

UK NEQAS FOR H&I SCHEME 3 - HLA SPECIFICITY ANALYSIS

Samples 301-05/2017 DESPATCHED ON 24TH JANUARY 2017

COMPLEMENT DEPENDENT CYTOTOXICITY (CDC) TECHNIQUE INFORMATION

Lab No	Technique	Cell source	Frozen trays	In-house/ Commercial	Panel		Complement source	Volume added (µl per well)				Incubation		Dead cell visualisation	Analysis Procedures	Software Manufacturer	Comments
					Composition	Size		Cells	Sera	Complement	DTT	Pre-C'	Post-C'				
20 42	NIH	B-cells	No		Random		Commercial	1	1	5	1	30+30	45	EB/AO	Computerised	In-house	
116 136	NIH	B-cells	Yes	Biorad	Selected	26	Biorad		2	5		60	140	EB/AO	Manual		
206 209 268	NIH	PBL	Yes	In-house	Selected		Cedarlane	1	1	6.6	1	30	90	Fluoroquench	Manual0		

EB - ethidium bromide
AO - acridine orange
PI - propidium iodide
Hb - haemoglobin
CFDA - carboxyfluorescein diacetate

FLOW CYTOMETRY TECHNIQUE INFORMATION

Lab No	Flow Cytometer Model	Cell		Conjugate	Manufacturer	Software	Manufacturer
		preparations (number)	source				
165 222	FACS Canto II	(32)	One Lambda FlowPRA™				

ELISA TECHNIQUE INFORMATION

Lab No	ELISA kit used	Batch No	Manufacturer	ELISA kit used	Batch No	Manufacturer	ELISA kit used	Batch No	Manufactu	Comments
117 165	LAT1288	007	One Lambda							

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LUMINEX TECHNIQUE INFORMATION

Lab No	Kit used	Used for All/Selected	Criteria	Batch no.	Bead vol (µl)	Serum vol (µl)	Negative Control Source	Serum Details	Details of +/- cut off	Kit used	Batch no.	Bead vol (µl)	Serum vol (µl)	Negative Control Source	Serum Details	Details of +/- cut off	Kit used	Batch no.	Bead vol (µl)	Serum vol (µl)	Negative Control Source	Serum Details	Details of +/- cut off
9																							
11																							
12																							
14																							
15																							
19																							
20																							
23																							
24	LS1PRA	ALL		016	3.5	14	Manufacturer		1000	LS2PRA	016	3.5	14	Manufacturer		1000	LSAI	08256N	20	5	Manufacturer		1500
25	LSAI	ALL		08256N	20	5	Manufacturer		1500	LSAII	09156A	20	5	Manufacturer		1500	LSAII	09156A	20	5	Manufacturer		1500
26																							
28																							
34																							
35	LS1PRA	Selected		016	2.5	20	Manufacturer		1000	LS2PRA	016	2.5	20	Manufacturer		1000							
36	LSAI	ALL		12255B			Manufacturer		750	LSAII	03296A			Manufacturer		750	LMX	3003946					1000
38																							
39																							
41																							
42																							
45																							
48	LM1	ALL		3003924	2	8	Manufacturer		None	LM2	3004223	2	8	Manufacturer		None							
51																							
54																							
58																							
62																							
100																							
112																							
114																							
116																							
117																							
119																							
120																							
133																							
136																							
142																							
143																							
145																							
147																							
149																							
157																							
160																							
162																							
164																							
165																							
169																							
181	LMX				5	12.5	Manufacturer	Manufacturer		LSAI		40	10	Manufacturer		ADJ. Val >2	LSAII		40	10	Manufacturer		ADJ. Val >2
185																							
195																							
197																							
204																							
206																							
209																							
214																							
216																							
218																							
222																							
227	LM1	ALL			5	12.5	Manufacturer	1000 Raw		LM2		5	12.5	Manufacturer		1000 Raw							
229																							
232																							
239																							
242																							
252																							
267																							
268																							
273																							
277																							
284																							
293																							
302																							
303																							
309																							
351																							
361																							

Lifecodes	One Lambda
LM1	Class I ID Kit
LM2	Class II ID Kit
LMX	Lifecodes Lifescreen Deluxe Kit
LSAI	Lifecodes Lifescreen SA Class I
LSAII	Lifecodes Lifescreen SA Class II
LSAI&II	Lifecodes Lifescreen SA Class I&II
LSAMIC	Lifecodes Lifescreen SA MICA
	LS1PRA
	LS2PRA
	LS12PRA
	LS1AD4
	LS2A01
	LSM12
	LSMUTR
	LABScreen PRA Class I
	LABScreen PRA Class II
	LABScreen PRA Class I&II
	LS SA Class I
	LS SA Class II
	LS Mixed Class I&II
	LABScreen Multi

