

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

Samples 306-310/2018 DESPATCHED ON 13 NOVEMBER 2018

**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	NIH	B-cells	Yes	Biorad	Selected	26	Biorad		2	5		60	140	EB/AO	Manual		
206	NIH	PBL	Yes	In-house	Selected		Cedarlane	1	1	6.6	1	30	90	Fluoroquench	Manual0		
268																	

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	LAT1288	007	One Lambda							
165										

165 - Denotes previous methodology/No methodology received



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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	LS1PRA	ALL		017	3.5	14	Manufacturer		1000	LS2PRA	016	3.5	14	Manufacturer		1000	LSAI	3005842	20	5	Manufacturer	1500, BCR 3, AD-BCR 4	
24	LSAI	ALL		08256N	20	5	Manufacturer		1500	LSAII	09156A	20	5	Manufacturer		1500							1500, BCR 3, AD-BCR 4
25	LSMUTR	Selected	Donors	008	5	20	Manufacturer		Varies														
34																							
35	LSAII	ALL		11236A	20	10	Manufacturer		1000														
38																							
39																							
41																							
42																							
45																							
48	LM1	ALL		3005614	2	8	Manufacturer		None	LM2	3005268	2	8	Manufacturer		None	LS2A01	012	2	8	Manufacturer	1500 (taken with clinical history)	
51																							
54																							
58	LSAI	ALL		3006129	20	10	Manufacturer		>750														
62																							
100																							
112																							
114																							
116																							
117																							
119																							
120																							
133																							
136																							
142																							
143																							
145																							
149																							
157																							
160																							
162																							
165																							
169																							
181																							
185	LMX	ALL			5	12.5	Manufacturer	Manufacturer		LSAI		40	10	Manufacturer		ADJ. Val >2	LSAII		40	10	Manufacturer	ADJ. Val >2	
194																							
195																							
197																							
206																							
212																							
214																							
216																							
218																							
222																							
227	LM1	ALL			5	12.5	Manufacturer	Raw 1000		LM2		5	12.5	Manufacturer		Raw 1000							
229																							
230																							
232																							
242																							
252																							
257																							
268	LS1PRA				5	20		2000		LS2PRA		5	20		2000								
273																							
277																							
284																							
292																							
293																							
294																							
302																							
303																							
309																							
351																							
361																							
392																							

Lifecodes  
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  
LSAII Lifecodes Lifescreen SA Class I & II  
LSAMIC Lifecodes Lifescreen SA MICA

One Lambda  
LS1PRA LABScreen PRA Class I  
LS2PRA LABScreen PRA Class II  
LS12PRA LABScreen PRA Class I & II  
LS1AD4 LS SA Class I  
LS2A01 LS SA Class II  
LSM12 LS Mixed Class I & II  
LSMUTR 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 UKNEQASHand@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		1.6 Wash step details		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?	1.5 Incubation times	Wash medium	Anything added	First wash step	Second wash step	Software	Min bead count	Min pos control bead MFI	High background criteria				
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	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	
20	Yes	Sensitised Patient	Yes		No		Yes	80	>48	Adsorption Beads, EDTA			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: 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	Mix of sensitised patient sera	Yes		Yes		Sometimes	Frozen/Fridge (varies)	24-48	Adsorption Beads, EDTA, Dilution			Manufacturer's Instructions	Supplied		5	200	5	200	Vacuum Manifold	Fusion 4.1	50	5000	NC >500	
28	Yes	Sensitised patient sera	Yes		Yes		Yes	Frozen (-20)	>48	N/A			Manufacturer's Instructions	Supplied	No	4	1 x 100	3	4	1 x 100	Vacuum Manifold	Fusion	30	500	NC >1500
34	Yes	Sensitised Patient sera for One Lambda kits	Yes		No	Different pos con for Labscreen and Sag kits from One Lambda	Always	40/1.4	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	Selected Samples	Modified Halifax protocol	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	Yes		Yes		Yes					Adsorption Beads/EDTA	All Samples		Manufacturer's Instructions	Supplied	No	4	200	4	200	In House Software		50	5000	500	
46	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/Heat inactivation	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	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	Samples giving high background are retested with Adsorb Out beads	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		
112	Yes	Patient sensitised sera with low MFI value	Yes		No	One with each class	Yes	20	>48	N/A			Manufacturer's Instructions	Supplied	No	1	100	3	250	Vacuum Manifold, Centrifuge	HLA Fusion, MatchIT	60 Immucor, 100 One Lambda		2000 with control serum	MFI > range for NC bead
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	

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120	No		No	No	No	Always	20	24-48	Adsorption Beads/Dilution	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	Manufacturer's Instructions	Supplied	No	3	1x150, 2x200	2	200	Centrifuge	Fusion 4.1	50	4000	NC >500
133	Yes	Manufacturer	Yes	Yes	Yes	Yes	20	>48	Adsorption Beads/Heat Inactivation		Manufacturer's Instructions	Supplied	No	3	250			Vacuum Manifold	MatchIT	60	1000, for single antigen - 2000	For screening - 1000 For single antigen - 300
136		Sensitised patient sera	No	No	No	No		<24	Adsorption Beads		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	1	100	3	250	Vacuum Manifold	MatchIT EXE	60	10000	High CON 1
149	Yes	Individuals shown to be alloimmunized to HLA antigens	Yes	No	According to current LOT number	Always	4	24-48	N/A		Manufacturer's Instructions	Supplied	No	1	100	3	250	Vacuum Manifold	MatchIT EXE	60	10000	High CON 1
157						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						Always	2-8	<24			Manufacturer's Instructions	Supplied	No	2	150	2	150	Centrifuge	HLA Fusion	100	500	
165	Yes	Sensitised patient sera	Yes	No		Always	2-8	<24			Manufacturer's Instructions	Supplied	No	2	150	2	150	Centrifuge	HLA Fusion	100	500	
169	Yes	Sensitised patient sera in House pool	Yes	Yes	Yes	Always	20	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
185	Yes		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
194																						
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																						
206						Yes	Frozen	<24	Dilution		Manufacturer's Instructions	Supplied	No	3	250			Vacuum Manifold	MatchIT	60	>10000	>1000
212				Yes																		
214																						
216	Yes	Manufacturer	Yes	No	Kit-specific controls used, supplied by the manufacturer	Sometimes	Frozen/Fridge	24-48/>48	N/A		Manufacturer's Instructions	Supplied	No	3	250	1	150	Vacuum Manifold	MatchIT	100	5000	
218	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																						
227	Yes	Manufacturer, sensitised patient sera	Yes	Yes		Always	40	>48	Adsorption/EDTA/ Dilution	Only samples with high background are treated with adsorption beads	Manufacturer's Instructions	Supplied	No	5	175	5	175	Vacuum Manifold	HLA Fusion	50	PC >500 PC/NC >5	NC >500
229																						
230																						
232	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
242	No					Always	20	>48	Adsorption Beads		Manufacturer's Instructions	Supplied	No	1	150	2	200	Centrifuge	HLA Fusion	100	13415	
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																						
273	Yes		Yes	Once for lot control	Yes	Always	4	24-48	N/A		Manufacturer's Instructions	Supplied	No	5	175	5	175	Vacuum Manifold	HLA Fusion	50	>500	>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																						
292																						
293	No					No																
294																						
302	Yes		Yes	Yes		Yes	40	>48			Manufacturer's Instructions	Supplied	No	3	250	3	250	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	Yes	Sensitised patient serum	Yes	Yes		Always	4	24-48	EDTA		Manufacturer's Instructions	Supplied	No	3	200	2	200	Centrifuge	HLA Fusion	50	6000	NC >1500
361	Yes	Manufacturer supplied	Yes	No	Each kit has own controls with different batches	Always	Fridge/Frozen	>48	N/A		Manufacturer's Instructions	Supplied	No	1	100	3	250	Vacuum Manifold	MatchIT	60	10000	When notified by software
392																						

- Denotes previous methodology/No methodology received