

**SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2017**

| 1. Cell preparation |   |               |                          |   |                |                  |         |   |            |  |                              |                       |
|---------------------|---|---------------|--------------------------|---|----------------|------------------|---------|---|------------|--|------------------------------|-----------------------|
| Lab Code            | Cell preparation method   | Cell vol (µl) | Cell concentration       | Diluent   | Serum vol (µl) | Used as supplied | Diluted | Local negative control                    | Replicates | Local positive control                       | No of pos control replicates | No of test replicates |
| 9                   | Density gradient centrifugation                                   | 200           | 2-3x10 <sup>6</sup>      | RPMI with 10% FCS                                     | 50             | Yes              | 1:2     | Biosera                                   | 5          | Pooled serum of sensitised patients          | 2                            | 4                     |
| 11                  |   | 30            | 5x10 <sup>6</sup>        | PBS/Azide (0.1%)                                      | 20             | Yes              |         | AB Serum (unsensitised male blood donors) | 4          | Pooled highly sensitised patient sera        | 1 weak, 1 strong             | 2                     |
| 14                  | Lymphoprep  | 30            | 10 <sup>7</sup>          | PBS/BSA/Azide   | 40             | Yes              |         | NIBSC                                     | 2          | In-house                                     | 2                            | 2                     |
| 15                  |   | T: 25, B:50   | 10x10 <sup>6</sup>       | FACS diluent  | T: 25, B: 50   | Yes              |         | In-house                                  | 2          | In-house                                     | 2                            | 2                     |
| 19                  |   |               |                          |   |                |                  |         |   |            |  |                              |                       |
| 20                  |   | 50            | 5x10 <sup>6</sup>        | PBS   | 50             | Yes              |         | In-house                                  | 4          | NIBSC  | 2                            | 4                     |
| 23                  | Lymphoprep  | 50            | 5x10 <sup>6</sup>        | PBS/1%FBS   | 50             | Yes              |         | NIBSC                                     | 3          | In-house                                     | 2                            | 3                     |
| 24                  | Lymphocyte separation medium (Lympholyte)                         | 50            | 2.5x10 <sup>6</sup>      | HBSS  | 50             | Yes              |         | NIBSC                                     | 2          | NIBSC and In-house                           | 1                            | 3                     |
| 25                  | Lymphocyte separation medium                                      | 50            | 5M                       | 1% PBSA   | 50             |                  |         | NIBSC                                     | 3          | In-House                                     | 2                            | 3                     |
| 28                  | Lymphocyte density gradient method                                | 30            | 16x10 <sup>6</sup>       | PBA   | 50             | Yes              |         | Biorad                                    | 3          | Campath                                      | 2                            | 3                     |
| 34                  | Carbonyl iron lympholyte + Dextran sulphate                       | 50            | 6x10 <sup>6</sup>        | TBS   | 50             | Yes              |         | Local                                     | 6          | Local  | 2                            | 3                     |
| 35                  | Ficoll density gradient   | 50            | 5x10 <sup>6</sup>        | PBS+1% FCS  | 50             | Yes              |         | NIBSC                                     | 3          | In-house                                     | 3                            | 3                     |
| 38                  | Density gradient centrifugation                                   | 30            | 5M                       | PBS azide   | 20             | Yes              |         | NIBSC                                     | 2          | In-House                                     | 4                            |                       |
| 39                  | Lympholyte  | 50            | 1x10 <sup>7</sup>        | RPMI  | 50             | Yes              |         | NIBSC                                     | 3          | In-House                                     | 3                            | 3                     |
| 41                  | Density gradient  | 25            | 10x10 <sup>6</sup>       | 2% FBS/PBS  | 50             | Yes              |         | Seralab                                   | 3          | In-house                                     | 3                            |                       |
| 42                  |   | 50            | 4x10 <sup>6</sup>        | 1% FCS/PBS  | 50             |                  |         |   | 3          |  | 3                            | 3                     |
| 45                  |   | 40            | 2.5x10 <sup>6</sup>      | TPM   | 10             | Yes              |         | Local and NIBSC                           | 2          | 2 Local and NIBSC                            | 2                            | 2                     |
| 48                  |   | 30            | 5x10 <sup>6</sup>        | Local flow diluent                                    | 20             | Yes              |         | NIBSC                                     | 2          | Local pool                                   | 2                            | 2                     |
| 51                  | Density gradient centrifugation                                   | 50            | 2x10 <sup>6</sup>        | PBS   | 50             | Yes              |         | Local                                     | 3          | Local  | 2                            | 2                     |
| 54                  | Ficoll Hypaque  | 100           | 2x10 <sup>6</sup>        | PBS 0.1% azide 1% normal goat + 1% normal mouse serum | 50             |                  |         | SLI                                       | 6          | In-House + Human monoclonal antibodies       | 1                            |                       |
| 58                  |   | 50            | 5.7x10 <sup>6</sup>      | PBS   | 50             | Yes              |         | NIBSC                                     | 4          | In-House positive pool                       | 3                            | 3                     |
| 62                  | Gradient centrifugation   | 50            | 5M                       | 1% NBCF in PBS (PBSCS)                                | 50             | Yes              |         | NIBSC                                     | 3          | In-House                                     | 2                            | 2                     |
| 101                 |   | 100           |                          |   | 50             |                  |         |   | 2          |  | 2                            | 2                     |
| 112                 | Concentration gradient-lympholyte-H + Pronase Treatment           | 25            | 10x10 <sup>6</sup>       | PBS   | 30             | No               |         | Human serum AB                            | 2          | Pool patients                                | 2                            |                       |
| 114                 | Density gradient separation                                       | 100           | 2.5x10 <sup>5</sup>      | AB sera   | 20             | No               |         | Pooled AB sera                            | 2          | Pooled sensitized patients                   | 2                            | 2                     |
| 115                 |   | 250,000       |                          |   | 25             | Yes              |         | NIBSC                                     | 2          | NIBSC and In-House                           | 1                            | 2                     |
| 117                 | Ficoll  | 200           | 200,000                  | RPMI with 20% FBS                                     | 50             | Yes              |         | NIBSC                                     | 3          | Local pool                                   | 2                            | 2                     |
| 118                 |   | 10            | 1x10 <sup>6</sup>        | PBS   | 100            | No               |         | Local + NIBSC                             | 4          | Local  | 2                            | 2                     |
| 119                 | Density gradient  | 100-120       | 200000                   | PBS   | 100            | Yes              |         | AB+ male                                  | 2          | Patient pool                                 | 3                            |                       |
| 120                 | Cell wash centrifugation suspended in Hanks                       | 100           | 5M                       | PBS1x -BSA1%  | 50             | Yes              |         | AB serum                                  | 2          | Hyperimmunised serum                         | 2                            | 2                     |
| 122                 |   | 50            | 3x10 <sup>5</sup>        | PBS+2% FCS  | 50             | Yes              |         | NIBSC                                     | 2          | Local  | 1 weak, 1 strong             | 2                     |
| 126                 |   |               |                          |   |                |                  |         |   |            |  |                              |                       |
| 130                 |   |               | 0.25-0.3x10 <sup>6</sup> |   | 50             | Yes              |         | In-House                                  | 2          | In-house                                     | 1 weak, 1 strong             | 2                     |
| 133                 | Buffy coat (without density gradient)                             | 50            | Visual check             | RPMI  | 50             | Yes              | 1/2     | AB serum                                  | 1          | PRA positive (>10000MFI or 100%+)            | 1                            | 2                     |
| 136                 | Ficoll hypaque separation   | 100           | 2.5x10 <sup>6</sup>      | RPMI  | 20             | No               |         | AB serum                                  | 3          | Hypersensitized sera pool                    | 2                            | 2                     |
| 138                 |   | 100           | 10 <sup>7</sup>          | PBS   | 80             | Yes              |         | AB serum                                  | 1          | Hypersensitized sera pool                    | 1                            | 1                     |
| 139                 |   | 50            | 6000                     | RPMI-SVF (5%)   | 50             | Yes              |         | AB serum                                  | 2          | Hyperimmunized patient pool                  | 1                            | 1                     |
| 142                 |   | 2000          | 6-15                     | PBS   | 20             | Yes              | 1:8     |   | 3          |  | 1                            | 1                     |
| 143                 | Ficoll layering (Lymphoprep) and magnetic nanoparticle separation | 40            | 3x10 <sup>6</sup>        | FBS   | 50             | Yes              |         | In-house                                  | 2          | Local pooled sera                            | 1                            | 2                     |
| 144                 | Ficoll separation   | 25            | 15x10 <sup>6</sup>       | PBS   | 25             | Yes              |         | Local                                     | 1          | Local  | 2                            | 2                     |
| 145                 | Ficoll  | 50            | 1x10 <sup>7</sup>        | PBS   | 50             | Yes              |         | Local                                     | 1          | Biorad                                       | 1                            | 1                     |
| 147                 | Ficoll  | 100           | 5000                     | RPMI  | 50             | Yes              |         | Human serum AB                            | 2          | Serum from polycy immunised patients         | 2                            | 2                     |
| 149                 | Ficoll  | 50            | 1x10 <sup>7</sup>        | PBS   | 50             | Yes              |         | Biorad                                    | 1          | Biorad                                       | 1                            | 1                     |
| 154                 |   | 20            | 15x10 <sup>6</sup>       | PBS BSA 1%  | 50             | Yes              |         | Commercial                                | 2          | Commercial                                   | 2                            |                       |
| 157                 |   | 15            | 1.5x10 <sup>5</sup>      | PBS   | 50             | Yes              |         | In-house                                  | 2          | Hyperimmunized patient pool                  | 2                            | 2                     |
| 159                 |   | 100           | 3000                     | PBS BSA 1%  | 50             | Yes              |         | Local                                     | 3          | Local  | 2                            | 2                     |
| 160                 | Cell wash centrifugation suspension in RPMI+2%FBS                 | 50            | 10 <sup>6</sup>          | RPMI+2%FBS  | 50             | Yes              | 2       | Local                                     | 2          | Biotest                                      | 1                            | 2                     |
| 163                 | Lymphocyte separation medium                                      | 0             | 0.2x10 <sup>6</sup>      |   | 50             | Yes              |         | NIBSC                                     | 2          | NIBSC  | 2                            | 2                     |
| 167                 |   |               |                          |   |                |                  |         |   |            |  |                              |                       |
| 169                 |   |               |                          |   |                |                  |         |   |            |  |                              |                       |
| 176                 | PBMC isolation (Lymphoprep)                                       | 100           | 2E6/ml                   | TBS   | 100            | Yes              |         | AB donor pool                             | 1          | Local serum                                  | 1                            | 1                     |
| 186                 | Ficoll  | 100           | 5M                       |   | 25             | Yes              |         | Pool sera for AB donors                   | 1          | Local sera from immunized patients           | 2                            | 1                     |
| 190                 | Ficoll  | 100           | 200000                   | NaCl  | 50             |                  |         | SAB male                                  | 3          | Pool hyperimmunised                          | 2                            | 3                     |
| 191                 | isolated cells sent were used. Washed with PBS                    | 100           | 2-3x10 <sup>5</sup>      | PBS   | 20             |                  |         | AB Negative, PRA negative sera            | 2          | Pooled PRA positive sera                     | 2                            | 2                     |
| 193                 | Manual  | 100           | 3000-5000                | RPMI  | 20             | Yes              |         | Pooled sera                               | 2          | 2 pooled sera                                | 2                            | 2                     |
| 194                 |   | 100           | 2400-3800                | PBS   | 20             | Yes              |         | In-house                                  | 2          | In-house                                     | 1                            | 2                     |
| 195                 |   | 25            | 20000                    | RPMI  | 50             | Yes              |         | NIBSC                                     | 3          | Local  | 1                            | 2                     |
| 201                 |   | 50            | 1-1.5x10 <sup>7</sup>    | 20% medium (IMDM+FCS)                                 | 50             | Yes              |         | Local NC                                  | 1          | Local PC                                     | 1                            | 1                     |
