26:01				A26(10)			
93	118	4.07	1	1.00	Negative	-220	1	-213.59	A*29:02				A29(19)			
78	119	4.02	1	1.00	Negative	-213	1	-217.57	A*30:01				A30(19)			
90	120	4.31	1	1.00	Negative	-234	1	-237.08	A*31:01				A31(19)			
88	121	3.73	1	1.00	Negative	-186	1	-190.96	A*32:01			Bw4	A32(19)			
73	122	3.89	1	1.00	Negative	-204	1	-221.26	A*33:01				A33(19)			
80	123	4.23	1	1.00	Negative	-231	1	-230.77	A*33:03				A33(19)			

88	124	3.82	1	1.00	Negative	-194	1	-199.79	A*34:02				A34(10)			
97	125	4.06	1	1.00	Negative	-215	1	-202.64	A*36:01				A36			
85	126	4.25	1	1.00	Negative	-217	1	-217.00	A*43:01				A43			
82	128	3.60	1	1.00	Negative	-167	1	-143.47	A*66:02				A66(10)			
100	129	3.70	1	1.00	Negative	-190	1	-192.31	A*68:01				A68(28)			
94	130	3.51	1	1.00	Negative	-168	1	-152.17	A*68:02				A68(28)			
90	134	4.61	1	1.00	Negative	-253	1	-233.61		B*07:02		Bw6	B7			
90	135	4.09	1	1.00	Negative	-196	1	-170.88		B*07:03		Bw6	B703			
88	136	3.71	1	1.00	Negative	-177	1	-144.61		B*08:01		Bw6	B8			
75	137	4.25	1	1.00	Negative	-214	1	-219.26		B*13:02		Bw4	B13			
92	140	3.59	1	1.00	Negative	-171	1	-138.46		B*15:01		Bw6	B62(15)			
68	142	3.82	1	1.00	Negative	-186	1	-149.88		B*15:03		Bw6	B72(70)			
94	143	3.95	1	1.00	Negative	-189	1	-149.76		B*15:12		Bw6	B76(15)			
72	144	4.17	1	1.00	Negative	-212	1	-200.19		B*15:13		Bw4	B77(15)			
65	145	4.32	1	1.00	Negative	-205	1	-169.28		B*15:16		Bw4	B63(15)			
92	146	3.92	1	1.00	Negative	-184	1	-148.51		B*15:18		Bw6	B71(70)			
92	150	3.56	1	1.00	Negative	-149	1	-107.27		B*27:08		Bw6	B2708			
79	151	4.17	1	1.00	Negative	-227	1	-228.83		B*35:01		Bw6	B35			
83	152	4.28	1	1.00	Negative	-233	1	-256.33		B*35:08		Bw6	B35			
78	153	3.78	1	1.00	Negative	-186	1	-161.46		B*37:01		Bw4	B37			
83	155	3.66	1	1.00	Negative	-167	1	-136.44		B*39:01		Bw6	B3901			
82	160	4.07	1	1.00	Negative	-208	1	-190.48		B*44:02		Bw4	B44(12)			
98	162	4.17	1	1.00	Negative	-214	1	-231.60		B*45:01		Bw6	B45(12)			
82	163	5.05	1	1.00	Negative	-303	1	-388.96		B*46:01			B46			
87	171	3.91	1	1.00	Negative	-194	1	-175.25		B*54:01		Bw6	B54(22)			
74	172	4.29	1	1.00	Negative	-239	1	-258.10		B*55:01		Bw6	B55(22)			
81	193	3.52	1	1.00	Negative	-268	1	-342.71			C*08:02				Cw8	
90	103	4.00	1	0.50	Negative	-217	0	-209.38	A*01:01				A1			
93	104	3.82	0	0.00	Negative	-204	0	-219.12	A*02:01				A2			
100	106	3.89	0	0.00	Negative	-204	0	-185.45	A*02:02				A2			
101	107	3.64	0	0.00	Negative	-198	0	-182.49	A*02:03				A203			
111	112	3.52	0	0.00	Negative	-174	0	-177.37	A*23:01			Bw4	A23(9)			
106	113	3.83	0	0.00	Negative	-189	0	-179.49	A*24:02			Bw4	A24(9)			
71	127	3.56	0	0.00	Negative	-168	0	-144.70	A*66:01				A66(10)			
106	131	4.19	0	0.00	Negative	-229	0	-275.57	A*69:01				A69(28)			
108	132	3.77	0	0.00	Negative	-191	0	-194.90	A*74:01				A74(19)			
103	133	3.99	0	0.00	Negative	-229	0	-220.40	A*80:01				A80			
85	141	3.98	0	0.00	Negative	-202	0	-186.18		B*15:02		Bw6	B75(15)			
73	154	3.76	0	0.00	Negative	-184	0	-154.36		B*38:01		Bw4	B38(16)			

Reviewer Comment:

Date:

Completed By:

Date:

Approved By:

Date:

Reviewed By:

Immucor, Inc.

MATCH IT! Antibody v1.3.1

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Sample ID: A3-PE 150

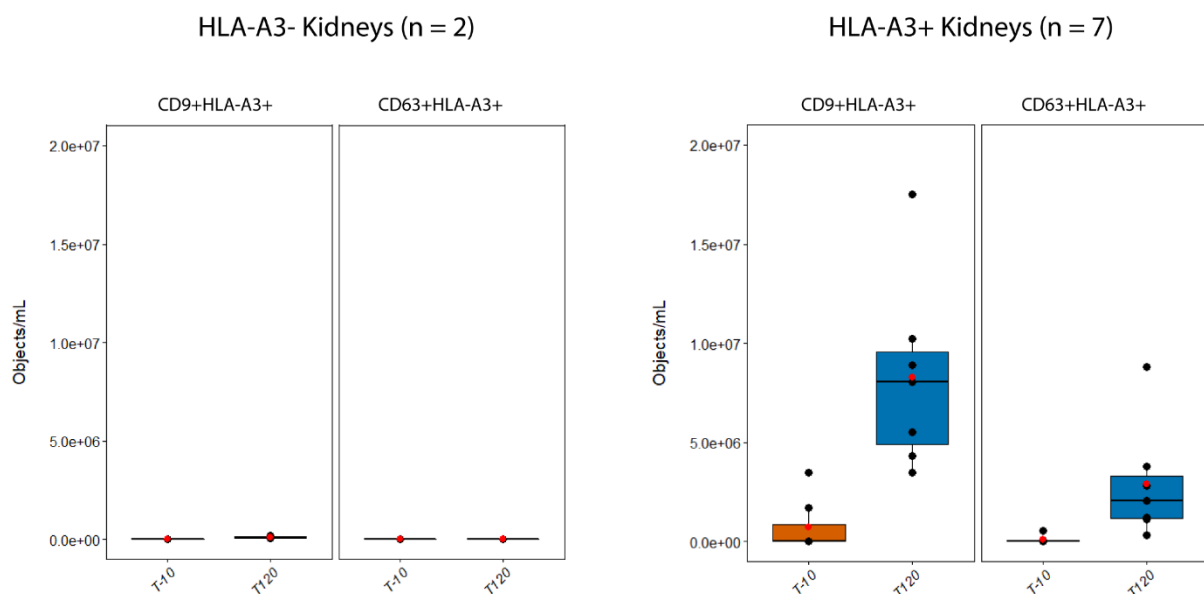
Patient Name: A3-PE 150

Draw Date:

Report Date: 24-08-2021

Supplementary Figure S1

Before transplantation, 9 kidneys were subjected to Normothermic Machine Perfusion (NMP). NMP is an (experimental) organ-preservation technique in which explanted kidneys are connected to a machine which pumps a 37 °C preservation fluid through the organ. Because of this, the (cells of) the kidney become metabolically active, and excrete/release various components into the perfusion fluids, including EVs ¹. This allows the analysis of kidney-derived EVs without interference of EVs from other sources. Perfusion fluids were labeled with 200 ng/mL anti-HLA-A3-BV421 (clone GAP-A3, 200 µg /mL, BD Biosciences, New York, USA) and either 200 ng/mL anti-CD9-APC (clone HI9a, 6 µg/mL, Biolegend, San Diego, USA) or 6.6 µg/mL anti-CD63-APC (clone H5C6, 200 µg /mL, Biolegend, San Diego, USA).



Supplementary Figure S1 – Co-localization of HLA-A3 with CD9 and CD63. Nine kidneys (2x HLA-A3-, 7x HLA-A3+) were subjected to NMP. Perfusate samples taken before (T-10) and 2 hours after (T120) NMP were labelled with anti-CD9 or anti-CD63 in combination with anti-HLA-A3. In perfusion fluids of HLA-A3+ kidneys, an approximate 3-fold higher concentration of CD9+HLA-A3+ EVs was found compared to CD63+HLA-A3+ EVs.

Reference

1. Woud WW, Arykbaeva AS, Alwayn IPJ, Baan CC, Minnee RC, Hoogduijn MJ, et al. Extracellular Vesicles Released During Normothermic Machine Perfusion are Associated with Human Donor Kidney Characteristics. *Transplantation*. 2022.