Note: UK NEQAS for H&I have used participant and manufacturer's information to aid consistency with methodological information. Please report any errors to Deborah Pritchard on UKNEQASHandl@wales.nhs.uk

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LUMINEX TECHNIQUE INFORMATION

Lab No	1.2 Positive control serum						1.3			1.4 Serum treatment			1.5 Incubation times	Wash medium	Anything added	1.6 Wash step details				Buffer removed by	1.7 Data analysis				
	Positive control serum for Luminex testing?	Source	Every run?	If no:	Same serum?	If no:	Samples stored prior to testing?	Conditions?	Storage time? (hours)	Methods used?	Extra Information	Criteria?				Washes	First wash step		Second wash step		Software	Min bead count	Min pos control bead MFI	High background criteria	
																	Volume (µl)	Washes	Volume (µl)						Washes
9	Yes	Sensitised Patient	No	Included once a week of in batches	Yes		Sometimes	-20	>48	Dilution	Selected Samples	High background, high NC, multispecific with high MFI values	Manufacturer's Instructions	Supplied	No	5	200	5	200	Vacuum Manifold	HLA Fusion	50	500	High NC	
11	Yes	Pooled sensitised patient sera	Yes		No	Separate controls for LSM12, SABS Class I and II	Always	-40	>48	Adsorption Beads/EDTA	Selected Samples	Adsorb out used on samples with NC >1000	Manufacturer's Instructions	Supplied	No	3	200	2	200	Centrifuge	HLA Fusion	50	1000	>1000	
12	Yes	Sensitised Patient	Yes		No	Positive control sera tested with mixed bead assay. Not single antigen assay	Sometimes	-25	24-48	Adsorption Beads, EDTA, Dilution	Selected Samples	Samples with high background not removed by treatment with adsorb out	Manufacturer's Instructions	Supplied	No	5	175	5	175	Vacuum Manifold	HLA Fusion, xPONENT	50	1000	NC >500	
14	Yes	Sensitised Patient	Yes		No	Sensitised patient control sera for each kit	Sometimes	-20	>48	Heat inactivation, EDTA			Manufacturer's Instructions	Supplied	EDTA	3	200	2	200	Centrifuge	HLA Fusion	50	5000	Neg MFI >500 or Pos/Neg Ratio <2	
15	Yes	Pooled HSP sera	Yes		Yes		Yes	4	>48	Adsorption Beads, EDTA	Selected Samples		Manufacturer's Instructions	Supplied	EDTA	5	200	5	200	Vacuum Manifold	HLA Fusion	50	PC/NC >2	NC >1500	
19	20	Yes	Sensitised Patient	Yes	No	Specific sera selected relevant to kit used	Yes	-80	>48	Adsorption Beads, EDTA		AO used when high background (MFI >500)	Manufacturer's Instructions	Supplied	No	5	175	5	175	Vacuum Manifold	HLA Fusion	50	2000	MFI >500	
23	Yes	Pool of sensitised patients diluted 1 in 8	Yes		Yes		Always	4	>48	Adsorption Beads, EDTA	Selected Samples	Adsorb if NC >1500	Manufacturer's Instructions	Supplied	No	2	200	3	200	LabXpress	HLA Fusion	50	500	1500	
24	Yes	Sensitised Patient	Yes		Yes		Sometimes	-20/2-8	>48	Adsorption Beads, EDTA	Selected Samples	Adsorb samples with high background, EDTA added only for single antigen testing	Manufacturer's Instructions	Supplied	No	One Lambda:5 Immucor:4	One Lambda:200 Immucor:1x100, 3x250	One Lambda:5	200	Vacuum Manifold	HLA Fusion, MatchIT	50	3000	NC >500	
25	Yes	Immucor positive control	No	Only included in Immucor SAB runs	No	Only included in Immucor SAB runs	Sometimes	-30	24-48	Adsorption Beads, EDTA	Selected Samples	Treatment with seraclean if NC >500	Manufacturer's Instructions	Supplied	No	5	220	5	220	Vacuum Manifold	HLA Fusion	50	>500 and at least twice the NC	NC >500	
26	Yes	Patient sera	Yes		Yes		Yes	Frozen/Fridge (vanes)	<24 (varies)	Adsorption Beads, EDTA			Manufacturer's Instructions	Supplied	EDTA	5	200	5	200	Vacuum Manifold	HLA Fusion	50	5000	NC >500	
28	34	Yes	Sensitised Patient sera for One Lambda kits	Yes	No	Different pos con for Labscreen and Sag kits from One Lambda	Always	-40/-4	24-48	EDTA			Manufacturer's Instructions	Supplied	EDTA	5	200	5	200	Vacuum Manifold	Fusion 3	20	5000	NC >500	
35	Yes	Sensitised Patient sera	Yes		Yes		No			Adsorption Beads/EDTA	Selected Samples		Manufacturer's Instructions	Supplied	EDTA	3	200	2	200	Centrifuge	Fusion 3	50	500	Adsorb Out	
38	No						Sometimes	-30	>48	EDTA			Manufacturer's Instructions	Supplied	No	2	200	2	200	Centrifuge	HLA Fusion, MatchIT	80	1500	500	
39	Yes		Yes		Yes		Always	2-8	>48	Adsorption Beads/EDTA	Selected Samples		Manufacturer's Instructions	Supplied	EDTA	5	200	5	200	Vacuum Manifold	HLA Fusion	50	5000	NC >500	
41	Yes	In house pooled sensitised sera	No	On receipt of new delivery	No	Neat used for single antigen beads, 1:400 dilution used for screening beads	Always	-80	>48	Adsorption Beads/EDTA	Selected Samples	NC >1500	Manufacturer's Instructions	Supplied	No	4	200	3	200	Vacuum Manifold	Fusion 3	50	5000	>1500	
42	45	Yes			Yes					Adsorption Beads/EDTA	All Samples		Manufacturer's Instructions	Supplied	No	4	200	4	200		In House Software	50	5000	500	
48	Yes	Pooled sensitised patient sera used for Labscreen Mixed kits, Manufacturer's positive control is used for all other kits	Yes		No	Pooled positive control is only used for Labscreen Mixed Class I and II antibody testing	Sometimes	-20	24-48	Adsorption Beads/EDTA	Selected Samples	High background - Seraclean beads Heat inactivation - for use on Labscreen single antigen beads only	Manufacturer's Instructions	Supplied	No	3	250	3	150, then 2x at 250 (Labscreen only)	Vacuum Manifold	HLA Fusion, MatchIT	50	1000 for Labscreen mixed and single antigen beads, 10000 for Lifecodes C1 and 2, 2000 for Lifecodes single antigen beads	Sample with a negative control over 1000 for Labscreen, and the users discretion taking into account the manufacturer's suggested ranges for Lifecodes	
51	54	Yes	Sensitised patient sera	No	Only used in LSM12 mixed bead assay, not used in single antigen assays		Yes	However, different pos control is used for IgM antibody testing	Always	-20	>48	Adsorption Beads/EDTA	Selected Samples	Manufacturer's Instructions	Supplied	No	3	200	3	200	Centrifuge	HLA Fusion	40	1500	>750
58	Yes	Manufacturer/In house sensitised patient sera	Yes		No	Labscreen mixed - manufacturer and in house patient sera, Labscreen SA manufacturer only	Always	-15 to -20	>48	EDTA, Foetal calf serum added to 5% of total serum volume		EDTA treatment used for all SA testing. Foetal calf serum used to treat high background serum samples on second round testing	Manufacturer's Instructions	Supplied	No	5	150	5	150	Vacuum Manifold	HLA Fusion	40	>5000	Labscreen Mixed >500, Labscreen SA >1500	
62	Yes	Pool of highly sensitised patient sera/control provided with kit (Immucor)	Yes		No	In house positive control for One Lambda kits and control provided with the Immucor kit	Always	-70/2-8	24-48	Adsorption Beads			Manufacturer's Instructions	Supplied	No	5	200	5	200	Vacuum Manifold	Fusion 3.0, MatchIT	50	5000	>500	
100	Yes	Manufacturer	Yes		No	Positive control is assay specific	Always	-20	>48				Manufacturer's Instructions	Supplied	No	3	250			Vacuum Manifold	MatchIT	50	2000 with control serum	MFI > range for NC bead	

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112	Yes	Patient sensitised sera with low MFI value	Yes	No	One with each class	Always	-20	>48		Manufacturer's Instructions	Supplied	No	1	100	3	250	Vacuum Manifold, Centrifuge	HLA Fusion, MatchIT	60 Immucor, 100 One Lambda	12000 Immucor	Bead NC >3000
114	Yes	Sensitised patient sera	Yes	Yes		Sometimes	-20	24-48	EDTA	Manufacturer's Instructions	Supplied	No	3	160	2	160	Centrifuge	HLA Fusion	50	PC >1500	NC >500
116	No					Always	-20	>48	Adsorption Beads	Manufacturer's Instructions	Supplied	No	3	200	2	200	Centrifuge	HLA Fusion	50		NC >800
117	No					Always	-65	>48	Adsorption Beads	Manufacturer's Instructions	Supplied	No	3	200	3	200	Centrifuge	HLA Fusion	15	500	NC: 300-400
119	No					Yes	-20	>48	Adsorption Beads/EDTA	Manufacturer's Instructions	Supplied	EDTA	3	200	3	200	Centrifuge	HLA Fusion	50	100MFI	NEG >500
120	No		No	No		Always	-20	24-48	Adsorption Beads/Dilution	Manufacturer's Instructions	Supplied	No	3	1x150, 2x200	2	200	Centrifuge	Fusion 4.1	50	4000	NC >500
133						Sometimes	-20			Adsorption carried out when NC >500 and/or PC <4000. Dilution 1/8 carried out when adsorption is not sufficient to allow test validation according to PC/NC quality values	Supplied										300
136	Yes	Manufacturer	Yes	Yes		Always	Frozen	>48		Adsorption Beads/Heat Inactivation/Dilution?	Supplied	No	3	250	1	250	Vacuum Manifold	MatchIT	60	10000	
138	No					Always	<24	<24		Manufacturer's Instructions	Supplied	No	3	150	2	150	Centrifuge	HLA Fusion	50	>1000	>500
142	Yes	Manufacturer	Yes	No	Anti-HLA Class I with LS1A04, Anti HLA Class II with LS2A01	Sometimes	-20	24-48	EDTA	Manufacturer's Instructions	Supplied	No	5	175	5	175	Vacuum Manifold	HLA Fusion	50	500	NC >1500
143	No					Always	-20	>48		Manufacturer's Instructions	Supplied	No	3	150/200/200	2	200	Centrifuge	HLA Fusion	100	3000MFI	NC >1500MFI
145	No					Yes	-20	>48		Manufacturer's Instructions	Supplied	No	4	1x100, 3x250			Vacuum Manifold	MatchIT EXE	60	10000	High CON 1
147	Yes	Individuals shown to be alloimmunized to HLA antigens	Yes	No	According to current LOT number	Always	4	24-48		Manufacturer's Instructions	Supplied	No	4	1x100, 3x250			Vacuum Manifold	MatchIT EXE	60	10000	High CON 1
157	No					Always	4-6	>48	Adsorption Beads	Manufacturer's Instructions	Supplied	No	3	150/200/200	2	200	Centrifuge	HLA Fusion	100	5000MFI	
160	No					Always	4-6	>48	Adsorption Beads	Manufacturer's Instructions	Supplied	No	3	150/200/200	2	200	Centrifuge	HLA Fusion	100	5000MFI	
162	Yes	Sensitised patient sera	Yes	Yes		Sometimes	5	>48	EDTA	Manufacturer's Instructions	Supplied	EDTA	5	175	5	175	Vacuum Manifold	HLA Fusion	50	>500	NC >1500
165	Yes	Sensitised patient sera	Yes	No		Always		<24		Manufacturer's Instructions	Supplied	No	2	150	2	150	Centrifuge	HLA Fusion	100	500	
181	Yes	in House pool	Yes	Yes		Always	2-8	24-48		Manufacturer's Instructions	Supplied	No	3	250			Vacuum Manifold	MatchIT	690	Not defined, relative to standard PC provided by manufacturer	Manufacturer ranges
185	Yes		Yes	Yes		Always	-25	24-48	Adsorption/EDTA	Manufacturer's Instructions	Supplied	No	3	250			Vacuum Manifold	MatchIT	690	Not defined, relative to standard PC provided by manufacturer	Manufacturer ranges
195	Yes	Sensitised patient sera	Yes	No		Yes	Frozen	>48		Manufacturer's Instructions	Supplied	No	5	175	5	175	Vacuum Manifold	HLA Fusion	100	5000	>500
197	Yes	Pool of sensitised patients	Yes	Yes		Always	-20	>48	Adsorption/EDTA	Manufacturer's Instructions	Supplied	No	4	175	4	175	Vacuum Manifold	Fusion 4.0.1 and homemade Excel file	50	PC >5000	NC >500
206	Yes	Sensitised patient sera	Yes	Yes		Yes	-30	>48	Adsorption/EDTA	Manufacturer's Instructions	Supplied	EDTA	5	175	5	175	Vacuum Manifold	Fusion	50	3000	NC >500
214	Yes	Manufacturer	Yes	No	Kit-specific controls used, supplied by the manufacturer	Yes	Frozen/Fridge	24-48/>48		Manufacturer's Instructions	Supplied	No	3	250			Vacuum Manifold	MatchIT	100	5000	
216	Yes	Manufacturer	Yes	Yes		Sometimes	-80	>48	Adsorption/Heat Inactivation	Manufacturer's Instructions	Supplied	No	4	100-200			Vacuum Manifold	MatchIT	100	>10000	High reaction on CON beads, high MFIs without the beads being called positive by the software, or when most of the beads in the well have high MFIs or high adjusted values
222	Yes	Manufacturer, sensitised patient sera	Yes	Yes		Always	-40	>48	Adsorption/EDTA/Dilution	Manufacturer's Instructions	Supplied	No	5	175	5	175	Vacuum Manifold	HLA Fusion	50	PC >500 PC/NC >5	NC >500
227	Yes					Always	-40	>48	Adsorption/EDTA/Dilution	Only samples with high background are treated with adsorption beads	Supplied	No	5	175	5	175	Vacuum Manifold	HLA Fusion	50	PC >500 PC/NC >5	NC >500
229	Yes	Sensitised patient sera	Yes	Yes		Always	-60	>48		Manufacturer's Instructions	Supplied	No	5	275	5	275	Vacuum Manifold	Fusion	80	5000	NC >2000
232	Yes					Always	4	>48		Manufacturer's Instructions	Supplied	No	4	1 x 100 x 250	3		Vacuum Manifold	MatchIT	60	PC >10000	>1000
239	Yes	Class I positive control for LMI, Class II positive control for LMII	Yes	No		Always	4	>48		Manufacturer's Instructions	Supplied	No	4	1 x 100 x 250	3		Vacuum Manifold	MatchIT	60	PC >10000	>1000
242	No					Always	-20	>48	Adsorption Beads	Manufacturer's Instructions	Supplied	No	1	150	2	200	Centrifuge	HLA Fusion	100	15000	

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252	Yes	Commercial	Yes		No	Each commercial kit has its own positive control	Yes	-20	>48		The sera are used undiluted	Manufacturer's Instructions	Supplied	No	1/3	100, 250			Vacuum Manifold	MatchIT		10000	
267	Yes	Provided with kit	Yes		No	Each commercial kit has positive serum included	Always	-30	>48	EDTA		Manufacturer's Instructions	Supplied	No					Vacuum Manifold	MatchIT			
268					Yes		Always	4	24-48			Manufacturer's Instructions	Supplied	No	5	175	5	175	Vacuum Manifold	HLA Fusion	50	>500	>1500
273	Yes		Yes		Yes		Always	-30	24-48	EDTA		Manufacturer's Instructions	Supplied	No	3	150, 200, 200	2	200	Centrifuge	xPonent 3/ Fusion 3	100	500; PC/NC >2	NC >1500
277	Yes	UK NEQAS sample, tested last year	No	Once for lot control	Yes			-30	24-48	EDTA		Manufacturer's Instructions	Supplied	No	3	150, 200, 200	2	200	Centrifuge	xPonent 3/ Fusion 3	100	500; PC/NC >2	NC >1500
284					Yes		Always	4	24-48			Manufacturer's Instructions	Supplied	No	5	175	5	175	Vacuum Manifold	HLA Fusion	50	>500	>1500
293	No		Yes		Yes		No	-40	>48			Manufacturer's Instructions	Supplied	No	3	200	3	200	Vacuum Manifold	MatchIT	5000		5000
302	Yes		Yes		Yes		Yes	-40	>48			Manufacturer's Instructions	Supplied	No	3	200	3	200	Vacuum Manifold	MatchIT	5000		5000
303	Yes	Sensitised patient sera	Yes		Yes		Always	-20	>48	EDTA/Adsorption Beads/Heat Inactivation		Manufacturer's Instructions	Supplied	No	3	200	3	200	Centrifuge	Fusion	50	5000 MFI	NC >500 MFI
309	Yes	Manufacturer	Yes		No		Always	4	>48			Manufacturer's Instructions	Supplied	No	3	250			Vacuum Manifold	MatchIT	60	10000	NC >500
351					No	Each kit has own controls with different batches	Always	Frozen	>48			Manufacturer's Instructions	Supplied	No	1	100	3	250	Vacuum Manifold	MatchIT	60	10000	When notified by software
361	Yes	Manufacturer supplied	Yes		No		Always	Frozen	>48			Manufacturer's Instructions	Supplied	No	1	100	3	250	Vacuum Manifold	MatchIT	60	10000	When notified by software