| 202                 |   |               |                          |   |                |                  |         |   |            |  |                              |                       |
| 204                 | Ficoll separation   | 50            | 6000                     | RPMI  | 50             | Yes              |         | Pool of AB sera from healthy blood donors | 2          | Pool of sera from hypersensitized recipients | 1                            | 1                     |
| 206                 | PBMC (Ficoll gradient)  | 25            | 10000                    | PBS+FCS   | 50             | Yes              |         | Local neg sera pooled                     | 1          | Local positive sera pooled                   | 1                            | 2                     |
| 209                 |   |               |                          |   |                |                  |         |   |            |  |                              |                       |
| 218                 | Ficoll  | 400           |                          |   | 200            |                  |         | Local                                     | 2          | Local  | 1                            | 1                     |
| 220                 | Pronase-treatment   | 15            | 10000                    | 2% FCS, 5% Na3-PBS                                    | 15             | Yes              |         | In-house                                  | 4          | In-house                                     | 2                            | 2                     |
| 227                 | STEMCELL  | 50            | 6x10 <sup>6</sup>        | NaCl 0.9%   | 50             | Yes              |         | In-house                                  | 2          | In-house                                     | 2                            | 2                     |
| 235                 | Ficoll hypaque  | 50            | 600000                   | RPMI+FCS  | 50             | Yes              | 1/2     | AB non sensitized donor                   | 2          | Hypersensitized patient                      | 1                            | 1                     |
| 238                 | Lymphocytes Separation Medium                                     | 50            | 10M                      | RPMI  | 50             | Yes              |         | Pool AB human sera                        | 2          | Pool hyperimmunised patient's sera           | 2                            | 2                     |
| 245                 | Whole blood   | 100           | 2500                     | PBS   | 75             | Yes              |         | NIBSC                                     | 3          | NIBSC  | 2                            | 2                     |
| 246                 | Ficoll  | 150           | 400000                   | RPMI  | 150            | Yes              |         | NIBSC                                     | 4          | NIBSC+BAG                                    | 1                            | 1                     |
| 252                 |   | 25            |                          |   | 25             |                  |         | AB + Healthy serum                        |            | PRA >80% serum                               |                              |                       |
| 260                 |   | 5             |                          |   | 20             |                  |         | Ab male                                   | 1          | Pooled positive sera                         | 1                            | 1                     |
| 262                 | Ficoll  | 50            | 500000                   | RPMI + Human SAB 10%                                  | 50             |                  |         | Human SAB                                 | 2          | Immunised patient                            | 1                            | 2                     |
| 271                 | Ficoll hypaque  | 250000        | 250000                   | PBS 2% FBS  | 25             | Yes              |         | AB NHS Invitrogen                         | 2          | In-house                                     | 1                            | 2                     |
| 284                 | Mononuclear cells isolated by density centrifugation over Ficoll  | 50            | 5x10 <sup>6</sup>        | RPMI  | 50             | Yes              |         | NIBSC                                     | 3          | Local + NIBSC                                | 2                            | 2                     |
| 293                 |   |               |                          |   |                |                  |         |   |            |  |                              |                       |
| 297                 |   | 50            |                          |   | 50             |                  |         | Local                                     | 2          | Local  | 1                            | 2                     |
| 341                 | Density gradient centrifugation using Ficoll                      | 50            | 5500                     | PBS+2% FCS  | 50             | Yes              | 1/16    | NC HLA Class I+II                         | 2          | PC HLA Class I+II                            | 1                            | 2                     |
| 351                 | Ficoll hypaque  | 200           | 2-2.5x10 <sup>6</sup>    |   | 20             | Yes              |         | Local                                     | 1          | Local  | 1                            | 2                     |
| 358                 | Density gradient centrifugation                                   | 100           | 5x10 <sup>6</sup>        | PBS +1% BSA   | 50             | Yes              |         | Inno-Train                                | 1          | Inno-Train                                   | 1                            | 1                     |

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| Lab Code | 2. Sensitisation Stage |           |                         |                            | 3. Detection Stage: |        |           |                   |           |   |                                  |  |          |                 |               |
|----------|------------------------|-----------|-------------------------|----------------------------|---------------------|--------|-----------|-------------------|-----------|---|----------------------------------|--|----------|-----------------|---------------|
|          | Time (mins)            | Temp (°C) | Manual Automated washer | Wash medium                | Vol / tube (ml)     | Washes | Temp (°C) | Manufacturer      | Raised in | Labelled with                               | Dilution                         | Diluted in   | Vol (µl) | Inc time (mins) | Inc temp (°C) |
| 9        | 30                     | 37        | Manual                  | PBS                        | 100                 | 3      | RT        | Life Technologies | Goat      | FITC/Fab2 fragment                          | 1:20                             | PBS  | 50       | 30              | 4             |
| 11       | 30                     | 37        | Manual                  | Cold PBS/Azide             | 4                   | 3      | 22        | Sigma             | Goat      | FITC/Fab2 fragment                          | 1/10                             | PBS/Azide  | 20       | 20              | 4             |
| 14       | 30                     | RT        | Manual                  |                            | 2                   | 2      | RT        | Sigma             | Goat      | FITC/Fab2 fragment                          | 1/23                             | PBS/BSA/Azide  | 115      | 20              | 4             |
| 15       | 30                     | RT        | Automated               | PBS                        | 3                   | 3      | RT        | Sigma             | Goat      | FITC/Fab2 fragment                          | 1:180                            | FACS diluent   | 100      | 15              | 4             |
| 19       |                        |           |                         |                            |                     |        |           |                   |           |   |                                  |  | 50       | 30              | 4             |
| 20       | 15                     | 37        | Automated               | PBS                        | 2                   | 2      | 2-8       | Beckman Coulter   | Goat      | FITC/Fab2 fragment                          | 1:20                             | CD3-PC5 and CD19-PE (detection cocktail)             | 5        | 15              | 2-8           |
| 23       | 30                     | 37        | Automated               | PBS/FBS                    | 1                   | 3      | 4         | Sigma             | Goat      | FITC/Fab2 fragment                          | 1:40                             | PBS  | 50       | 30              | 4             |
| 24       | 30                     | 37        | Automated               | BD cell wash               | BD LWA              | 4ml x3 | RT        | Sigma             | Goat      | FITC/Fab2 fragment                          | 1/15                             | PBS  | 5        | 30              | 2-8           |
| 25       | 15                     | 37        | Automated               | 1% PBSA                    | 1                   | 3      | RT        | Sigma             | Goat      | FITC/Fab2 fragment                          | 1/50                             | 1% PBSA  | 5        | 15              | 4             |
| 28       | 30                     | 20        | Manual                  | PBA                        | 2.5                 | 2      | 4         | DAKO              | Rabbit    | FITC/Fab2 fragment                          | 1:20                             | PBA  | 100      | 30              | 4             |
| 34       | 30                     | 37        | Automated               | TBS                        | 5                   | 2      | 4         | Sigma             | Goat      | FITC/Fab2 fragment                          | 1/32                             | TBS  | 50       | 30              | 4             |
| 35       | 30                     | 37        | Manual                  | PBS+1% FCS                 | 1.5                 | 3      | 4         | Sigma             | Goat      | FITC  | 1:300                            | dH2O   | 50       | 30              | RT            |
| 38       | 30                     | 22        | Manual                  | PBS azide                  | 4                   | 2      | 22        | Southern Biotech  | Goat      | FITC/Fab2 fragment                          | 1/50                             | PBS azide  | 100      | 20              | 4             |
| 39       | 30                     | 37        | Manual                  | 1% FBS in PBS buffer       | 4                   | 3      | RT        | Sigma             | Goat      | FITC/Fab2 fragment                          | 1:20                             | 1% FBS in PBS buffer                                 | 4        | 20              | 4-8           |
| 41       | 20                     | 22        | Automated               |                            | 4                   | 3      | 22        | Sigma             | Goat      | FITC/Fab2 fragment                          | 1:2000                           | 2% FBS/PBS   | 90       | 10              | 22            |
| 42       | 30                     | 22        | Manual                  | 1% FCS/PBS                 | 2                   | 2      | 22        |                   | Goat      | FITC/Fab2 fragment                          | 1/10                             | PBS  | 10       | 15              | 22            |
| 45       | 30                     | RT        | Manual                  | PBA                        | 1                   | 2      | RT        | BioSciences       | Goat      | FITC/Fab2 fragment                          | 1/50                             | PBA  | 50       | 45              | Ice           |
| 48       | 30                     | 21        | Manual                  | Local flow diluent         | 2                   | 3      | 21        |                   | Rabbit    | FITC/Fab2 fragment                          | Neat                             | Neat   | 4        | 30              | 4             |
| 51       | 30                     | RT        | Manual                  | PBS                        | 3                   | 2      | RT        | DAKO              | Rabbit    | FITC/Fab2 fragment                          | 1:10                             | PBS  | 5        | 30              | 4             |
| 54       | 30                     | 22        | Yes                     | PBS 0.1% azide             | 4                   | 2      | 4         | Sigma             | Goat      | FITC/whole IgG                              | 1:20                             | PBS 0.1% azide 1% normal goat + 1% normal mous serum | 100      | 30              | 4             |
| 58       | 30                     | 37        | Manual                  | PBS                        | 4                   | 3      | 4         | Sigma             | Goat      | FITC/Fab2 fragment                          | 1:50                             | PBS  | 5        | 30              | 4             |
| 62       | 30                     | 37        | Manual                  | PBSCS                      | 4                   | 3      | 22        | DAKO              | Rabbit    | FITC/Fab2 fragment                          |                                  |  | 12       | 30              | 22            |
| 101      | 30                     | Ambient   | Manual                  | PBS                        | 2-3                 | 2      | Ambient   | Life Technologies | Goat      | FITC/Fab2 fragment                          | Lot dependent                    | PBS  | 10       | 30              | 4             |
| 112      | 30                     | 4         | Automated               | PBS                        | 0.8                 | 2      | Ambient   | Invitrogen        | Goat      | FITC/Fab2 fragment                          | 1/60                             | PBS  | 80       | 10              | 4             |
| 114      | 30                     | RT        | Manual                  | DPBS without Ca++          | 150                 | 3      | RT        | DAKO              | Rabbit    | FITC/Fab2 fragment                          | 1/30                             | PBS  | 20       | 20              | 4             |
| 115      | 30                     | 4         | Manual                  | PBS+2% FBS                 | 0.4                 | 3      | 4         | Jackson           | Goat      | FITC/Fab2 fragment                          | 1:160                            |  | 20       | 30              | 4             |
| 117      | 30                     | 24        | Manual                  | PBS                        | 2                   | 3      | 24        | BD                |           | FITC/Fab2 fragment                          |                                  |  | 10       | 30              | 4             |
| 118      | 30                     | RT        | Manual                  | PBS                        | 3                   | 4      | RT        | BD                | Goat      | Streptavidin FITC/Fab2 fragment/ Biotin IgG | 1/50, 1/100                      | PBS  | 200      | 1.15, 2.10      | RT            |
| 119      | 30                     | RT        | Manual                  | PBS                        | 2                   | 3      | RT        | Jackson           | Goat      | FITC/Fab2 fragment                          | Optimum dilution after titration | H2O distilled 1ml                                    | 10       | 30              | RT            |
| 120      | 30                     | RT        | Manual                  | PBS1x - BSA1%              | 2                   | 3      | RT        |                   | Goat      | FITC/Fab2 fragment                          | 1/80                             | PBS1x - BSA1%  | 50       | 30              | 4             |
| 122      | 30                     | 22        | Manual                  | PBS+2% FCS                 | 1                   | 3      | 22        | Invitrogen        | Goat      | FITC/Fab2 fragment                          | 1:50                             | PBS+2% FCS   | 50       | 30              | 4             |
| 126      |                        |           |                         |                            |                     |        |           |                   |           |   |                                  |  | 50       | 20              | 4-8           |
| 130      | 30                     | RT        | Manual                  | Washing buffer             | 2                   | 3      | RT        | Jackson           | Goat      | FITC/Fab2 fragment                          | 1/80                             | Washing buffer                                       | 25       | 20              | Ice           |
| 133      | 30                     | 22        | Manual                  | PBS                        | 2                   | 2      | 22        | DAKO              | Rabbit    | FITC/Fab2 fragment                          | 1/20                             | PBS  | 50       | 40              | 4             |
| 136      | 30                     | RT        | Manual                  | PBS/azide/FCS 3%           | 200                 | 4      | RT        | DAKO              | Rabbit    | FITC/Fab2 fragment                          | 1/50                             | Gelatine/PBS/azide                                   | 20       | 20              | 4             |
| 138      | 30                     | 2         | Manual                  | PBS                        | 4                   | 1      | 4         |                   | Rabbit    | FITC/Fab2 fragment                          |                                  |  | 20       | 20              | 5             |
| 139      | 30                     | 22        | Manual                  | PBS                        | 0.2                 | 3      | 22        | Coulter           | Goat      | FITC/Fab2 fragment                          | 1/40                             | PBS  | 10       | 30              | 4             |
| 142      | 30                     | 4         | Manual                  | PBS                        | 0.2                 | 3      | 4         |                   | Goat      | FITC/Fab2 fragment                          |                                  |  | 0.5      | 20              | 4             |
| 143      | 20                     | 24        | Manual                  | BD pharmingen stain buffer | 1.5                 | 2      | 2-4       | Jackson           | Goat      | FITC/Fab2 fragment                          |                                  |  | 10       | 15              | 2-4           |
| 144      | 30                     | RT        | Manual                  | PBS                        | 4                   | 3      | RT        | DAKO              | Rabbit    | FITC/Fab2 fragment                          | 1:25                             | PBS  | 25       | 30              | 4             |
| 145      | 30                     | RT        | Manual                  | PBS                        | 3                   | 2      | RT        | Sigma             | Goat      | FITC/Fab2 fragment                          | 1:40                             | PBS  | 50       | 20              | 4             |
| 147      | 30                     | 4         | Manual                  | PBS                        | 2.5                 | 3      | 22        | Biorad            | Goat      | FITC/Fab2 fragment                          | 1/500                            | PBS  | 100      | 30              | 4             |
| 149      | 30                     | RT        | Manual                  | PBS                        | 3                   | 2      | RT        | Sigma             | Goat      | FITC/Fab2 fragment                          | 1:40                             | PBS  | 50       | 20              | 4             |
| 154      | 30                     | 20        | Manual                  | PBS-BSA1%                  | 1                   | 2      | 20        | Beckman Coulter   | Goat      | FITC/Fab2 fragment                          | 1:250                            | PBS-BSA1%  | 50       | 30              | 20            |
| 157      | 30                     | 4         | Manual                  | PBS 0.02% NaN3 2% FBS      | 2                   | 2      | 4         | DAKO              | Rabbit    | FITC/Fab2 fragment                          | 1:3                              | PBS  | 20       | 20              | 4             |
| 159      | 30                     | 22        | Manual                  | PBS-BSA1%                  | 1                   | 3      | 4         | Biorad            | Sheep     | FITC/Fab2 fragment                          | 1/500                            | PBS-BSA1%  | 100      | 30              | 4             |
| 160      | 30                     | 37        | Manual                  | BD cell wash               | 2                   | 2      | RT        | Sigma             | Goat      | FITC/Fab2 fragment                          | 1:80                             | PBS  | 50       | 30              | 4             |
| 163      | 30                     | Ambient   | Manual                  | PBS 1X/BSA 0.5%            | 2                   | 3      | 20        | Jackson           | Goat      | FITC/Fab2 fragment                          | 1:200                            | PBS-BSA1%  | 20       | 30              | 4             |
| 167      |                        |           |                         |                            |                     |        |           |                   |           |   |                                  |  | 20       | 30              | 4             |
| 169      |                        |           |                         |                            |                     |        |           |                   |           |   |                                  |  |          |                 |               |
| 176      | 30                     | 20        | Manual                  | TBS                        | 3                   | 3      | 18        | DAKO              | Rabbit    | FITC/Fab2 fragment                          |                                  |  | 5        | 20              | 20            |
| 186      | 30                     | 4         | Manual                  | PBS                        | 2                   | 2      | 4         | Beckman Coulter   | Goat      | FITC/Fab2 fragment                          | 1/200                            | PBS + FBS 8%   | 50       | 30              | 4             |
| 190      | 30                     | 20        | Manual                  | PBS/BSA/Azide              | 0.2                 | 4      | 20        | Jackson           | Goat      | FITC/Fab2 fragment                          |                                  |  | 40       | 30              | 4             |
| 191      | 30                     | RT        | Automated               | PBS                        | 5                   | 2      | RT        | Jackson           | Rabbit    | Phycoerythrin                               | 1/40                             | PBS  | 5        | 30              | RT            |
| 193      | 30                     | 22        | Manual                  | PBS-BSA1%                  | 2                   | 3      | RT        | Jackson           | Goat      | FITC/Fab2 fragment                          | 1/60                             | PBS-BSA1%  | 50       | 30              | 4             |
| 194      | 30                     | 22        | Manual                  | PBS-BSA                    | 2                   | 2      | 22        | Beckman Coulter   | Goat      | FITC/Fab2 fragment                          | 1/100                            | PBS  | 50       | 30              | 5             |
| 195      | 30                     | 22        | Manual                  | PBS                        | 4                   | 2      | 22        | DAKO              | Rabbit    | FITC/Fab2 fragment                          | 1/50                             | PBS  | 100      | 30              | 22            |
| 201      | 30                     | 22        | Manual                  | PBS                        | 4                   | 3      | 22        | Jackson           | Goat      | FITC/Fab2 fragment                          | 1:100                            | PBS  | 5        | 30              | 22            |
| 202      |                        |           |                         |                            |                     |        |           |                   |           |   |                                  |  |          |                 |               |
| 204      | 30                     | 22        | Manual                  | PBS                        | 0.2                 | 4      | 22        | Beckman Coulter   | Goat      | FITC/Fab2 fragment                          | 1/400                            | PBS  | 10       | 30              | 22            |
| 206      | 30                     | 20        | Manual                  | PBS+FCS                    | 0.2                 | 2      | 20        | DAKO              | Rabbit    | FITC/Fab2 fragment                          | 1/100                            | PBS  | 100      | 20              | 4             |
| 209      |                        |           |                         |                            |                     |        |           |                   |           |   |                                  |  |          |                 |               |
| 218      | 60                     | 37        | Manual                  | PBS                        | 5                   | 2      | RT        | Abcam             | Goat      |   |                                  |  | 5        | 30              | RT            |
| 220      | 30                     | 4         | Manual                  | 2% FCS0, 5% NaN3-PBS       | 200                 | 2      | 4         | Jackson           | Goat      | Phycoerythrin/Fab2 fragment                 | 512                              | PBS-CD3-CD19   | 50       | 30              | 4             |
| 227      | 30                     | 22        | Manual                  | PBS                        | 0.2                 | 3      | 22        | Jackson           | Goat      | Phycoerythrin/Fab2 fragment                 | 1/10                             | PBS  | 10       | 30              | 4             |
| 235      | 30                     | 4         | Manual                  | PBS                        | 1                   | 2      | 4         | DAKO              | Rabbit    | Phycoerythrin/Fab2 fragment                 | 1/10                             | PBS  | 25       | 30              | 4             |
| 238      | 30                     | 22        | Manual                  | PBS                        | 4                   | 2      | 22        | Biorad            | Sheep     | Phycoerythrin/Fab2 fragment                 | 1/80                             | PBS  | 100      | 20              | 22            |
| 245      | 30                     | 22-24     | Manual                  | PBS + SVF                  | 3                   | 2      | 22-24     | DAKO              | Rabbit    | FITC/Fab2 fragment                          |                                  |  | 10       | 30              | 22-24         |
| 246      | 30                     | 20        | Manual                  | FACS Flow                  | 10                  | 3      | 20        |                   | Mouse     | FITC/whole IgG                              |                                  |  | 5        | 10              | 20            |
| 252      | 30                     | RT        | Manual                  | PBS x1                     | 1                   | 3      | RT        |                   | Rabbit    | FITC/Fab2 fragment                          | 1x                               | PBS x1   | 50       | 30              | RT            |
| 260      | 30                     | 22        | Manual                  | Cell wash BD               | 1                   | 2      | 22        | Jackson           | Goat      | FITC/Fab2 fragment                          | 1.5                              | dH2O   | 5        | 30              | 22            |
| 262      | 30                     | 20-25     | Manual                  | PBS                        | 3                   | 2      | 20-25     | Biorad            | Sheep     | FITC/Fab2 fragment                          | 1/100                            | PBS  | 100      | 20              | 4             |
| 271      | 30                     | 4         | Manual                  | PBS 2% FBS                 | 0.5                 | 3      | 20-25     | Jackson           | Goat      | FITC/Fab2 fragment                          | 1/100                            | PBS 2% FBS   | 20       | 20              | 4             |
| 284      | 30                     | 22        | Manual                  | Cell wash BD               | 2                   | 2      | 22        | DAKO              | Rabbit    | FITC/Fab2 fragment                          |                                  |  | 10       | 30              | 4             |
| 293      |                        |           |                         |                            |                     |        |           |                   |           |   |                                  |  |          |                 |               |
| 297      | 30                     | 22        | Manual                  | PBS                        | 0.2                 | 3      | 22        |                   | Goat      | FITC/Fab2 fragment                          | 1/40                             | PBS  |          | 30              | 4             |
| 341      | 30                     | 22-25     | Manual                  | PBS+2%FCS                  | 1                   | 3      | 22-25     | Invitrogen        | Goat      | FITC/Fab2 fragment                          | 1000                             | PBS+2%FCS  | 50       | 30              | 4             |
| 351      | 30                     | 4         | Manual                  | Cold PBS Azide             | 1                   | 5      | 4         | DAKO              | Rabbit    | FITC/Fab2 fragment                          |                                  |  | 3        | 30              | 4             |
| 358      | 30                     | RT        | Manual                  | PBS+1% BSA                 | 1                   | 3      | RT        | Jackson           | Goat      | FITC  | 1:100                            | PBS+1%BSA  | 50       | 30              | RT            |

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| 4. Anti-T cell reagent |                 |                                    |   |           |                            |                |                  |           |  |                         |     |               |         |                      |          |                |                 |               |
|------------------------|-----------------|------------------------------------|---|-----------|----------------------------|----------------|------------------|-----------|--|-------------------------|-----|---------------|---------|----------------------|----------|----------------|-----------------|---------------|
| Lab Code               | Manufacturer    | IgG added with anti-T cell reagent | Wash step before adding anti-T cell reagent | Wash step | Wash medium                | Vol/ tube (ml) | Number of washes | Temp (°C) | Anti-T cell reagent added with the anti-B cell reagent | Labelled with           | CD3 | Other         | Diluted | Diluent              | Dilution | Vol/ tube (µl) | Inc time (mins) | Inc temp (°C) |
| 9                      | Beckman Coulter | Yes                                |   | Manual    | PBS                        | 100            | 3                |           |  | Phycoerythrin-cyanine 5 | Yes |               | Yes     | PBS                  | 1:30     | 50             | 30              | 4             |
| 11                     |                 | No                                 |   | Manual    | Cold PBS/Azide             | 4              | 1                | RT        | Yes  | PerCp                   | Yes |               | No      |                      |          | 3              | 20              | 4             |
| 14                     | BD              | Yes                                |   |           |                            |                |                  |           | Yes  | PE                      | Yes |               | Yes     |                      | 1/11.5   | 115            | 20              | 4             |
| 15                     | DAKO            | No                                 | Yes   | Automated |                            | 3              | 1                | RT        | No   |                         | Yes |               | Yes     | FACS diluent         | 1:60     | 100            | 15              | 4             |
| 19                     |                 |                                    |   |           |                            |                |                  |           |  |                         |     |               | No      |                      |          | 10             | 30              | 4             |
| 20                     | Beckman Coulter | Yes                                |   |           |                            |                |                  |           | Yes  | Phycoerythrin-cyanine 5 | Yes |               | Yes     | Detection cocktail   | 1:2      | 5              | 15              | 2-8           |
| 23                     | BD              | Yes                                |   |           |                            |                |                  |           | No   | PE                      | Yes |               | No      |                      |          | 5              | 30              | 4             |
| 24                     | BD              | Yes                                |   |           |                            |                |                  |           | Yes  | Phycoerythrin           | Yes |               | No      |                      |          | 5              | 30              | 2-8           |
| 25                     | BD              | No                                 | Yes   | Automated | 1% PBSA                    | 1              | 3                | RT        | Yes  |                         | Yes | PE            | No      |                      |          | 5              | 15              | 4             |
| 28                     | BD              | Yes                                |   |           |                            |                |                  |           | Yes  |                         | Yes |               | No      |                      |          | 5              | 30              | 4             |
| 34                     | BD              | Yes                                |   |           |                            |                |                  |           | No   | Phycoerythrin           | Yes |               | No      |                      |          | 5              | 30              | 4             |
| 35                     | DAKO            | No                                 | Yes   | Manual    |                            | 1.5            | 3                | 4         | No   | R-Phycoerythrin         | Yes |               | No      |                      |          | 10             | 20              | 2-8           |
| 38                     | Beckman Coulter | No                                 | Yes   | Manual    | PBS azide                  | 4              | 1                | 4         | Yes  | Phycoerythrin-cyanine 5 | Yes |               | Yes     | PBS azide            | 1/20     | 100            | 30              | 4             |
| 39                     | DAKO            | No                                 | Yes   | Manual    | 1% FBS in PBS buffer       | 4              | 1                | RT        | Yes  | RPE                     | Yes |               | Yes     |                      |          | 4              | 20              | 4-8           |
| 41                     | BD              | Yes                                |   |           |                            |                |                  |           | Yes  |                         | Yes | APC           | Yes     | 2% FBS/PBS           | 1:20     | 4.75           | 10              | 22            |
| 42                     | DAKO            | No                                 | Yes   | Manual    | 1% FCS/PBS                 |                |                  |           | Yes  | RPE                     | Yes |               | No      |                      |          | 5              | 15              | 22            |
| 45                     | Beckman Coulter | No                                 | Yes   | Manual    |                            | 1              | 1                | RT        |  |                         | Yes |               | Yes     | PBA                  | 1/10     | 50             | 15              | Ice           |
| 48                     | DAKO            | Yes                                |   |           |                            |                |                  |           | Yes  |                         | Yes | PE            | Yes     |                      |          | 4              | 30              | 4             |
| 51                     | Beckman Coulter | Yes                                |   | Manual    | PBS                        | 3              | 2                | 4         | Yes  | ECD                     | Yes |               | Yes     | PBS                  | 1:10     | 5              | 30              | 4             |
| 54                     | BD              | No                                 | No  |           |                            |                |                  |           | No   |                         | Yes | PE            | Yes     |                      |          | 5              | 15              | 4             |
| 58                     |                 | Yes                                |   |           |                            |                |                  |           | Yes  | Phycoerythrin-cyanine 5 | Yes |               | No      |                      |          | 5              | 30              | 4             |
| 62                     | Beckman Coulter | No                                 | Yes   | Manual    | PBSCS                      | 4              | 2                | 22        | Yes  | Phycoerythrin-cyanine 5 | Yes |               | No      |                      |          | 5              | 15              | 22            |
| 101                    | Beckman Coulter | Yes                                |   |           |                            |                |                  |           | Yes  | A750                    | Yes |               | No      |                      |          | 5              | 30              | 4             |
| 112                    | Iotest          | No                                 | No  |           |                            |                |                  |           | Yes  | PE                      | Yes |               | No      |                      |          | 5              | 20              | 4             |
| 114                    | Beckman Coulter | Yes                                |   | Manual    | DPBS without Ca++          | 150            | 1                | 4         | Yes  | Phycoerythrin           | Yes |               | No      |                      |          | 20             | 20              | 4             |
| 115                    | BD              | Yes                                |   | Manual    | PBS+2% FBS                 | 0.4            | 1                | 4         | Yes  | PerCp                   | Yes |               | Yes     | PBS+2% FBS           | 1:4      | 20             | 30              | 4             |
| 117                    | BD              | No                                 | No  |           |                            |                |                  |           | Yes  | Phycoerythrin           | Yes |               | No      |                      |          | 10             | 30              | 4             |
| 118                    | BD              | No                                 | No  | Manual    | PBS                        | 3              | 2                | RT        | No   | PE                      | Yes |               | Yes     | PBS                  | 1/20     | 200            | 15              | RT            |
| 119                    | Beckman Coulter | Yes                                | No  | Manual    | PBS                        | 2              | 3                | RT        | Yes  | Phycoerythrin-PE        | Yes |               | No      |                      |          | 10             | 30              | RT            |
| 120                    | Beckman Coulter | Yes                                |   | Manual    |                            | 2              | 3                | RT        | Yes  |                         | Yes | PC7           | No      |                      |          | 10             | 30              | 4             |
| 122                    | Immunostep      | Yes                                |   |           |                            |                |                  |           | Yes  |                         | Yes | PerCp         | Yes     | PBS+2% FCS           | 1:16     | 50             | 30              | 4             |
| 126                    |                 |                                    |   |           |                            |                |                  |           | No   |                         | Yes |               | No      |                      |          | 2.5            | 20              | 4-8           |
| 130                    | BD              | Yes                                |   | Manual    | Washing buffer             | 2              | 2                | RT        | No   |                         | Yes | Phycoerythrin | Yes     | Washing buffer       | 1/4      | 25             | 20              | Ice           |
| 133                    | BD              | Yes                                |   |           |                            |                |                  |           | Yes  | PerCp                   | Yes |               | No      |                      |          | 15             | 40              | 4             |
| 136                    | DAKO            | Yes                                |   |           |                            |                |                  |           | Yes  | PE - Phycoerythrin      | No  | CD2           | No      |                      |          | 5              | 20              | 4             |
| 138                    |                 | No                                 | Yes   | Manual    | PBS                        | 4              | 1                | 4         | Yes  | APC                     | Yes |               | No      |                      |          | 5              | 15              | 4             |
| 139                    | Coulter         | Yes                                |   |           |                            |                |                  |           | Yes  | APC                     | Yes |               | No      |                      |          | 10             | 30              | 4             |
| 142                    |                 | Yes                                |   | Manual    |                            | 0.2            | 2                | 4         | Yes  | APC-CD3                 | Yes |               | No      |                      |          | 5              | 20              | 4             |
| 143                    | BD              | No                                 | Yes   | Manual    | BD pharmingen stain buffer | 20             | 1                | 2-4       | No   |                         | Yes | PE            | Yes     |                      |          | 20             | 15              | 2-4           |
| 144                    | BD              | Yes                                |   |           |                            |                |                  |           | Yes  | PE                      | Yes |               | Yes     | PBS                  | 1:25     | 25             | 30              | 4             |
| 145                    | BD              | Yes                                |   | Manual    | PBS                        | 3              | 1                | RT        | No   | PE                      | Yes |               | No      |                      |          | 10             | 20              | 4             |
| 147                    | BD              | Yes                                | No  | Manual    | PBS                        | 2.5            | 3                | 22        | Yes  |                         | Yes | PE            | No      |                      |          | 10             | 30              | 4             |
| 149                    | Beckman Coulter | Yes                                |   | Manual    | PBS                        | 3              | 1                | RT        | No   | Phycoerythrin           | Yes |               | No      |                      |          | 10             | 20              | 4             |
| 154                    | Beckman Coulter | Yes                                |   | Manual    | PBS-BSA1%                  | 1              | 2                | 20        | Yes  | Phycoerythrin           | Yes | PE            | No      |                      |          | 15             | 30              | 20            |
| 157                    | BD              | No                                 | No  | Manual    | PBS 0.02% NaN3 2% FBS      | 2              | 1                | 4         | Yes  | PerCp                   | Yes |               | Yes     | PBS                  | 1:10     | 20             | 20              | 4             |
| 159                    | Beckman Coulter | Yes                                |   | Manual    |                            | 1              | 3                | 4         | Yes  |                         | No  | PC7           | No      |                      |          | 10             | 30              | 4             |
| 160                    | BD              | No                                 | Yes   | Manual    | BD cell wash               | 2              | 2                | RT        | No   | Phycoerythrin           | Yes |               | Yes     | BD cell wash         | 1:10     | 50             | 30              | 4             |
| 163                    | Beckman Coulter | No                                 | No  |           |                            |                |                  |           | Yes  | PE                      | Yes |               | No      |                      |          | 4              | 30              | 4             |
| 167                    |                 |                                    |   |           |                            |                |                  |           |  |                         |     |               | No      |                      |          | 5              | 30              | 4             |
| 169                    |                 |                                    |   |           |                            |                |                  |           |  |                         |     |               |         |                      |          |                |                 |               |
| 176                    | BD              | No                                 | Yes   | Manual    | TBS                        | 3              | 1                | 18        | Yes  | Phycoerythrin           | Yes |               | No      |                      |          | 10             | 20              | 20            |
| 186                    | Beckman Coulter | Yes                                |   | Manual    | PBS + FBS 8%               | 2              | 1                | 4         | Yes  | PE                      | Yes |               | No      |                      |          | 10             | 30              | 4             |
| 190                    | Beckman Coulter | Yes                                |   |           |                            |                |                  |           | Yes  | Phycoerythrin-cyanine 5 | Yes |               | No      |                      |          | 5              | 30              | 4             |
| 191                    | BD              | Yes                                |   | Automated | PBS                        | 5              | 2                | RT        | Yes  | PerCP                   | Yes |               | No      |                      |          | 5              | 30              | RT            |
| 193                    | BD              | No                                 | Yes   | Manual    | NH4CL                      | 2              | 1                | 4         | Yes  | Phycoerythrin           | Yes |               | No      |                      |          | 20             | 15              | 4             |
| 194                    | Beckman Coulter | Yes                                |   | Manual    |                            | 2              | 2                | 22        | Yes  | PE                      | Yes |               | No      |                      |          | 5              | 30              | 5             |
| 195                    |                 | Yes                                |   |           |                            |                |                  |           | Yes  | V450                    | Yes |               | No      |                      |          | 5              | 30              | 22            |
| 201                    | BD              | Yes                                | No  |           |                            |                |                  |           | Yes  | PE                      | Yes |               | No      |                      |          | 5              | 30              | 22            |
| 202                    |                 |                                    |   |           |                            |                |                  |           |  |                         |     |               |         |                      |          |                |                 |               |
| 204                    | Beckman Coulter | Yes                                | No  |           |                            |                |                  |           | Yes  | APC                     | Yes |               | No      |                      |          | 10             | 30              | 4             |
| 206                    | BD              | Yes                                |   | Manual    | PBS+FCS                    | 0.2            | 2                |           | Yes  | Phycoerythrin-cyanine 5 | Y   |               | Yes     | PBS                  | 3/100    | 100            | 20              | 4             |
| 209                    |                 |                                    |   |           |                            |                |                  |           |  |                         |     |               |         |                      |          |                |                 |               |
| 218                    | BD              | No                                 | Yes   | Manual    | PBS                        | 5              | 2                | RT        | Yes  | APC                     | Yes |               | No      |                      |          | 5              | 15              | RT            |
| 220                    | eBioscience     | Yes                                |   |           |                            |                |                  |           | Yes  | 450nm                   | Yes |               | Yes     | 2% FCS0, 5% NaN3-PBS | 512      | 50             | 30              | 4             |
| 227                    | Beckman Coulter | Yes                                |   |           |                            |                |                  |           | Yes  | ECD                     | Yes |               | No      |                      |          | 10             | 30              | 4             |
| 235                    | BD              | Yes                                |   | Manual    | PBS                        | 1              | 2                | 4         | No   | Phycoerythrin           | Yes |               | No      |                      |          | 3              | 30              | 4             |
| 238                    | Beckman Coulter | Yes                                |   |           |                            |                |                  |           | Yes  | Phycoerythrin           | Yes |               | No      |                      |          | 5              | 20              | 22            |
| 245                    | Beckman Coulter | Yes                                |   | Manual    |                            | 3              | 2                | 22-24     | Yes  | APC                     | Yes |               | No      |                      |          | 5              | 30              | 22-24         |
| 246                    | BD              | Yes                                |   |           |                            |                |                  |           | Yes  | PerCp                   | Yes |               | No      |                      |          | 5              | 10              | 20            |
| 252                    | BD              | Yes                                |   |           |                            |                |                  |           | Yes  | Phycoerythrin-cyanine 5 | Yes |               | No      |                      |          | 5              | 30              | RT            |
| 260                    | BD              | Yes                                |   | Manual    | Cell wash BD               | 1              | 1                | 22        | Yes  | Phycoerythrin           | Yes |               | No      |                      |          | 5              | 30              | 22            |
| 262                    | BD Biosciences  | Yes                                |   | Manual    | PBS                        | 3              | 1                | 20-25     | Yes  | Phycoerythrin           | Yes |               | No      |                      |          | 5              | 20              | 4             |
| 271                    | BD              | Yes                                | No  | Manual    |                            | 0.5            | 1                | RT        | Yes  | PerCp                   | Yes |               | Yes     | PBS 2% FBS           | 1/4      | 20             | 20              | 4             |
| 284                    | BD              | Yes                                |   | Manual    | Cell wash BD               | 2              | 2                | 22        | Yes  | APC                     | Yes |               | No      |                      |          | 10             | 30              | 22            |
| 293                    |                 |                                    |   |           |                            |                |                  |           |  |                         |     |               |         |                      |          |                |                 |               |
| 297                    |                 | Yes                                |   |           |                            |                |                  |           | Yes  |                         | Yes | PC7           | No      |                      |          | 5              | 30              | 4             |
| 341                    | BD              | Yes                                |   |           |                            |                |                  |           | Yes  | PerCp                   | Yes |               | Yes     | PBS+2%FCS            | 10       | 5              | 30              | 4             |
| 351                    | BD              | Yes                                |   | Manual    | Cold PBS Azide             | 1              | 5                | 4         | Yes  | Phycoerythrin-cyanine 5 | Yes |               | No      |                      |          | 4              | 30              | 4             |
| 358                    | Miltenyi        | Yes                                |   | Manual    | PBS+1%BSA                  | 1              | 2                |           | Yes  | PerCP-Vio700            | Yes |               | Yes     | PBS+1%BSA            | 1:10     | 50             | 30              | RT            |

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| 5. Anti-B cell reagent |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
|------------------------|-----------------|------------------------------------|---|-----------|-----------------------|-----------------|------------------|-----------|-------------------------|------|------|-------|---------|--------------------|----------|-----------------|-----------------|---------------|--|
| Lab Code               | Manufacturer    | IgG added with anti-B cell reagent | Wash step before adding anti-B cell reagent | Wash step | Wash medium           | Vol / tube (ml) | Number of washes | Temp (°C) | Labelled with           | CD19 | CD20 | Other | Diluted | Diluent            | Dilution | Vol / tube (µl) | Inc time (mins) | Inc temp (°C) |  |
| 9                      |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
| 11                     |                 | No                                 | Yes   | Manual    | Cold PBS/Azide        | 4               | 1                | 22        | Phycoerythrin           | Yes  |      |       | No      |                    |          | 3               | 20              | 4             |  |
| 14                     | DAKO            | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin-cyanine 5 | Yes  |      |       | Yes     | PBS/BSA/Azide      | 1/23     | 115             | 20              | 4             |  |
| 15                     |                 | No                                 | Yes   | Automated |                       | 3               | 1                | RT        |                         | Yes  |      |       | Yes     | FACS diluent       | 1:50     | 100             | 15              | 4             |  |
| 19                     |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       | No      |                    |          | 5               | 30              | 4             |  |
| 20                     |                 | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | Yes     | Detaction cocktail | 1:2      | 5               | 15              | 2-8           |  |
| 23                     | BD              | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | No      |                    |          | 5               | 30              | 4             |  |
| 24                     | BD              | Yes                                |   |           |                       |                 |                  |           | Allophycocyanin         | Yes  |      |       | No      |                    |          | 5               | 30              | 4             |  |
| 25                     | BD              | No                                 | Yes   | Automated | 1% PBSA               |                 |                  |           | APC                     | Yes  |      |       | No      |                    |          | 5               | 15              | 4             |  |
| 28                     | BD              | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | No      |                    |          | 5               | 30              | 4             |  |
| 34                     | BD              | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           |      | Yes  |       | No      |                    |          | 5               | 30              | 4             |  |
| 35                     |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
| 38                     | Beckman Coulter | No                                 | Yes   | Manual    | PBS azide             | 4               | 1                | 4         | Phycoerythrin           | Yes  |      |       | Yes     | PBS azide          | 1/20     | 100             | 30              | 4             |  |
| 39                     | DAKO            | No                                 | Yes   | Manual    | 1% FBS in PBS buffer  | 4               | 1                | RT        | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 4               | 20              | 4-8           |  |
| 41                     | BD              | Yes                                | No  |           |                       |                 |                  |           | BV241                   | Yes  |      |       | Yes     | 2% FBS/PBS         | 1:80     | 0.25            | 10              | 22            |  |
| 42                     | DAKO            |                                    |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | No      |                    |          | 5               | 15              | 22            |  |
| 45                     |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
| 48                     | DAKO            | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin-cyanine 5 | Yes  |      |       |         |                    |          | 4               | 30              | 4             |  |
| 51                     | Beckman Coulter | Yes                                |   | Manual    | PBS                   | 3               | 2                | 4         | Phycoerythrin           | Yes  |      |       | Yes     | PBS                | 1:10     | 5               | 30              | 4             |  |
| 54                     | BD              | No                                 | No  | Manual    |                       |                 |                  |           | Phycoerythrin           |      | Yes  |       | No      |                    |          | 10              | 15              | 4             |  |
| 58                     |                 | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | No      |                    |          | 5               | 30              | 4             |  |
| 62                     | Beckman Coulter | Yes                                | No  |           |                       |                 |                  |           | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 5               | 15              | 22            |  |
| 101                    | Beckman Coulter | Yes                                |   |           |                       |                 |                  |           | ECD                     | Yes  |      |       | No      |                    |          | 5               | 30              | 4             |  |
| 112                    | Iotest          | No                                 | No  |           |                       |                 |                  |           | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 7               | 20              | 4             |  |
| 114                    | BD              | Yes                                |   | Manual    | DPB without Ca++      | 150             | 1                | 4         | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 5               | 20              | 4             |  |
| 115                    | BD              | Yes                                |   | Manual    | PBS+2% FBS            | 0.4             | 1                | 4         | PE                      | Yes  |      |       | Yes     | PBS+2% FBS         | 1:4      | 20              | 30              | 4             |  |
| 117                    | BD              | No                                 | No  |           |                       |                 |                  |           | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 10              | 30              | 4             |  |
| 118                    | DAKO            | Yes                                |   | Manual    |                       | 3               | 1                | RT        | Phycoerythrin           | Yes  |      |       | Yes     | PBS                | 1/200    | 200             | 15              | RT            |  |
| 119                    | Beckman Coulter | Yes                                | No  | Manual    | PBS                   | 2               | 3                | RT        | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 10              | 30              | RT            |  |
| 120                    | Beckman Coulter | Yes                                |   | Manual    |                       | 2               | 3                | RT        | Phycoerythrin           | Yes  |      |       | No      |                    |          | 10              | 30              | 4             |  |
| 122                    | Beckman Coulter | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | Yes     | PBS+2% FCS         | 1:16     | 50              | 30              | 4             |  |
| 126                    |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          | 2.5             | 20              | 4-8           |  |
| 130                    | BD              | Yes                                |   | Manual    | Washing buffer        | 2               | 2                | RT        | Phycoerythrin           | Yes  |      |       | Yes     | Washing buffer     | 1/2      | 25              | 20              | Ice           |  |
| 133                    | BD              | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | No      |                    |          | 15              | 40              | 4             |  |
| 136                    |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
| 138                    |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
| 139                    | Coulter         | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | No      |                    |          | 10              | 30              | 4             |  |
| 142                    |                 | Yes                                |   | Manual    |                       | 0.2             | 2                | 4         | Phycoerythrin           | Yes  |      |       | No      |                    |          | 10              | 20              | 4             |  |
| 143                    |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
| 144                    | BD              | Yes                                |   |           |                       |                 |                  |           | APC                     | Yes  |      |       | Yes     | PBS                | 1:25     | 25              | 30              | 4             |  |
| 145                    | Immunotech      | Yes                                |   | Manual    | PBS                   | 3               | 1                | RT        | Phycoerythrin           | Yes  |      |       | No      |                    |          | 10              | 20              | 4             |  |
| 147                    | BD              | Yes                                |   | Manual    |                       | 2.5             | 3                | 22        | APC                     | Yes  |      |       | No      |                    |          | 10              | 30              | 4             |  |
| 149                    | Beckman Coulter | Yes                                |   | Manual    | PBS                   | 3               | 1                | RT        | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 10              | 20              | 4             |  |
| 154                    | Beckman Coulter | Yes                                |   | Manual    | PBS-BSA1%             | 1               | 2                | 20        | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 10              | 30              | 20            |  |
| 157                    | BD              | No                                 | No  | Manual    | PBS 0.02% NaN3 2% FBS | 2               | 1                | 4         | Phycoerythrin           | Yes  |      |       | Yes     | PBS                |          | 20              | 20              | 4             |  |
| 159                    | Beckman Coulter | Yes                                | No  | Manual    |                       | 1               | 3                | 4         | Phycoerythrin           | Yes  |      |       | No      |                    |          | 20              | 30              | 4             |  |
| 160                    | BD              | No                                 | Yes   | Manual    | BD cell wash          | 2               | 2                | RT        | Phycoerythrin           | Yes  |      |       | Yes     | BD cell wash       | 1:10     | 50              | 30              | 4             |  |
| 163                    | Beckman Coulter | No                                 | No  |           |                       |                 |                  |           | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 2               | 30              | 4             |  |
| 167                    |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       | No      |                    |          | 5               | 30              | 4             |  |
| 169                    |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
| 176                    | BD              | No                                 | Yes   | Manual    | TBS                   | 3               | 1                | 18        | PerCp                   |      | Yes  |       |         |                    |          | 10              | 20              | 20            |  |
| 186                    | Beckman Coulter | Manual                             |   | Manual    | PBS + FBS 8%          | 2               | 1                | 4         | Phycoerythrin           | Yes  |      |       | No      |                    |          | 10              | 30              | 4             |  |
| 190                    |                 | Yes                                |   |           |                       |                 |                  |           |                         | Yes  |      |       |         |                    |          | 5               | 30              | 4             |  |
| 191                    | BD              | Yes                                |   | Automated | PBS                   | 5               | 2                | RT        |                         | Yes  |      |       | No      |                    |          | 5               | 30              | RT            |  |
| 193                    | BD              | No                                 | Yes   | Manual    | NH4CL                 | 2               | 1                | 4         | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 20              | 15              | 4             |  |
| 194                    | Beckman Coulter | Yes                                |   | Manual    |                       | 2               | 2                | 22        | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 5               | 30              | 5             |  |
| 195                    |                 | Yes                                |   |           |                       |                 |                  |           | Per-Cp Cy5.5            | Yes  |      |       | No      |                    |          | 20              | 30              | 22            |  |
| 201                    | Beckman Coulter | Yes                                | No  |           |                       |                 |                  |           | Phycoerythrin-cyanine 5 | Yes  |      |       | No      |                    |          | 5               | 30              | 22            |  |
| 202                    |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
| 204                    | Beckman Coulter | Yes                                | No  |           | PBS                   |                 |                  |           | Phycoerythrin           | Yes  |      |       | No      |                    |          | 10              | 30              | 4             |  |
| 206                    | BD              | Yes                                |   | Manual    | PBS+FCS               | 0.2             | 2                | 20        | Phycoerythrin           | Yes  |      |       | Yes     | PBS                | 2/100    | 100             | 20              | 4             |  |
| 209                    |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
| 218                    | Beckman Coulter | No                                 | Yes   | Manual    | PBS                   | 5               | 2                | RT        | Phycoerythrin           | Yes  |      |       | No      |                    |          | 10              | 15              | RT            |  |
| 220                    | eBioscience     | Yes                                |   |           |                       |                 |                  |           | APC                     | Yes  |      |       | Yes     | PBS-CD3-IgG        | 512      | 50              | 30              | 4             |  |
| 227                    | Beckman Coulter | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | No      |                    |          | 10              | 30              | 4             |  |
| 235                    | BD              | Yes                                |   | Manual    | PBS                   | 1               | 2                | 4         | Phycoerythrin           | Yes  |      |       | No      |                    |          | 3               | 30              | 4             |  |
| 238                    | Beckman Coulter | Yes                                |   |           |                       |                 |                  |           | APC                     | Yes  |      |       | No      |                    |          | 5               | 20              | 22            |  |
| 245                    | Beckman Coulter | Yes                                |   | Manual    |                       | 3               | 2                | 22-24     | PC7                     | Yes  |      |       | No      |                    |          | 5               | 30              | 22-24         |  |
| 246                    |                 | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | No      |                    |          | 5               | 10              | 20            |  |
| 252                    |                 | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | No      |                    |          | 5               | 30              | RT            |  |
| 260                    | BD              | Yes                                |   | Manual    | Cell wash BD          | 1               | 1                | 22        | APC                     | Yes  |      |       |         |                    |          | 5               | 30              | 22            |  |
| 262                    | Beckman Coulter | Yes                                |   | Manual    | PBS                   | 3               | 1                | 4         | APC                     | Yes  |      |       | No      |                    |          | 5               | 20              | 4             |  |
| 271                    | BD              | Yes                                | No  | Manual    | PBS 2% FBS            | 0.5             | 1                | 4         | Phycoerythrin           | Yes  |      |       | Yes     |                    | 1/4      | 20              | 20              | 4             |  |
| 284                    | BD              | Yes                                |   | Manual    |                       | 2               | 2                | 22        | BV421                   | Yes  |      |       | No      |                    |          | 2.5             | 30              | 4             |  |
| 293                    |                 |                                    |   |           |                       |                 |                  |           |                         |      |      |       |         |                    |          |                 |                 |               |  |
| 297                    |                 | Yes                                |   |           |                       |                 |                  |           | APC                     | Yes  |      |       | No      |                    |          | 5               | 30              | 4             |  |
| 341                    | BD              | Yes                                |   |           |                       |                 |                  |           | Phycoerythrin           | Yes  |      |       | Yes     | PBS+2%FCS          | 10       | 5               | 30              | 4             |  |
| 351                    | BD              | Yes                                |   | Manual    | Cold PBS Azide        | 1               | 5                | 4         | R-Phycoerythrin         | Yes  |      |       | No      |                    |          | 3               | 30              | 4             |  |
| 358                    | Miltenyi        | Yes                                |   | Manual    | PBS+1% BSA            | 1               | 2                | RT        | Phycoerythrin           | Yes  |      |       | Yes     | PBS+1%BSA          | 1:10     | 50              | 30              | RT            |  |

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| 6. Final wash |           |                            |                 |                  |           |                            |                 |
|---------------|-----------|----------------------------|-----------------|------------------|-----------|----------------------------|-----------------|
| Lab Code      | Wash step | Wash medium                | Vol / tube (ml) | Number of washes | Temp (°C) | Resuspension of cells in   | Vol / tube (ul) |
| 9             | Manual    | PBS                        | 100             | 3                | RT        | Phosphate buffered saline  | 400             |
| 11            | Manual    | PBS/Azide                  | 4               | 1                | 22        | Phosphate buffered saline  | 400             |
| 14            | Manual    |                            | 2               | 2                | RT        | PBS/BSA/Azide              | 300             |
| 15            | Automated | PBS                        | 3               | 1                | RT        | FACS diluent               | 200             |
| 19            |           |                            |                 |                  |           |                            |                 |
| 20            | Automated | PBS                        | 2               | 1                | 2-8       | Phosphate buffered saline  | 300             |
| 23            | Automated | PBS/FBS                    | 1               | 3                | 4         | Fixative                   | 300             |
| 24            | Automated | BD cell wash               | BD LWA          | 4ml x1           | RT        | BD cell wash               | 300             |
| 25            | Automated |                            | 1               | 3                | RT        | 1% PBSA                    | 500             |
| 28            | Manual    | PBA                        | 2.5             | 2                | 4         | Sheath fluid               | 250             |
| 34            | Automated |                            | 5               | 1                | 4         | TBS                        | 280             |
| 35            | Manual    |                            | 2               | 3                | 4         | Phosphate buffered saline  | 150             |
| 38            | Manual    | PBS azide                  | 4               | 1                | 4         | PBS azide                  | 500             |
| 39            | Manual    | 1% FBS in PBS buffer       | 4               | 1                | RT        | 1% FBS in PBS buffer       | 250             |
| 41            | Automated |                            | 4               | 1                | 22        | 2% FBS/PBS                 | 300             |
| 42            | Manual    | 1% FCS/PBS                 | 2               | 2                | 22        | 1% FCS/PBS                 | 280             |
| 45            | Manual    |                            | 1               | 1                | RT        | PBA                        | 220             |
| 48            | Manual    |                            | 4               | 1                | 21        | Local flow diluent         | 500             |
| 51            |           |                            |                 |                  |           |                            |                 |
| 54            | Manual    | PBS 0.1% azide             | 4               | 1                | 4         | Sheath fluid               | 100             |
| 58            | Manual    |                            | 4               | 1                | 4         | Phosphate buffered saline  |                 |
| 62            | Manual    | PBSCS                      | 4               | 1                | 22        | PBSCS                      | 0.5             |
| 101           | Manual    | PBS                        | 2-3             | 2                | Ambient   | Phosphate buffered saline  |                 |
| 112           | Automated | PBS                        | 0.8             | 2                | Ambient   | Fixative                   | 450             |
| 114           | Manual    | DPBS without Ca++          | 150             | 1                | 4         | Fixative                   | 150             |
| 115           | Manual    | PBS+2% FBS                 | 0.4             | 1                | 4         | Fixative                   | 300             |
| 117           | Manual    | PBS                        | 2               | 1                | 24        | Phosphate buffered saline  | 650             |
| 118           | Manual    | PBS                        | 3               | 1                | RT        | Phosphate buffered saline  | 200             |
| 119           | Manual    | PBS                        | 2               | 3                | RT        | Phosphate buffered saline  | 500             |
| 120           | Manual    |                            | 2               | 1                | RT        | PBS1x - BSA1%              | 400             |
| 122           | Manual    | PBS+2% FCS                 | 1               | 2                | 22        | Fixative                   | 350             |
| 126           |           |                            |                 |                  |           |                            |                 |
| 130           |           |                            |                 |                  |           | Fixative                   | 100             |
| 133           | Manual    |                            | 2               | 2                | RT        | Phosphate buffered saline  |                 |
| 136           | Manual    | PBS/azide/FCS 3%           | 200             | 1                | RT        | Phosphate buffered saline  | 400             |
| 138           | Manual    | PBS                        | 4               | 1                | 4         | Phosphate buffered saline  | 500             |
| 139           | Manual    | PBS                        | 0               | 3                | 22        | Phosphate buffered saline  | 450             |
| 142           | Manual    |                            |                 |                  |           | Phosphate buffered saline  | 400             |
| 143           | Manual    | BD phatmingen stain buffer | 1.5             | 1                | 2-4       | Stain buffer               | 600             |
| 144           | Manual    |                            | 4               | 2                | 4         | Fixative                   | 100             |
| 145           | Manual    | PBS                        | 3               | 1                | RT        | Fixative                   | 500             |
| 147           | Manual    |                            | 2.5             | 3                | 22        | Phosphate buffered saline  | 250             |
| 149           | Manual    | PBS                        | 3               | 1                | RT        | Fixative                   | 500             |
| 154           | Manual    |                            | 1               | 2                | 20        | Fixative                   |                 |
| 157           | Manual    | PBS 0.02% NaN3 2% FBS      | 2               | 1                | 4         | Phosphate buffered saline  | 100             |
| 159           | Manual    |                            | 1               | 3                | 4         | Phosphate buffered saline  | 400             |
| 160           | Manual    | BD cell wash               | 2               | 2                | RT        | BD cell wash               | 350             |
| 163           | Manual    | PBS 1X/BSA 0.5%            | 2               | 2                | 20        | Phosphate buffered saline  | 100/150         |
| 167           |           |                            |                 |                  |           |                            |                 |
| 169           |           |                            |                 |                  |           |                            |                 |
| 176           | Manual    | TBS                        | 3               | 1                | 18        | Sheath fluid               | 500             |
| 186           | Manual    | PBS + FBS 8%               | 2               | 1                | 4         | Phosphate buffered saline  | 300             |
| 190           | Manual    |                            | 0.2             | 4                | 20        | Phosphate buffered saline  | 300             |
| 191           | Automated | PBS                        | 5               | 2                | RT        | Phosphate buffered saline  | 100             |
| 193           | Manual    | PBS-BSA1%                  | 2               | 1                | 4         | Phosphate buffered saline  | 250             |
| 194           | Manual    |                            | 2               | 2                | 22        | Fixative                   | 300             |
| 195           | Manual    | PBS                        | 4               | 1                | 22        | Phosphate buffered saline  | 250             |
| 201           | Manual    | PBS                        |                 |                  |           |                            |                 |
| 202           |           |                            |                 |                  |           |                            |                 |
| 204           | Manual    | PBS                        | 0.2             | 4                | 22        | Phosphate buffered saline  | 450             |
| 206           | Manual    | PBS+FCS                    | 0.2             | 2                | 20        | Phosphate buffered saline  | 400             |
| 209           |           |                            |                 |                  |           |                            |                 |
| 218           | Manual    |                            | 5               | 1                | RT        | Phosphate buffered saline  | 300             |
| 220           | Manual    | 2% FCS0, 5% NaN3-PBS       | 200             | 2                | 4         | 2% FCS0, 5% NaN3-PBS+7-AAD | 120             |
| 227           | Manual    |                            | 0.2             | 3                | 22        | Phosphate buffered saline  | 450             |
| 235           | Manual    | PBS                        | 1               | 2                | 4         | Phosphate buffered saline  | 500             |
| 238           | Manual    | PBS                        | 4               | 1                | 22        | Phosphate buffered saline  | 200             |
| 245           | Manual    | PBS + SVF                  | 3               | 3                | 22-24     | Phosphate buffered saline  | 300             |
| 246           | Manual    | FACS Flow                  | 10              | 3                | 20        | Sheath fluid               | 300             |
| 252           | Manual    | PBS x1                     | 1               | 2                | RT        | Phosphate buffered saline  | 500             |
| 260           | Manual    | Cell wash BD               | 1               | 1                | 22        | Fixative                   | 500             |
| 262           | Manual    |                            | 3               | 1                | 20-25     | Phosphate buffered saline  | 250             |
| 271           | Manual    | PBS 2% FBS                 | 0.5             | 1                | 20-25     | PBS 2% FBS                 | 300             |
| 284           | Manual    |                            | 2               | 2                | 22        | Cell wash BD               | 500             |
| 293           |           |                            |                 |                  |           |                            |                 |
| 297           | Manual    | PBS                        |                 | 3                | 22        | Phosphate buffered saline  | 500             |
| 341           | Manual    | PBS                        | 1               | 2                | 22-25     | Phosphate buffered saline  | 300             |
| 351           | Manual    |                            | 1               | 5                | 4         | Fixative                   |                 |
| 358           | Manual    | PBS+1%BSA                  | 1               | 2                | RT        | Fixative                   | 100             |

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| 7. Data Acquisition |                                |            |                        |   |  |              |        |        |                 |         |           |  |                             |
|---------------------|--------------------------------|------------|------------------------|---|--|--------------|--------|--------|-----------------|---------|-----------|--|-----------------------------|
| Lab Code            | Flow cytometer                 | Live gated | Calibration beads used | Used for  | Type of beads                                | Total events |        | Events |                 | B-cells |           | Parameter used to assess peak performance  | Scale used                  |
|                     |                                |            |                        |   |  | Min          | Max    | Min    | Max             | Min     | Max       |  |                             |
| 9                   | Beckman Coulter                | Yes        | Yes                    | Alignment check, Fluorescence check   | Flowcheck, Flow set                          | 3000         | 5000   | 3000   | 5000            |         |           | Median   | Log channel                 |
| 11                  | BD FACS Canto II               | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | CST  |              |        |        |                 | 3000    | 1000      | Median   | Log channel                 |
| 14                  | FACS Canto II                  | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | 7 colour set-up                              |              |        | 3000   |                 |         |           | Median   | Log channel                 |
| 15                  | BD FACS Canto II               | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | CST  |              |        | 300    | 7500            | 300     | 7500      | Median   | Log channel                 |
| 19                  |                                |            |                        |   |  |              |        |        |                 |         |           |  |                             |
| 20                  | Beckman Coulter FC500          | No         | Yes                    | Alignment check, Laser check, Fluorescence check                            | Flowcheck, Flow set, Immunobrite             |              | 200000 | 250    | 6000            | 100     | 5000      | Median   | Log channel                 |
| 23                  | BD FACS Calibur                | No         | Yes                    | Alignment check, Laser check, Fluorescence check                            | Rainbow 8 peak, Calibrite                    |              |        |        | 10000           |         | 10000     | Median   | Log channel                 |
| 24                  | BD FACS Canto                  | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | CST, 7 colour set-up                         |              |        | 500    |                 | 200     |           | Median   | Linear channel              |
| 25                  | BD FACS Canto II               | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | CST, 7 colour set-up                         | 10000        |        |        |                 | 1000    |           | Median   | Log channel                 |
| 28                  | BD FACS Calibur                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | BD Calibrite-3 colour kit                    |              |        | 1000   |                 | 1000    |           | Median   | Log channel                 |
| 34                  | Beckman Coulter                | No         | Yes                    | Alignment check, Fluorescence check   | Flowcheck, Immunobrite                       |              | 10000  | 100    |                 |         |           | Median   | Log channel                 |
| 35                  | BD FACS Canto                  | Yes        | Yes                    | Laser check, Fluorescence check   | CST, 7 colour set-up                         | 500          |        | 300    |                 |         |           | Median   | Log channel                 |
| 38                  | Beckman Coulter 500 MPL        | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Flowcheck, Flow set                          |              |        |        |                 | 500     |           | The median fluorescence value is converted in to a linear channel value. The mean linear channel value of the negative controls are subtracted from the mean linear channel of each sample to give a linear channel shift. | Log channel                 |
| 39                  | Beckman Coulter - FC500/Navios | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Flowcheck, Flow set, Immunobrite             |              |        | 500    | 5000            | 500     | 5000      | Median   | Log channel                 |
| 41                  | BD                             | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | CST  |              |        | 50     | 5000            | 50      | 500       | Median   | Log channel                 |
| 42                  | BD FACS Canto I                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | 7 colour set-up                              |              |        | 1000   | >1000           | 1000    | >1000     | Median   | Log channel                 |
| 45                  | BD FACS Canto II               | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Flow check, Flow set                         |              |        | 3000   | 4000            |         |           | Median   | Log channel                 |
| 48                  | Beckman Coulter                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation |  |              |        | 5000   |                 | 1500    |           | Median   | Log channel                 |
| 51                  |                                |            |                        |   |  |              |        |        |                 |         |           |  |                             |
| 54                  | BD FACS Canto II               | No         | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | CST, DAKO fluorospheres                      |              |        | 5000   | 5000            | 20000   | 20000     | Median   | Log channel                 |
| 58                  | Beckman Coulter FC500          | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Flowcheck, Flow set, Immunobrite             |              |        | 5000   |                 |         | 1000      | Median   | Log channel                 |
| 62                  | Beckman Coulter                | Yes        | Yes                    | Alignment check, Laser check  | Fluorospheres                                | 10000        |        |        |                 |         |           | Median   | Log channel                 |
| 101                 | Beckman Coulter                | Yes        | No                     |   |  |              |        |        |                 |         |           | Mean   | Log channel                 |
| 112                 | Beckman Coulter FC500          | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Flowcheck, Flow set, Quantum-MESF            |              |        |        |                 | 1000    |           | Median   | Log channel                 |
| 114                 | FACS Calibur                   | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Calibrite 3                                  | 5000         |        |        |                 | 1000    |           | Median   | Linear channel              |
| 115                 | FACS Calibur                   | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Calibrite + Rainbow Cal. Part.               |              |        |        | 20000 or 120sec |         |           | Median   | Log channel                 |
| 117                 | BD                             | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Calibrite 3                                  |              |        |        |                 | 350     | 1500      | Median   | Log channel                 |
| 118                 | Calibur                        | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            |  |              |        | 5000   |                 | 1000    |           | Median   | Linear channel              |
| 119                 | Coulter Epics-XL               | No         | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Flowcheck, Flow set, Quick-comb, Immunobrite |              |        | 5000   | 15000-20000     | 400     | 1000      | Mean   | Log channel                 |
| 120                 | FACS Canto                     | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Calibrite 3                                  |              |        |        |                 | 3000    |           | Median   | Log channel                 |
| 122                 | BD FACS Canto II               | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | 7 colour set-up                              |              |        | 6000   | 10000           | 800     | 1000      | Geomean  | Linear channel              |
| 126                 |                                |            |                        |   |  |              |        |        |                 |         |           |  | Linear channel              |
| 130                 | FACS Scan                      | Yes        | Yes                    | Fluorescence check, Fluorescence quantitation                               | Sphero rainbow calibration                   | 2000         |        |        |                 |         |           | Mean   | Linear channel              |
| 133                 |                                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Calibrite 3 beads                            | 20000        | 50000  |        |                 |         |           | Median   | Log channel                 |
| 136                 | FACS Calibur                   | No         | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Calibrite                                    |              |        | 5000   | 10000           |         |           | Geomean  | Linear channel              |
| 138                 |                                | No         |                        | Alignment check, Laser check, Fluorescence check                            | CST, Rainbow                                 | 30000        |        |        |                 |         |           | Kolmogorov   | Log channel                 |
| 139                 | Beckman Coulter                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Flow check pro, Flow set pro                 | 10000        | 15000  | 5000   | 10000           | 1000    | 10000     | Mean   | Log channel                 |
| 142                 | BD FACS Canto II               | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | CST  |              |        | 50000  | 100000          | 50000   | 100000    | Median   | Log channel                 |
| 143                 | FACS Calibur                   | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Calibrite 3 and APC                          | 5000         | 10000  | 3000   | 5000            |         |           | Median   | Linear channel              |
| 144                 | BD                             | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            |  |              |        | 1000   |                 | 500     |           | Median, Mean   | Log channel                 |
| 145                 | Beckman Coulter                | Yes        | Yes                    | Alignment check, Fluorescence check, Fluorescence quantitation              | Flowcheck, Flow set, Immunobrite             | 10000        | 15000  | 5000   | 10000           | 500     | 1000      | Median   | Log channel                 |
| 147                 | FC500                          | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Fluorocheck, Fluoroset                       |              |        | 100000 |                 |         | 2000      | Median   | Linear channel              |
| 149                 | Beckman Coulter                | Yes        | Yes                    | Alignment check, Fluorescence check, Fluorescence quantitation              | Flowcheck, Flow set, Immunobrite             | 10000        | 15000  | 5000   | 10000           | 500     | 1000      | Median   | Log channel                 |
| 154                 | FACS Canto II                  | No         | Yes                    | Alignment check, Laser check, Fluorescence check                            | CST  |              |        | ≥2000  |                 | >2000   |           | Median   | Log channel                 |
| 157                 | FACS Calibur                   | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Calibrite                                    |              | 10000  |        |                 | 1000    |           | Median   | Linear channel              |
| 159                 | BD                             | No         | No                     | Alignment check, Laser check, Fluorescence check                            | Calibrite 3                                  | 12000        | 10000  | 10000  |                 | 1000    |           | Mean   | Linear channel              |
| 160                 | BD FACS Calibur                | No         | Yes                    | Alignment check, Laser check, Fluorescence check                            | Calibrite                                    | 3000         | 30000  | 1000   | 5000            | 300     | 1000      | Mean   | Log channel                 |
| 163                 | BD FACS Canto II               | No         | Yes                    | Alignment check, Laser check, Fluorescence check                            | CST  |              | 20000  |        |                 |         |           | Geometric mean   | Linear channel              |
| 167                 |                                |            |                        |   |  |              |        |        |                 |         |           |  | Log channel                 |
| 169                 |                                |            |                        |   |  |              |        |        |                 |         |           |  |                             |
| 176                 | CD Canto II                    | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | CST, Rainbow                                 |              |        |        |                 | 200     |           | Median   | Log channel                 |
| 186                 |                                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Flowcheck, Flow set                          |              |        |        |                 | 3500    | 1500      | Mean   | Log channel                 |
| 190                 | Beckman Coulter Navios         | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation |  | 12000        |        | 15000  |                 |         | 2000-5000 | Mean   | Linear channel, Log channel |
| 191                 | BD FACS Calibur                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Calibrite                                    | 2000         |        |        |                 |         |           | Mean   | Log channel                 |
| 193                 | FACS Calibur                   | No         | Yes                    | Alignment check, Laser check, Fluorescence check                            | Calibrite                                    | 10000        |        | 250    |                 |         |           | Mean   | Log channel                 |
| 194                 |                                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Flowcheck, Flow set                          | 3000         |        |        |                 |         |           | Median   | Log channel                 |
| 195                 | BD                             | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Rainbow                                      | 20000        | 50000  |        |                 |         |           | Median   | Log channel                 |
| 201                 | FC500                          | Yes        | Yes                    | Laser check   | Flow check                                   | 10000        |        | 1000   |                 | 100     |           | Mean   | Log channel                 |
| 202                 |                                |            |                        |   |  |              |        |        |                 |         |           |  |                             |
| 204                 | Beckman Coulter FC500          | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Flowcheck, Flow set                          |              | 10000  |        | 3000            |         |           | Median/Mean  | Linear channel, Log channel |
| 206                 | MACSQuant/Miltenyi             | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Miltenyi Biotec                              |              | <10000 |        |                 | >1000   |           | Median   | Log channel                 |
| 209                 |                                |            |                        |   |  |              |        |        |                 |         |           |  |                             |
| 218                 | LSR II                         | No         | Yes                    | Alignment check, Laser check, Fluorescence check                            | CST  |              | 300000 |        |                 |         |           | Mean   | Log channel                 |
| 220                 | BD FACS Canto II               | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | CST  |              |        |        |                 | 2000    |           | Median   | Linear scale                |
| 227                 | Beckman Coulter                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | Flow set                                     | 1000         | 20000  | 500    |                 | 500     |           | Mean   | Log channel                 |
| 235                 |                                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | BD Calibrite                                 | 30000        | 100000 | 10000  |                 | 1000    |           | Median   | Log channel                 |
| 238                 |                                | Yes        | Yes                    | Alignment check, Fluorescence check   | Immuno-brite                                 | 40000        | 100000 |        |                 |         |           | Median   | Log channel                 |
| 245                 | Beckman Coulter Navios         | No         | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Flowcheck, Flow set                          |              |        | 2500   | 5000            | 500     | 2000      | Median   | Linear channel              |
| 246                 | BD FACS Canto                  | Yes        | Yes                    |   |  |              |        | 100    | 1000            | 100     | 1000      | Median   | Log channel                 |
| 252                 |                                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | BD Calibrite bead                            |              |        |        |                 |         |           | Median   | Log channel                 |
| 260                 |                                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Unlabeled, FITC, PE, APC                     |              |        | 5000   | 10000           | 500     | 2000      | Mean   | Log channel                 |
| 262                 | 3000 events on B-cells         | No         | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | CST, Immunobrite                             |              |        |        |                 | 500     | 3000      | Median   | Log channel                 |
| 271                 | FACS Canto II                  | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | CST  |              |        |        |                 | 250     | 1000      | Median   | Log channel                 |
| 284                 | BD FACS Canto II               | No         | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | CST  |              |        |        |                 | 1000    |           | Median   | Log channel                 |
| 293                 |                                |            |                        |   |  |              |        |        |                 |         |           |  |                             |
| 297                 |                                | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | CST  |              |        | 1000   | 10000           | 300     | 10000     | Median   | Log channel                 |
| 341                 | FACS Canto II                  | Yes        | Yes                    | Alignment check, Laser check, Fluorescence check                            | CST, 7 colour set-up                         |              |        | 2000   | 10000           | 500     | 1000      | Geomean  | Linear channel              |
| 351                 | BD FACS Canto II               | Yes        | Yes                    | Fluorescence check, Fluorescence quantitation                               | Fluorospheres                                | 5000         | 10000  | 5000   | 10000           | 5000    | 10000     | Median   | Log channel                 |
| 358                 | Cube 6                         | Yes        | Yes                    |   |  |              |        |        |                 |         |           | Median   | Log channel                 |

**SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2017**

| 8. Data Analysis |   |  |   | 9. Use of XM by flow cytometry                                    |   |
|------------------|---|--|---|---|---|
| Lab Code         | Parameter used to assess difference in fluorescence of anti-human IgG reagent | Minimum value for valid positive control | Discriminator value taken to distinguish negative from positive | Discriminator value to distinguish clinically significant binding |   |
|                  | T-cell  | B-cell                                   |   |   |   |
| 9                | Median difference   | pos/neg trimmed mean value >3            | >1.5 x trimmed mean   |   | Pre-transplant crossmatching  |
| 11               | Median difference   | >Mean AB +2SD                            | >1.6 RMF  |   | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 14               | Median difference   | 4x neg                                   | 4x neg  | 1.5x mean neg   | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 15               | Median difference   | 20x neg                                  | 10x neg   | NEQAS RMF >1.5  | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching   |
| 19               |   |  |   |   |   |
| 20               | Median difference   | 60                                       | 60  | 40  | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching   |
| 23               | Median difference   | +3SD for mean of AB                      | +2SD  | T: +3SD, B: +2SD  | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring, Renal transplant   |
| 24               | Median difference   | 1.5x neg                                 | 2x neg  | T: 1.5x neg, B: 2x neg  | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 25               | Median difference   |  |   | RMF >1.3  | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 28               | Ratio of median fluorescence of test sample to negative control               | ratio ≥3.1                               | ratio ≥3.6  | T-cell ≥1.6<br>B-cell ≥2.0  | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring (if antibody screening is not possible) |
| 34               | Mean difference   | 2x neg                                   | 2x neg  | +2SD  | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 35               | Median difference   |  |   | Mean +3SD   | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 38               | Linear channel shift  | >46 channels                             | >63 channels  | T-cells: >46 channels<br>B-cells: >63 channels                    | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring   |
| 39               | Peak shift, Median difference   | >1.5 RMF                                 | >2.0 RMF  | T: 2SD, B:3SD   | Pre-transplant crossmatching  |
| 41               | Mean difference   | 10x neg                                  | 10x neg   | T: RMF <1.3, B: RMF<1.5   | Pre-transplant crossmatching  |
| 42               | Mean difference   | MCS -30, RMF >1.3                        | MCS -45, RMF >1.5   | 40 channel shift  | Pre-transplant crossmatching  |
| 45               | Mean difference   | 40 channel shift                         | 4x neg  | 1.3x neg  | Pre-transplant crossmatching  |
| 48               | Relative median fluorescence  |  |   |   | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring   |
| 51               |   |  |   |   |   |
| 54               | Test of neg control MESF ratio  | a test/neg MESF ratio of >10             | ≥1.2 MESF ratio   | ≥1.5 MESF ratio   | Pre-transplant crossmatching  |
| 58               | Median difference   | 1.3x mean                                | 1.3x mean   | 1.3x mean   | Pre-transplant crossmatching  |
| 62               | Median difference   | RMF >1.5                                 | RMF >3.0  |   | Pre-transplant crossmatching  |
| 101              | Mean difference   |  |   |   | Pre-transplant crossmatching  |
| 112              | Ratio   | >1.5 ratio                               | >2.0 ratio  | T-cell: >1.5 ratio<br>B-cell: >2.0 ratio                          | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 114              | Median difference   | 40 channel                               | 70 channel  | 50  | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 115              | Median difference   | >40                                      | >60   | >40 channel shift   | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 117              | Median difference   |  | RMF >4  | T: RMF >1.3, B: RMF >1.5  | Clinical/Diagnostic, Post-transplant monitoring   |
| 118              | Median difference   |  |   | T: Med neg +66 ch, B: Med neg +25.5 ch                            | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring   |
| 119              | Mean difference   | ratio >1.285                             | ratio >1.7  | T-cells: ratio >1.285<br>B-cells: ratio >1.7                      | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 120              | Median difference   | ratio >13                                | ratio >12   | T-cells: ratio >1.6<br>B-cells: ratio >2                          | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 122              | Fluorescence index  | FI >1.5                                  | FI >2.0   | 3SD   | Clinical/Diagnostic, Research/Development, Antibody screening, Pre-transplant crossmatching, Post-transplant monitoring                     |
| 126              |   |  |   |   |   |
| 130              | Mean difference   |  |   | T: >40 channels, B: >80 channels                                  | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 133              | Peak shift, Median difference, Mode difference                                | >x4 of NC                                | >x4 of NC   |   | Pre-transplant crossmatching  |
| 136              |   |  |   | >+50% of neg, >+100% of neg<br>NR >200                            | Pre-transplant crossmatching, Post-transplant monitoring  |
| 138              |   |  |   |   |   |
| 139              | Mean difference   |  |   | 1.5 or 2 (T or B cells) of neg mean log channel                   | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching   |
| 142              | Peak shift  |  |   | Mean +3SD   | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 143              | Mean difference, Median difference  | 40                                       | >500  | 40  | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring   |
| 144              | Mean difference, Median difference  | 1.76                                     | 2.5   | Ratio   | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 145              | Ratio median log channel test/Median log channel neg                          | Mean ratio +2SD                          | Mean ratio +2SD   | Mean ratio +2SD   | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 147              | % shift   | Tpos-Tneg=200                            | t: >40 channel, B: >60 channel                                  | None  | Pre-transplant crossmatching, Post-transplant monitoring  |
| 149              | Ratio median log channel /median log channel neg control                      | Mean ratio +2SD                          | Mean ratio +2SD   | Mean ratio +2SD   | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 154              | Median difference   |  |   | 150% of neg control and shape of the curve                        | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 157              | Median difference   | 150 channel shift                        | 250 channel shift   | 2SD from neg control  | >10 channel shift over cutoff   |
| 159              | Median difference   | 100 channel shift                        | 100 channel shift   | T: 40 channel shift, B: 100 channel shift                         | Pre-transplant crossmatching, Post-transplant monitoring  |
| 160              | Median difference   | 100 mean log channel                     | 170 mean log channel  | >Mean of neg sera +2SD  | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 163              | Ratio of geometric mean   | 2.3                                      | 1.7   | T: 2.3, B: 1.7  | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 167              |   |  |   |   |   |
| 169              |   |  |   |   |   |
| 176              | MFI of test serum/MFI of neg control serum                                    | 0.5                                      | 0.22  | T: 0.50, B:0.22   | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring   |
| 186              | Mean difference   |  |   | NA  | Pre-transplant crossmatching  |
| 190              | Mean difference   |  |   | T <1.2Neg >1.5Pos<br>B <1.2Neg >2.5Pos<br>T: >30, B: >100         | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 191              | Mean difference   | 100                                      | 300   |   | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 193              | Geometric mean  |  |   | Neg geometric mean x2.5   | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 194              | Median difference   | ratio >0.7                               | ratio >1  |   | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 195              | % shift   |  |   | ratio sample/neg control, T:1.3, B: 1.6                           | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 201              | Ratio x-mean sample/NC  | 2  | 2.5   | NA  | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 202              |   |  |   |   |   |
| 204              |   | 1.5x                                     | 2x  | T-cells: 1.5x the local neg<br>B-cells: 2x the local neg          | Depend on autocrossmatch done in parallel   |
| 206              | 4 Ratio MFI (sample/neg control)  | Ratio >2                                 | