

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

Samples 3 01-05/2016 DESPATCHED ON 26TH JANUARY 2016

**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	NIH	B-cells	No		Random	40	Cedarlane	1	1	5	1	60	45	EB/AO	Manual/Computerised	In-House	
28	CDC	CI panels of selected lymphocytes	Yes	One Lambda	Selected	30	Cedarlane		1	5		30	60	Fluoroquen	Manual		
35	NIH	T-cells	Yes	One Lambda	Selected	28	Cedarlane					30	60	Fluoroquen	Manual		
42	Extended NIH	PBL	Yes	In-House	Random	40	TCS Biosciences	1	1	5	1 in 10% DTT			Fluoroquen	Manual		
116	NIH	B-cells	Yes	Biorad	Selected	26	Biorad		2	5		60	140	EB/AO	Manual		
136	NIH	PBL	No		Mixture	45	In-House	1	1	5		30	60	EB/AO	Computerised	One Lambda	
139	NIH	PBL	Yes	Gentrak	Selected	30	Cedarlane	1	2	5		30	60	Fluoroquen	Manual		
147		PBL	Yes	In-House	Random	37	In-House	1	1	5	1µl DTT - 19µl sera	30	60	Fluoroquen	Computerised	One Lambda	
159																	
190	LCT	T-cells	Yes	One Lambda	Mixture	28	Servibio		1	5		30	60	Fluoroquen	Manual		
194	CDC	PBL	No		Selected	42	In-House	1	1	5		30	60	Fluoroquen	Computerised	One Lambda	
209	AGH	T-cells	Yes	GenTrak	Selected	30	GenTrak		2				75	Fluoroquen	Manual		
284	With and without DTT	PBL	No		Selected	30	Cedarlane	1	1	5	1	45	75	EB/AO	Manual, Computerised	Lymhoe	
294	AGH	T-cells	Yes	Commercial	Selected	60	Gentrak		2	5		30	60	Fluoroquen	Manual		

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							

**ELISA TECHNIQUE INFORMATION**

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



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206	LS1A04	All		009	5	20	In house	LS-NC	1000 MFI	LS2A01	All		011	5	20	In house	LS-NC	1000 MFI																						
209	LS1A04	All		009	2.5	20	Manufacturer		Normalised MFI >500	LS2A01	All		011	2.5	20	Manufacturer		Normalised MFI >500																						
218	LSA1	All			40	10	Manufacturer		Reactivity pattern	LSA11	All			40	10	Manufacturer		Reactivity pattern																						
227	LS1A04	All		009	5	20	Manufacturer		1000 baseline	LS2A01	All		011	5	20	Manufacturer		1000 baseline	LSM12	All				5	20	Manufacturer												3.6 NGB Ratio		
232	LS1A04	All		001	5	20	Manufacturer		1000 MFI	LS2A01	All		001	5	20	Manufacturer		1000 MFI	LSM12	All																		1000 MFI		
235	LS1PRA	All					Manufacturer		Positivity software	LS2PRA	All					Manufacturer		Positivity software	LSM1	All					001	5	20	Manufacturer												
238	LSA1	All					Manufacturer			LSA11	All					Manufacturer																								
245	LS1A04	All		009	4	20	No negative control		Positive >1000	LS2A01	All		011	4	20	No negative control		Positive >1000																						
262	LSA1	All		08245C	40	10	Manufacturer		Negative <500	LSA11	All		01166S	40	10	Manufacturer		Negative <500																						
267	LSA1	All		08245C	40	10	Manufacturer		AD BCR >=5	LSA11	All		09115A	40	10	Manufacturer		AD BCR >=5	LMX	All					3003153	5	12.5	Manufacturer											Software default criteria >1500 MFI	
268	LSA1	All		08245C - 08155F	5	12	Manufacturer		BCM >500	LSA11	All		08205C - 09115A	5	12	Manufacturer		BCM >500	LM1	All				3002915 - 30002878	5	12														
273	LS1A04	All		009	5	20	Manufacturer		>1500 MFI	LSA11	All		011	5	20	Manufacturer		>1500 MFI																						
277	LS1A04	Selected	Prescription by Dr	009	4	16	Manufacturer	LS-NC	1500 MFI or > score 4	LS2A01	Selected	Prescription by Dr	011	4	16	Manufacturer	LS-NC	1500 MFI or > score 4																						
284	LS1A04	All		6654S	5	20	Manufacturer	OLI LS-NC	>1000 MFI	LS2A01	All		6768S	5	20	Manufacturer	OLI LS-NC	>1000 MFI	LSM12	All				63524	5	20	In house	Sera from AB+ man without transfusion										Ratio >3		
292	LS1A04	Selected		009	3	18	Manufacturer		500 MFI	LS2A01	Selected		011	3	18	Manufacturer		500 MFI	LSM12	All																				
293	LS1A04	Selected	Mixed positive for Class I	009	5	20	Manufacturer		500 MFI	LS2A01	Selected	Mixed positive for Class II	011	5	20	Manufacturer		500 MFI	LSM12	All																				
294	LSA1	All		08245C	40	10	Manufacturer	LS-NC	Manufacturer criteria	LSA11	All		09115A	40	10	Manufacturer	LS-NC	Manufacturer criteria	LMX	All					3002973	5	12.5											Manufacturer criteria		
297	LS1A04	All		009	3	18	Manufacturer		1500 MFI	LS2A01	All		011	3	18	Manufacturer		1500 MFI																						
303	LS1A04	All		02296M	2.5	20	Manufacturer		>1000 MFI	LS2A01	All		01166S	2.5	20	Manufacturer		>1000 MFI	LM1	All																				
309	LSA1	All	Immucor only	02296M	40	10	Manufacturer		Software preferences	LSA11	All	Immucor only	01166S	40	10	Manufacturer		Software preferences																					Software preferences	
311	LS1A04	All		009	5	18	Manufacturer		1500	LS2A01	All		010	5	18	Manufacturer		1500																						
315	LS1A04	All		009	5	20	Manufacturer	Negative patient sera known	>2000 MFI	LS2A01	All		011	5	20	Manufacturer	Negative patient sera known	>2000 MFI																						
323	LS1A04	All		009	5	20	Manufacturer	Negative patient sera known	Establish with known patients results and quality control	LS2A01	All		011	5	20	Manufacturer	Negative patient sera known	Establish with known patients results and quality control																						

