

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

Samples 306-310/2017      DESPATCHED ON 13TH JUNE 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<br>42          | NIH       | B-cells     | No           |                      | Random      |      | Commercial        | 1                          | 1    | 5          | 1   | 30+30      | 45      | EB/AO                   | Computerised        | In-house              |          |
| 116<br>136        | NIH       | B-cells     | Yes          | Biorad               | Selected    | 26   | Biorad            |                            | 2    | 5          |     | 60         | 140     | EB/AO                   | Manual              |                       |          |
| 206<br>209<br>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<br>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<br>165 | LAT1288        | 007      | One Lambda   |                |          |              |                |          |           |          |



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

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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                   |          |           |               |                |                         |               |                        |
| 38     | LSAI     | ALL                   |          | 12255B    |               |                | Manufacturer            |               | 750                    | LSAII    | 03296A    |               |                | Manufacturer            |               | 750                    | LMX      | 3003946   | 2.5           | 20             | Manufacturer            |               | 1000                   |
| 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                     | LS1PRA LABScreen PRA Class I     |
| LM2       | Class II ID Kit                    | LS2PRA LABScreen PRA Class II    |
| LMX       | Lifecodes Lifescreen Deluxe Kit    | LS12PRA LABScreen PRA Class I&II |
| LSAI      | Lifecodes Lifescreen SA Class I    | LS1A04 LS SA Class I             |
| LSAII     | Lifecodes Lifescreen SA Class II   | LS2A01 LS SA Class II            |
| LSAI&II   | Lifecodes Lifescreen SA Class I&II | LSM12 LS Mixed Class I&II        |
| LSAMIC    | Lifecodes Lifescreen SA MICA       | LSMJTR 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**