Immucor  
LM1 Class I ID Kit  
LM2 Class II ID Kit  
LMX Lifecodes Lifescreen Deluxe Kit  
LSA1 Lifecodes Lifescreen SA Class I  
LSA11 Lifecodes Lifescreen SA Class II  
LSA11I Lifecodes Lifescreen SA Class II&I  
LSAMC Lifecodes Lifescreen SA MICA

One Lambda  
LS1PRA  
LS2PRA  
LS12PRA  
LS1A04  
LS2A01  
LSM12  
LSMUTR

LABScreen PRA Class I  
LABScreen PRA Class II  
LABScreen PRA Class II&I  
LS SA Class I  
LS SA Class II  
LS Mixed Class II&I  
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

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

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**LUMINEX TECHNIQUE INFORMATION**

Lab No	LUMINEX kit(s) used	Used for All/Selected	Criteria	Batch No	Bead Volume (µl)	Serum Volume (µl)	Negative Control Source	Serum Details	Details of +/- cut off	LUMINEX kit(s) used	Used for All/Selected	Criteria	Batch No	Bead Volume (µl)	Serum Volume (µl)	Negative Control Source	Serum Details	Details of +/- cut off	LUMINEX kit(s) used	Used for All/Selected	Criteria	Batch No	Bead Volume (µl)	Serum Volume (µl)	Negative Control Source	Serum Details	Details of +/- cut off									
9																																				
11																																				
12	LS1PRA			016	2	8	In house		1000 MFI	LS2PRA			015	2	8	In house		1000 MFI																		
14																																				
15																																				
19																																				
20																																				
23	LS2PRA	All		015	3.5	14	Manufacturer	015	1000 MFI	LSAI	All		05265D	20	5	Manufacturer		Manufacturer defaults	LSAII	All		05285A	20	5	Manufacturer	Manufacturer defaults	LMX	All		3002973 / 3002956	22.5	6	Manufacturer	Manufacturer defaults		
24																																				
25	LSAI	All		082450	20	5		Addition of EDTA	1000 MFI	LSAII	All		09115A	20	5		Addition of EDTA	1000 MFI																		
26																																				
28																																				
34																																				
35																																				
38	LSAI			02105C	2.5	20	Manufacturer			LSAII	All		05285A	2.5	20	Manufacturer			LMX	All		3002973	2.5	20	Manufacturer											
39																																				
41																																				
42																																				
45																																				
48	LSAI	All	Lab alternates between manufacturers	08245C	20	8	Manufacturer	Adjustments to manufacturer's instructions for use.	None	LSAII	All	Lab alternates between manufacturers	05285A	20	8	Manufacturer	Adjustments to manufacturer's instructions for use.	None	LM1	All	Lab alternates between manufacturers	3002701	2	8	Manufacturer	Adjustments to manufacturer's instructions for use.	None	LM2	All	Lab alternates between manufacturers	3002807	2	8	Manufacturer	Adjustments to manufacturer's instructions for use.	None
51	LSAII	Selected			20	10	Manufacturer		Not pre-defined																											
54																																				
58	LSAII	All			20	10	Manufacturer			LMX	All		2973	2.5	6.25	Manufacturer		2/3 adjusted ratios (v con1, 2 or 3) > 0.0																		
62																																				
100																																				
101																																				
112																																				
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160																																				
162																																				
163																																				
189																																				
185	LM1	Selected	1st LMX reactive & later in case of doubt of LMX or LSA	09115A_08205C	5	12.5	Manufacturer			LM2	Selected	1st LMX reactive & later in case of doubt of LMX or LSA		5	12.5	Manufacturer																				
186	LSAII	All			40	10	Manufacturer		1000																											
190																																				
193																																				
194																																				
195																																				
197																																				
204																																				

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235																																																							
238																																																							
245																																																							
262																																																							
267																																																							
268	LM2	All	3003072 - 3003007	5	12																																																		
273																																																							
277																																																							
284																																																							
292																																																							
293																																																							
294																																																							
297																																																							
299																																																							
303	LM2	All	Immucor only	5	12.5	Manufacturer																																																	
309																																																							
311																																																							
315																																																							
323																																																							

Immucor						One Lambda																																																	
LM1	Class I ID Kit					LS1PRA																																																	
LM2	Class II ID Kit					LS2PRA																																																	
LMX	Lifecodes Lifescreen Deluxe Kit					LS12PRA																																																	
LSA1	Lifecodes Lifescreen SA Class I					LS1A04																																																	
LSAII	Lifecodes Lifescreen SA Class II					LS2A01																																																	
LSAIIII	Lifecodes Lifescreen SA Class I&II					LSM12																																																	
LSAMC	Lifecodes Lifescreen SA MICA					LSMJTR																																																	

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DESPATCHED ON 26TH JANUARY 2016

**LUMINEX TECHNIQUE INFORMATION**

Lab No	Positive control serum for Luminex testing?	Source	Every run?	1.2 Positive control serum		1.3			1.4 Serum treatment		Criteria?	
				If no:	Same serum?	if no:	Samples stored prior to testing?	Conditions?	Storage time? (hours)	Methods used?		Extra Information
9	Yes	Sensitised patient sera	No	Once a week on batches >15	Yes		Yes - sometimes	Frozen (-20°C)	>48	Dilution	Selected samples, AB serum, 1 in 4, 1 in 8	High background, high NC, multispecific with very high MFI values
11	Yes	Sensitised patient sera	Yes		No	Pooled control for LSM12; known positive patient serum for Class I and SABs	Yes - always	Frozen (-40°C)	>48	Adsorption beads	Selected samples, Adsorb Out, 1:10	
12	No						Yes - sometimes	Frozen (-20°C)	24-48	Adsorption beads, EDTA	Beads: Selected samples, Adsorb Out, 1:10 EDTA: All samples, Added to sera, EDTA K2 Salt Dihydrate 6% w/v solution, 5µl EDTA in 95 µl sera/buffer, No minimum incubation time, Stored sometimes - aliquoted into EDTA at sample processing, stored -20°C until testing	Adsorb out: only for samples with High Background (NC MFI >500)
14	Yes	Sensitised PT sera	Yes		No	Sensitised PT control sera for each kit chosen by antibody profile	Yes	Frozen	>48	Heat Inactivation, EDTA	Heat Inactivation: All samples, 30 mins at 56°C EDTA: All samples, Added to wash buffer, 4.5mM, 0.436µl EDTA in 260µl sera/buffer, No minimum incubation time, Not stored	
15	Yes	Pooled HSP sera	Yes		Yes		Yes	Fridge (5°C)	>48	Adsorption beads, EDTA	Beads: Selected samples, Adsorb Out, 1:10 EDTA: 0.1M, 13.5µl EDTA in 300µl sera/buffer, No minimum incubation time, Not stored	
19	Yes	Sensitised patient sera	Yes		Yes		Yes	Room temperature	24-48	EDTA	EDTA: All samples, Added to wash buffer, 4.5mM	
20	Yes	Sensitised patient sera	Yes		No		Yes - always	Frozen (-80°C)	>48	EDTA	All samples, Added to sera, 6%, 20µl EDTA in 380µl sera/buffer, No minimum incubation time, Stored sometimes - prepared samples are stored with EDTA following testing, If a repeat test is required this stored sample is retrieved for testing	
23	Yes	Positive patient sera diluted 1:2	Yes		Yes		Yes - always	Frozen (-35°C)	>48	N/A		
24	Yes	Manufacturer or sensitised patient serum	Yes		No	For ID and single antigen runs using One Lambda we have an in-house pos control which is a pool of highly sensitised patients. For Lifecodes SA I and II we use the positive control provided with the kits Only for batch acceptance	Yes - always	Frozen (-30°C)	>48	Adsorption beads, EDTA	Beads: Selected samples, Adsorb Out + Sera Clean, 1:10 EDTA: All samples, Added to sera, 6%, 3µl EDTA in 57µl sera/buffer, No minimum incubation time, Stored sometimes - Are often EDTA treated and adsorbed the day before being tested, Stored overnight at -30°C	High background samples are adsorbed.
25	Yes	Sensitised patient sera	No	Only for batch acceptance	No		Yes	Frozen	>48	Adsorption beads, EDTA	Beads: Selected samples, Sera Clean, 1:5 EDTA: All samples, Added to sera, 6% EDTA, 20µl EDTA in 380µl sera/buffer, No minimum incubation time, Stored - frozen after EDTA addition	High background prescreens NC value greater than 500
26	Yes	Yes	Yes		Yes		Yes - sometimes	Fridge (4°C)	<24	EDTA	Selected samples, Added to buffer, 30 mins minimum incubation time, Not stored	
28	Yes	Sensitised patient sera	Yes		Yes		Yes	Frozen (-20°C)	>48			
34	Yes	Sensitised patient sera	Yes		Yes		Yes - sometimes	Fridge (2-8°C)	24-48	EDTA	All samples, Added to buffer, 4.5µl EDTA in 100µl sera/buffer, No minimum incubation time, Not stored	
35	Yes	Yes	Yes		Yes		Yes	Fridge (2-8°C)	24-48	EDTA	All samples, Added to buffer, 30 mins minimum incubation time, Not stored	
38	Yes	Sensitised patient sera	No	Used for LSM	No	Manufacturers pos control used for LifeCodes	Yes - always	Frozen (-40°C)	>48	EDTA	Selected samples, 6%, 10µl EDTA in 90µl sera/buffer, No minimum incubation time, Not stored	
39	Yes	For every run: 1) an in house positive control is included sourced from pooled sensitised patient sera as well as 2) in house performance monitoring control selected from a sensitised patient serum	Yes		Yes		Yes - always	Frozen (-40°C)	>48	Adsorption beads, EDTA	Beads: Selected samples, Adsorb Out, 1:10 EDTA: All samples, Added to buffer, 0.1M EDTA, 23ml EDTA in 520ml buffer, No minimum incubation time, Not stored	Samples with high background are treated with adsorb out beads
41	Yes	Pooled sensitised patient sera	No	Every labscreen mixed run consignment validation of SA beads	No	1/400 dilution used for labscreen mixed neat serum used for consignment validation	Yes - always	Frozen (-40°C)	>48	Adsorption beads	Adsorb out, 1:9	
42	Yes	Sensitised patient sera	No	Used in a run where a new lot is being used or when a new delivery for previous lot is being used	Yes		Yes - always	Frozen (-40°C)	24-48	Adsorption beads, EDTA	Adsorb out, 4:40, sera, 6% EDTA solution, 10µl EDTA in 90µl sera/wash buffer, Minimum incubation time 10 min, Stored following treatment at -40 until tested	
45	Yes	Pooled multiple sensitised patient sera	Yes		Yes		Yes - always	Frozen	>48	Adsorption beads, EDTA	Beads: Selected samples, Adsorb Out, 1:1 EDTA: All samples, Added to sera, 6%, 5µl EDTA in 95µl sera/buffer, No minimum incubation time, Not stored	
48	Yes	Pooled sensitised patient sera	No	Not used for single antigen testing. Currently only used for LabScreen Mixed Class I and II antibody testing. In this setting it would be used on every run	No	Only used for LabScreen Mixed Class I and II antibody testing	Yes - sometimes	Frozen (-20°C)	>48	Adsorption beads, Heat Inactivation	Beads: Selected samples, SeraClean, 4:20 Heat Inactivation: Selected samples, 30 mins @ 56°C	High background
51	Yes	Pooled sensitised patient sera	Yes		Yes		Yes	Frozen (-40°C)	>48	Adsorption beads, EDTA, Dilution	Beads: Selected samples EDTA: All samples, Added to sera, 0.5µl EDTA in 10µl sera/buffer, No minimum incubation time, Not stored	
54	Yes	Pooled sensitised patient sera	Yes		No	Not tested with single antigen kits as patient samples provide suitable positive control	Yes - always	Frozen (-20°C)	>48	Adsorption beads	Dilution: All samples, diluted in wash buffer, 1 in 2 Selected samples, Adsorb Out, 1:10	Samples with high NC value (>500)
58	Yes	Manufacturer / in house sensitised patient sera	Yes		No	Lifecodes screen manufacturer and in house patient sera Labscreen manufacturer and in house patient sera Labscreen SAB manufacturer only Lifecodes SAB manufacturer only	Yes - always	Frozen (-15 to -20°C)	>48	EDTA, Foetal Cal Serum	EDTA: Selected sample, Added to sera, 6%, 2.5µl EDTA in 47.5µl sera/buffer, No minimum incubation time, Not stored FCS: Added to 5% of total serum volume, Selected samples	EDAT treatment used for all SAB testing. Foetal calf serum used to treat high background serum on second round of testing
62	Yes	Tepnal Lifecodes	Yes		Yes		Yes - always	Frozen (-70°C)	>48	N/A		
100	Yes	Manufacturer	Yes		No	Positive control is assay specific	Yes - always	Frozen (-20°C)	>48	N/A		
101	Yes	Sensitised patient sera	Yes		Yes		Yes - always	Frozen (-20°C)	>48	Adsorption beads, DTT	Beads: Selected samples DTT: Selected samples	High background for adsorption; complement and IgM interference detection for DTT
112	Yes	Sensitised patient sera aliquot	Yes		Yes		Yes - always	Frozen (-15 to -30°C)	>48	Adsorption beads	Adsorb out, 25µl serum/5µl adsorption beads (mmucor)	High background and/or irregular or atypical profile results in MatchIT analysis software
114	No						Yes - sometimes	Frozen (-20°C)	24-48	Adsorption beads, EDTA	Beads: Selected samples, Adsorb Out, 1:10 EDTA: All samples, Added to sera, 6%, 2.5µl EDTA in 47.5µl sera/buffer, No minimum incubation time, Not stored	
116	No						Yes - always	Frozen (-20°C)	>48	Adsorption beads	Selected samples, Adsorb Out, 1:10	Samples with high background
117	No						Yes - always	Frozen (-65°C)	>48	Adsorption beads	Selected samples, Adsorb Out, 1:10	Samples with high background are retested with Adsorb Out
119	No						Yes - always	Frozen (-20°C)	24-48	Adsorption beads, EDTA, Dilution	Beads: Selected samples, Adsorb Out, 10% EDTA: All samples, Added to sera, 0.1M pH 7.5, 2µl EDTA in 18µl sera/buffer, 15 mins minimum incubation time, Not stored Dilution: Selected samples, PBS, 1/8	- Adsorption is carried out when Negative control beads NC baseline >500 and/or Positive control beads PC baseline <400. - Dilution 1/8 is carried out when adsorption is not sufficient to allow test validation according to PC/NC quality values
133	Yes	Manufacturer (Immucor)	Yes		Yes		Yes - always	Frozen (-20°C)	>48	Adsorption beads	Selected samples, SeraClean, 1/2,5	We treat with adsorption beads if we get a high background
136	Yes	Sensitised patient sera	No	Only kit validation	No	Different lot	Yes - always	Fridge (4°C)	<24	Adsorption beads	Selected samples, Adsorb Out, 1:10	
139	Yes	Sensitised patient sera	Yes		No	Class I positive control and Class II positive control	Yes - always	Frozen (-30°C)	>48	Adsorption beads	Selected samples, Adsorb Out, 500	
142	Yes	Sensitised patient sera	Yes		Yes		Yes - sometimes	Frozen (-20°C)	24-48	Adsorption beads, EDTA	Beads: Selected samples, Adsorb Out, 5µl beads/50µl serum EDTA: All samples, Added to sera, 0.2M, 5µl EDTA in 50µl sera/buffer, No minimum incubation time, Not stored	Adsorption beads are used for samples with high background
143	No						Yes - always	Frozen (-20°C)	>48	N/A		
145	No						Yes - sometimes	Up to 2 weeks at 4°C. Longer periods are stored at -30°C or -80°C as we do for routine samples	>48	N/A		
147	Yes		No		No		Yes	Fridge (4°C)	>48	Adsorption beads, EDTA	Beads: Selected samples, Adsorb Out, 3µl beads/30µl sera EDTA: Selected samples, Added to sera, 0.1 mol/l, 2µl EDTA in 18µl sera/buffer, 10 mins minimum incubation time, Not stored	Discrepant results between screening and identification or inexplicable result
149	Yes	Manufacturer	Yes		No	We use positive control serum supplied with every kit	Yes - always	Frozen	>48	Adsorption beads, Dilution	Beads: Selected samples, SeraClean, 4:20 Dilution: Selected samples, Wash buffer, 1 in 4	High Background

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157	Yes		Yes		Yes - always	Fridge (4°C)	24-48	EDTA	All samples, Added to sera, 6%, 1µl EDTA in 10µl sera/buffer, No minimum incubation time, Not stored		
159	No				Yes - always	Fridge (4-6°C)	>48	Adsorption beads	All samples, Adsorb Out, 5-20		
160	No				Yes - always	Frozen (-20°C)	24-48	EDTA	All samples, Added to sera, 6%, 10µl EDTA in 190µl sera/buffer, No minimum incubation time, Stored - always, -20°C for 24-48h		
162	No				Yes - always	Frozen (-30°C)	>48	Adsorption beads, EDTA	Beads: Selected samples, Adsorb Out, 500		
163	Yes	Sensitised patient sera	Yes	No	A sensitised patient sera for Labscreen mixed, another sensitised patient sera for Labscreen SA	Fridge (5°C)	>48	N/A	EDTA: All samples, Added to sera, 0.1M, 2µl EDTA in 18µl sera/buffer, 10 mins minimum incubation time, Not stored		
164	Yes	Sensitised patient sera	Yes	Yes		Fridge (5°C)	>48	N/A			
165	No		No		Yes - always	Frozen (-20°C)	>48				
169	No		No		Always	-20	>48	Adsorption beads	Adsorb out, 1 in 10. Dilution in AB serum, 1 in 10 or 1 in 5		
181	Yes	Sensitised patients pool	Yes	No	One for class I, one for class II	Always	-25	24-48	Adsorption beads, EDTA	SeraClean, 16 beads + 80 serum, Sera, 0.2M but only to LM & LSA tests, 12µl EDTA in 225µl sera/wash buffer. Most are stored at least overnight; urgent samples may be tested directly without storage. This has been validated and makes no difference	LMX tests are not tested; dilution is only very rarely performed but in routine every Luminex positive sample for organ transplantation is tested with CDC.
185	Yes	Kit dependent	Yes	Yes		Always	-25	24-48	Adsorption beads, EDTA	Sera, 0.1M, 2µl EDTA in 18µl sera/wash buffer, minimum EDTA incubation time 10 min, Dilution in NaCl, 1 in 10 or 1 in 50	
186	Yes	Manufacturer	Yes	No	Only with LMX kit	Yes	Frozen	>48	Adsorption beads	Adsorb out	
190	Yes		Yes	No	Kit dependent	Always	-40	>48	EDTA	Sera, 0.1M, 2µl EDTA in 18µl sera/wash buffer, minimum EDTA incubation time 10 min, Dilution in NaCl, 1 in 10 or 1 in 50	
193	Yes	Sensitised patients sera	Yes	Yes		Always	-35	>48			
194	Yes	Sensitised patients sera	Yes	Yes		Sometimes	Frozen	>48			
195	Yes	Sensitised patients sera	Yes	Yes		Yes	4-6	>48	Adsorption beads	Adsorb out, 3µl in 30µl	
197	No		No			Always	-20	>48	Adsorption beads, EDTA	Adsorb out, 1/10, Sera, 0.1M, 2µl EDTA in 18µl sera/wash buffer, minimum EDTA incubation time 10 min	Adsorption beads: Samples with high background
204	Yes	Sensitised patients pool	Yes	Yes	Kit dependent	Yes	<-30	>48	Adsorption beads, EDTA	Adsorb out, NC<500MFI, sera, 0.1M, 10µl EDTA in 90µl sera, minimum EDTA incubation time 15 min	
206	Yes	Sensitised patients sera	Yes	Yes		Always	-30	0->48	Adsorption beads, EDTA	Adsorb out, sera, 0.1625M, 3µl EDTA in 57µl sera/wash buffer, minimum EDTA incubation time 10 min	
209	Yes	Sensitised patients pool from NIBSC	Yes	No	A specific control is used in screening Luminex assays	Always	-80	>48	Adsorption beads, Heat inactivation	SeraClean, 56° for 30min	
218	Yes	Kit dependent	Yes	No	Kit dependent	Always	-40	>48	Adsorption beads, Heat inactivation EDTA	Sera, 0.1M, 10µl EDTA in 90µl sera/wash buffer, stored after treatment +5 or -40, Dilution in wash buffer 1 in 10	All samples were also tested without any treatment
227	Yes	Sensitised patients sera	Yes	Yes		Always	-60, 5	>48			
232	Yes	Kit dependent	Yes	Yes	Kit dependent	Yes	-20	24-48	Adsorption beads	Adsorb out, 1/10, Dilution in PBS 1 in 8	
235	No		No			Yes	-20	>48			
238	Yes	Sensitised patient	Yes	Yes		Yes	-20	>48			
245	No		No			Always	-25	>48	Adsorption beads, EDTA	Adsorb out, 3µl per 30µl, sera, 0.1M, 20µl EDTA in 180µl sera/wash buffer, minimum EDTA incubation time 10 min	Samples with high background
262	Yes	Human immunized platelet donor	Yes	Yes		No		24-48			
267	Yes	Kit dependent	Yes	No	Kit dependent	Always	-30	>48	Adsorption beads	SeraClean, 1:4	Sensitised patient sera & high background sera
268											
273	Yes	Sensitised patients	Yes	Yes		Always	4	24-48			
277	Yes	Previous NEQAS samples	No	Yes	Kit dependent	Yes	≤-30	24-48	Adsorption beads, EDTA	Adsorb out, Neg bead: ratio ≥1500, sera, 6%, 5µl EDTA in 95µl sera	
284	Yes	Sensitised patient sera	Yes	Yes		Always	-30	>48	Adsorption beads, EDTA	Adsorb out, NC<500, Sera, 0.1 mol/l, 3µl EDTA in 27µl sera/wash buffer, minimum EDTA incubation time 10 min on agitation, Another frozen cycle	If NC<500 - adsorb out treatment If PC<5000 - supplemental frozen cycle
292	Yes	Sensitised patient sera pool	Yes	No	Kit dependent	Always	-30	>48	Adsorption beads, EDTA	Adsorb out, sera, 2µl EDTA in 18µl sera/wash buffer, minimum EDTA incubation time 10 min	Only samples with high background are treated with adsorption beads
293	No		No			Sometimes	-70		Adsorption beads, Heat inactivation	Adsorb out, 3µl/30µl (0.1), 30min at 56°c	High MFI value in NC beads
294	Yes	Kit dependent	Yes	Yes		Always	Frozen	24-48	Adsorption beads	SeraClean	
297	Yes	Sensitised patient	Yes	No		Yes	Frozen	>48	Adsorption beads, EDTA	1/10, Sera, 2µl EDTA in 18µl sera, minimum EDTA incubation time 10 min	
303	Yes	Sensitised patient	Yes	Yes		Always	-20	>48	Adsorption beads, Heat inactivation, EDTA	Adsorb out, 1:10, 30min 56°c, Sera, 6M, 1µl EDTA in 20µl sera/wash buffer, minimum EDTA incubation time 5 min	Samples with NC beads<500MFI are treated with Adsorb out samples with PC bead <5000MFI are heat inactivated
309	Yes	Kit dependent	Yes	No	Kit dependent						
311	Yes	Patient sera	Yes	Yes		Always	-80	>48	Adsorption beads, EDTA	Sera, 0.1M, 2µl EDTA in 18µl sera/wash buffer, minimum EDTA incubation time 10 min	
315	Yes	Sensitised patient sera	Yes	Yes		Yes	2-4	24-48	Adsorption beads, EDTA	Adsorb out, 1/10, Sera, 0.1M, 10µl EDTA in 90µl sera/wash buffer, minimum EDTA incubation time 15 min	
323	Yes	Sensitised patient sera	Yes	Yes		Yes	-80	>48	EDTA	Sera, 0.1M, 2µl EDTA in 18µl sera/wash buffer, minimum EDTA incubation time 10 min, Dilution in sera 1 in 10	

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Lab No	1.5 Incubation times	1.6 Wash step details							1.7 Data analysis				
		Wash medium	Anything added	First wash step		Second wash step		Buffer removed by	Software	Min bead count	Min pos control bead MFI	High background criteria	
				Washes	Volume (µl)	Washes	Volume (µl)						
9	As per manufacturer's instruction	Manufacturer supplied	No	5	200	5	200	Vacuum manifold	HLA Fusion	50	500	High NC, all beads positive with no negative specificities	
11	As per manufacturer's instruction	Manufacturer supplied	No	3	200	2	200	Centrifuge, "flick and blot"	HLA Fusion	50	1000	>1000	
12	As per manufacturer's instruction	Manufacturer supplied	No	5	175	5	175	Vacuum manifold	HLA Fusion	50	Pos control bead fluorescence value should be >500 and at least twice the NC value	Test sera with neg control bead values between 500-1500 should be reviewed with reference to patient sensitisation history. Test sera with neg control bead values over 1500 MFI will be treated with Adsorb Out beads.	
14	As per manufacturer's instruction	Manufacturer supplied	EDTA	3	200	2	200	Centrifuge, "flick and blot"	HLA Fusion	50	5000	Neg MFI >500 or Pos/Neg Ratio <2	
15	As per manufacturer's instruction	Manufacturer supplied	EDTA	5	200	5	200	Vacuum manifold	HLA Fusion	50	PC/NC >2	NC >1500	
19	As per manufacturer's instruction	Manufacturer supplied	EDTA	5	200	5	200	Vacuum manifold	HLA Fusion	50	5000	1000-1500	
20	As per manufacturer's instruction	Manufacturer supplied	No	5	200	4	200	Vacuum manifold	HLA Fusion	50	2000	Sample NC of >500 MFI	
23	As per manufacturer's instruction	Manufacturer supplied	No	2	200	2	200	LabXpress	HLA Fusion	50	500	>1500 MFI	
24	As per manufacturer's instruction	Manufacturer supplied	No	OL = 5, LC = 4	OL = 200, LC = 1x100, 3x250	OL = 5, LC = 0	200	Vacuum manifold	HLA Fusion and MatchIT	50	3000	NC >500	
25	As per manufacturer's instruction	Manufacturer supplied	No	x5	220	x5	220	Vacuum manifold	HLA Fusion	50	1500 or 2x negative control	MFI for NC bead greater than 1500	
26	As per manufacturer's instruction	Manufacturer supplied	No	5	200	5	200	Vacuum manifold	HLA Fusion	50	5000	NC >750	
28	As per manufacturer's instruction	Manufacturer supplied	No	4	100, 275	4	100, 275	Vacuum manifold	Fusion	30	500	NC >500	
34	As per manufacturer's instruction	Manufacturer supplied	EDTA	5	200	5	200	Vacuum manifold	HLA Fusion	50	>500 + *2*NC	Negative control >500	
35	As per manufacturer's instruction	Manufacturer supplied	EDTA	1	150	3	200	Centrifuge, "flick and blot"	HLA Fusion	50			
38	LABScreen rapid method	Manufacturer supplied	No	3	200	2	200	Centrifuge, "flick and blot"	HLA Fusion/Match IT	70	1500	500	
39	As per manufacturer's instruction	Manufacturer supplied	EDTA	5	200	5	200	Vacuum manifold	HLA Fusion	50	5000	Samples with an internal Negative Control >500 bead MFI are classified as having "High Background"	
41	As per manufacturer's instruction	Manufacturer supplied	No	4	200	3	200	Vacuum manifold	Fusion	50	5000	1500	
42	As per manufacturer's instruction	Manufacturer supplied	No	5	200	5	200	Vacuum manifold	Fusion	50	5000	NC >1500	
45	As per manufacturer's instruction	Manufacturer supplied	No	4	200	4	200	Vacuum manifold	HLA Fusion and in house software	50	5000	500	
48	As per manufacturer's instruction	Manufacturer supplied	No	3	250	3	150, then 2 at 250 for Labscreen only	Vacuum manifold	HLA Fusion/Match IT	50	1000 for Labscreen Mixed beads and Single Antigen beads, 10,000 for Lifecodes C1 and 2 ID's and 2000 for Lifecodes Single Antigen beads	Sample with a negative control over 1000 for Labscreen and at the user's discretion taking into account the manufacturer's suggest ranges for Lifecodes	
51	As per manufacturer's instruction	Manufacturer supplied	No	5	250	5	250	Vacuum manifold	HLA Fusion/Match IT	50 for analysis	5000	>500	
54	As per manufacturer's instruction	Manufacturer supplied	No	3	150	3	150	Centrifuge, "flick and blot"	HLA Fusion	40	1000	NC <500	
58	As per manufacturer's instruction	Manufacturer supplied	No	As per manufacturer instruction	Labscreen - 150µl Lifecodes - 250µl	As per manufacturer instruction	Labscreen - 150µl Lifecodes - 0	Vacuum manifold	HLA Fusion/Match IT	40	Labscreen - >500 Lifecodes - >3500	Labscreen screen - NC raw >500 Labscreen SAB - NC raw >1500 Lifecodes screen - Pos controls (1, 2 or 3) > lot specific specified range	
62	As per manufacturer's instruction	Manufacturer supplied	No	5	200	5	200	Vacuum manifold	HLA Fusion	50	500	>500	
100	As per manufacturer's instruction	Manufacturer supplied	No	3	250	N/A		Vacuum manifold	MatchIT	50	2000 with control sera	MFI greater value than the range for negative control bead	
101	As per manufacturer's instruction	Manufacturer supplied	No					Centrifuge, "flick and blot"	HLA Fusion	100	4000	>500	
112	As per manufacturer's instruction	Manufacturer supplied	No	3	250			Automated bottom suction by biotek ELU50	MatchIT	60	10000	NC <kit specification, irregular or atypical graphs or reactivity profiles	
114	As per manufacturer's instruction	Manufacturer supplied	EDTA	3	160	2	160	Centrifuge, "flick and blot"	HLA Fusion	50		Negative control >500	
116	As per manufacturer's instruction	Manufacturer supplied	No	3	200	2	200	Centrifuge, "flick and blot"	HLA Fusion	50		Negative control in LSM >800	
117	As per manufacturer's instruction	Manufacturer supplied	No	3	200	3	200	Centrifuge, "flick and blot"	HLA Fusion	15	500	MFI of negative control 300-400	
119	As per manufacturer's instruction	Manufacturer supplied	No	3	First = 150µl Two others = 200µl	2	200µl	Centrifuge, "flick and blot"	HLA Fusion	50	4000	NC >500 MFI	
133	As per manufacturer's instruction	Manufacturer supplied	No	3	250	N/A		Vacuum manifold	MatchIT	60	Screening: 10,000 Single Antigen: 2000	Screening: 1000 Single Antigen: 300 MFI >500	
136	As per manufacturer's instruction	Manufacturer supplied	No	3	250	2		Centrifuge, "flick and blot"	HLA Fusion	50	>1000		
139	As per manufacturer's instruction	Manufacturer supplied	No	5	175	5	175	Vacuum manifold	HLA Fusion	50	5000	NC >1500	
142	As per manufacturer's instruction	Manufacturer supplied	No	5	175	5	175	Vacuum manifold	HLA Fusion	50	5000	NC bead >1500 MFI	
143	As per manufacturer's instruction	Manufacturer supplied	No	3	150/200/200	2	200/200	Centrifuge, "flick and blot"	HLA Fusion	100	3000	Negative control >1500 MFI	
145													
147	As per manufacturer's instruction	Manufacturer supplied	EDTA	4	250	4	250	Vacuum manifold	HLA Fusion	50	2000	>500	
149	As per manufacturer's instruction	Manufacturer supplied	No	3	250			Vacuum manifold	MatchIT	60	2000	High MFI for Negative Control Sera (BCM >300 MFI)	



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157	As per manufacturer's instruction	Manufacturer supplied		3	200	3	200	Centrifuge, "flick and blot"	HLA Fusion	50		4000	Negative control >500
159	As per manufacturer's instruction	Manufacturer supplied	No	3	1st 150, 2nd and 3rd 200	2	200	Centrifuge, "flick and blot"	HLA Fusion	100	5000		
162	As per manufacturer's instruction	Manufacturer supplied	EDTA	3	200	2	200	Centrifuge, "flick and blot"	HLA Fusion	50		1500	NC bead >1500
163	As per manufacturer's instruction	Manufacturer supplied	EDTA	4	175	4	175	Vacuum manifold	HLA Fusion	50		400	500
164	As per manufacturer's instruction	Manufacturer supplied	No	5	175	5	175	Vacuum manifold	HLA Fusion	50		>500	Negative Control >1500
165	As per manufacturer's instruction	Manufacturer supplied		2	150	2	150	Centrifuge, "flick and blot"	Fusion	100		5000MFI	
181	Manufacturer's instruction	Manufacturer supplied	No	5	175	5	175	Vacuum manifold	Fusion	50		5000	High NC
185	Manufacturer's instruction	Manufacturer supplied	No	3	250			Vacuum manifold	MatchIT	60	Not defined. Relative to the standard PC as provided by the manufacturer		cf manufacturer's ranges
186	Manufacturer's instruction	Manufacturer supplied	No	3	250	3	250	Vacuum manifold	MatchIT	?		10000	NC bead
190	Manufacturer's instruction	Manufacturer supplied	EDTA	3	200	2	200	Centrifuge, flick and blot	Fusion	50		500	R<2 or NC<1500
193	Manufacturer's instruction	Manufacturer supplied	No	3	200	2	200	Centrifuge, flick and blot	Fusion	100		raw data PC>1000	raw data NC>500
194	Manufacturer's instruction	Manufacturer supplied						Vacuum manifold	Fusion				
195	Manufacturer's instruction	Manufacturer supplied	No	5	175	5	175	Vacuum manifold	Fusion	50		5000	NC<500
197	Manufacturer's instruction	Manufacturer supplied	No	4	175	4	175	Vacuum manifold	Fusion	50		Accepted with a PC<5000	High background when NC>500
206	Manufacturer's instruction	Manufacturer supplied	EDTA	5	175	5	175	Vacuum manifold	Fusion	50		3000	NC<500
209	Manufacturer's instruction	Manufacturer supplied	No	5	175	5	175	Vacuum manifold	Fusion	50		3000	Sera with NC bead MFI over 500 are adsorbed. Adsorbed sera with remaining NC bead MFI over 1500 are considered having "high background"
218	Manufacturer's instruction	Manufacturer supplied	No	4	150-200			Vacuum manifold	MatchIT	100			High MFIs, non-specific binding, high adjusted ratios
227	Manufacturer's instruction	Manufacturer supplied	No	5, 3	175, 100	5	175	Vacuum manifold	Fusion, MatchIT	50		1500 raw	up to 500 raw
232	Manufacturer's instruction	Manufacturer supplied	No	4	275	4	275	Vacuum manifold	Fusion	80		5000	Kit dependent
235	Manufacturer's instruction	Manufacturer supplied	No	2	200	2	200	Centrifuge, flick and blot	Fusion	100		5000	NC<500
238	Manufacturer's instruction	Manufacturer supplied	No	4	100, 200			Vacuum manifold	MatchIT	50 if only one of two beads are <100		3500 but mostly >10000	NC<350
245	Manufacturer's instruction	Manufacturer supplied	No	5	175	5	175	Vacuum manifold	Fusion	50		500	NC<1500
262	Manufacturer's instruction	Manufacturer supplied	No	3	250			Vacuum manifold	MatchIT	60		10000	NC bead>600
267	Manufacturer's instruction	Manufacturer supplied	No	3	250			Vacuum manifold	MatchIT	Kit dependent	Kit dependent		MFI of NC beads are out of range
268	Manufacturer's instruction	Manufacturer supplied	No	4	100, 250			Vacuum manifold	MatchIT	60		3500	Kit dependent
273	Manufacturer's instruction	Manufacturer supplied	No	5	175	5	175	Vacuum manifold	Fusion	50		>500	>1500
277	Manufacturer's instruction	Manufacturer supplied	No	3	150, 200	2	200	Centrifuge, flick and blot	Porant, Fusion	100		500, PC/NC ratio ≥2	Our criteria is the NC bead ≥1500
284	Manufacturer's instruction	Manufacturer supplied	EDTA	4	175	4	175	Vacuum manifold	Fusion	50		5000	500
292	Manufacturer's instruction	Manufacturer supplied	No	3	200	3	200	Centrifuge, flick and blot	Fusion, Pr Teupine table	50		5000	NC<500
293	Manufacturer's instruction	Manufacturer supplied	No	3	1000	2	1000	Centrifuge, flick and blot	Fusion	100		PC beads should be higher than 500 or at least twice the NC value	High MFI value in NC bead
294	Manufacturer's instruction	Manufacturer supplied	No	3	250			Vacuum manifold	MatchIT	60		>10000 for Pos and Neg control, >3500 for patient sample	CON>Range
297	Manufacturer's instruction	Manufacturer supplied		4	175	4	175	Vacuum manifold	Fusion	50		1500	NC<500
303	Manufacturer's instruction	Manufacturer supplied	No	3	200	3	200	Centrifuge, flick and blot	Fusion	50		5000MFI	NC bead >500 MFI
309	Manufacturer's instruction	Manufacturer supplied	No	3	250			Vacuum manifold	MatchIT	60		3600 for human samples, 1000 for control serum of the kit	when lowest reaction bead is >500MFI
311	Manufacturer's instruction	Manufacturer supplied	EDTA	5	175	5	175	Vacuum manifold	Fusion	50		4000	NC<500
315	Manufacturer's instruction	Manufacturer supplied	EDTA	4	175	4	175	Vacuum manifold	Fusion	50		>500	<1500
323	Manufacturer's instruction	Manufacturer supplied	No	5	175			Vacuum manifold	Fusion	50		500	Neg and Pos sera MFI as they are known