Samples 306-310/2017 DESPATCHED ON 13TH JUNE 2017

**LUMINEX TECHNIQUE INFORMATION**

| Lab No | Positive control serum for Luminex testing? | Source                                                                                                                   | 1.2 Positive control serum |                                                                        | Same serum? | If no:                                                                                   | 1.3                                |                        |                       | 1.4 Serum treatment                                       |                   | Criteria?                                                                                                                     | 1.5 Incubation times        | Wash medium | Anything added | 1.6 Wash step details  |                              |              |                                      | Buffer removed by           | Software            | Mn bead count                         | 1.7 Data analysis                                                                                                        |                                                                                                                                                                             |
|--------|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|----------------------------|------------------------------------------------------------------------|-------------|------------------------------------------------------------------------------------------|------------------------------------|------------------------|-----------------------|-----------------------------------------------------------|-------------------|-------------------------------------------------------------------------------------------------------------------------------|-----------------------------|-------------|----------------|------------------------|------------------------------|--------------|--------------------------------------|-----------------------------|---------------------|---------------------------------------|--------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|        |                                             |                                                                                                                          | Every run?                 | If no:                                                                 |             |                                                                                          | Included once a week of in batches | Conditions?            | Storage time? (hours) | Methods used?                                             | Extra information |                                                                                                                               |                             |             |                | First wash step        | Second wash step             | Washes       | Volume (µl)                          |                             |                     |                                       | Washes                                                                                                                   | Volume (µl)                                                                                                                                                                 |
| 9      | Yes                                         | Sensitised Patient                                                                                                       | No                         |                                                                        | 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                                   | Selected Samples  |                                                                                                                               | 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                                    | Selected Samples  | 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  | Adsorbed 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-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                          | Selected Samples  |                                                                                                                               | 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                                                       | Selected Samples  |                                                                                                                               | Manufacturer's Instructions | Supplied    | No             | 4                      | 1 x 100                      | 3            | 4                                    | 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/-4                 | 24-48                 | EDTA                                                      | Selected Samples  |                                                                                                                               | 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 EDTA                                | Selected Samples  |                                                                                                                               | Manufacturer's Instructions | Supplied    | EDTA           | 3                      | 200                          | 2            | 200                                  | Centrifuge                  | Fusion 3            | 50                                    | 500                                                                                                                      | Adsorb Out                                                                                                                                                                  |
| 38     | No                                          |                                                                                                                          |                            |                                                                        |             |                                                                                          | Sometimes                          | -30                    | >48                   |                                                           | Selected Samples  |                                                                                                                               | Manufacturer's Instructions | Supplied    | No             | 2                      | 200                          | 2            | 200                                  | Centrifuge                  | HLA Fusion, MatchIT | 80                                    | 1500                                                                                                                     | 500                                                                                                                                                                         |
| 39     | Yes                                         | Sensitised Patient sera                                                                                                  | 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                                                                                                                                                                         |
| 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/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 single antigen beads |
| 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 | Selected Samples  | 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                                          | Selected Samples  |                                                                                                                               | 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 One with each class                                   | Always                             | -20                    | >48                   |                                                           | Selected Samples  |                                                                                                                               | Manufacturer's Instructions | Supplied    | No             | 3                      | 250                          |              | Vacuum Manifold                      | MatchIT                     | 50                  | 2000 with control serum 12000 Immucor | MFI > range for NC bead                                                                                                  |                                                                                                                                                                             |
| 112    | Yes                                         | Patient sensitised sera with low MFI value                                                                               | Yes                        |                                                                        | No          |                                                                                          | Yes                                | -20                    | >48                   | N/A                                                       | Selected Samples  |                                                                                                                               | Manufacturer's Instructions | Supplied    | No             | 1                      | 100                          | 3            | 250                                  | Vacuum Manifold, Centrifuge | HLA Fusion, MatchIT | 60 Immucor, 100 One Lambda 50         | 50                                                                                                                       | Bead NC >3000                                                                                                                                                               |
| 114    | Yes                                         | Sensitised patient sera                                                                                                  | Yes                        |                                                                        | Yes         |                                                                                          | Sometimes                          | -20                    | 24-48                 | EDTA                                                      | Selected Samples  |                                                                                                                               | Manufacturer's Instructions | Supplied    | No             | 3                      | 160                          | 2            | 160                                  | Centrifuge                  | HLA Fusion          | 50                                    | PC >1500                                                                                                                 | NC >500                                                                                                                                                                     |
| 116    | No                                          |                                                                                                                          |                            |                                                                        |             |                                                                                          | Always                             | -20                    | >48                   | Adsorption Beads                                          | Selected Samples  |                                                                                                                               | Manufacturer's Instructions | Supplied    | No             | 3                      | 200                          | 2            | 200                                  | Centrifuge                  | HLA Fusion          | 50                                    |                                                                                                                          | NC >800                                                                                                                                                                     |
| 117    | No                                          |                                                                                                                          |                            |                                                                        |             |                                                                                          | Always                             | -65                    | >48                   | Adsorption Beads                                          | Selected Samples  |                                                                                                                               | 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                                     | Selected Samples  |                                                                                                                               | Manufacturer's Instructions | Supplied    | EDTA           | 3                      | 200                          | 3            | 200                                  | Centrifuge                  | HLA Fusion          | 50                                    | 100MFI                                                                                                                   | NEG >500                                                                                                                                                                    |

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

|     |     |                                                       |     |                      |                                                                     |           |               |                                         |                                                                                                                                                                            |                             |          |      |     |               |   |     |                 |                                      |      |                                                               |                                                                                                                                                                             |
|-----|-----|-------------------------------------------------------|-----|----------------------|---------------------------------------------------------------------|-----------|---------------|-----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|----------|------|-----|---------------|---|-----|-----------------|--------------------------------------|------|---------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 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                                                                 | 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<br>For single antigen - 300                                                                                                                            |
| 136 |     | Sensitised patient sera                               | 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   | 3   | 150/200/200   | 2 | 200 | Centrifuge      | HLA Fusion                           | 100  | 3000MFI                                                       | NC >1500MFI                                                                                                                                                                 |
| 147 |     |                                                       |     |                      | Yes                                                                 | 20        | >48           |                                         |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 3   | 150/200/200   | 2 | 200 | Centrifuge      | HLA Fusion                           | 100  | 3000MFI                                                       | NC >1500MFI                                                                                                                                                                 |
| 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                                                       |                                                                                                                                                                             |
| 162 |     | 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                                                                                                                                                                    |
| 164 | Yes |                                                       | Yes |                      |                                                                     |           |               |                                         |                                                                                                                                                                            | 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                                                           |                                                                                                                                                                             |
| 181 | Yes | In House pool                                         | Yes | Yes                  | Always                                                              | 20        | 24-48         | Adsorption/EDTA                         |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 3   | 250           |   |     | Vacuum Manifold | MatchIT                              | 60   | 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                              | 60   | 5000                                                          | >500                                                                                                                                                                        |
| 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 |     |                                                       |     |                      |                                                                     |           |               |                                         |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 4   | 175           | 4 | 175 | Vacuum Manifold | Fusion 4.0.1 and homemade Excel file | 50   | PC >5000                                                      | NC >500                                                                                                                                                                     |
| 204 | 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 |     |                                                       |     |                      |                                                                     |           |               |                                         |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | EDTA | 5   | 175           | 5 | 175 | Vacuum Manifold | Fusion                               | 50   | 3000                                                          | NC >500                                                                                                                                                                     |
| 209 | Yes | Sensitised patient sera                               | Yes | Yes                  | Yes                                                                 | Frozen    | <24           | Dilution                                |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 3   | 250           |   |     | Vacuum Manifold | MatchIT                              | 60   | >10000                                                        | >1000                                                                                                                                                                       |
| 214 |     |                                                       |     |                      |                                                                     |           |               |                                         |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 3   | 250           | 1 | 150 | Vacuum Manifold | MatchIT                              | 100  | 5000                                                          |                                                                                                                                                                             |
| 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 |     |                                                       |     |                      |                                                                     |           |               |                                         |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 5   | 175           | 5 | 175 | Vacuum Manifold | HLA Fusion                           | 50   | PC >500 PC/NC >5                                              | NC >500                                                                                                                                                                     |
| 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 |     |                                                       |     |                      |                                                                     |           |               |                                         |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 5   | 275           | 5 | 275 | Vacuum Manifold | Fusion                               | 80   | 5000                                                          | NC >2000                                                                                                                                                                    |
| 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                                                                                                                                                                    |
| 239 | Yes |                                                       | Yes | No                   | Class I positive control for LMI, Class II positive control for LMI | 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  | 13415                                                         |                                                                                                                                                                             |
| 252 | Yes | Commercial                                            | Yes | No                   | Each commercial kit has its own positive control                    | Yes       | 20            | >48                                     |                                                                                                                                                                            | 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 |     |                                                       |     |                      |                                                                     |           |               |                                         |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 5   | 175           | 5 | 175 | Vacuum Manifold | HLA Fusion                           | 50   | >500                                                          | >1500                                                                                                                                                                       |
| 273 | 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 | No  |                                                       |     |                      | No                                                                  | 40        | >48           |                                         |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 3   | 250           | 3 | 250 | Vacuum Manifold | MatchIT                              | 5000 | 5000                                                          |                                                                                                                                                                             |
| 302 | Yes |                                                       | Yes | Yes                  | Yes                                                                 | 20        | >48           | EDTA/Adsorption Beads/Heat Inactivation |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 3   | 200           | 3 | 200 | Centrifuge      | Fusion                               | 50   | 5000 MFI                                                      | NC >500 MFI                                                                                                                                                                 |
| 303 | Yes | Sensitised patient sera                               | Yes | Yes                  | Always                                                              | 20        | >48           | EDTA/Adsorption Beads/Heat Inactivation |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 3   | 250           |   |     | Vacuum Manifold | MatchIT                              | 60   | 10000                                                         | NC >500                                                                                                                                                                     |
| 309 | Yes | Manufacturer                                          | Yes | No                   | Always                                                              | 4         | >48           |                                         |                                                                                                                                                                            | Manufacturer's Instructions | Supplied | No   | 3   | 200           | 2 | 200 | Centrifuge      | HLA Fusion                           | 50   | 6000                                                          | NC >1500                                                                                                                                                                    |
| 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                                                                                                                                                   |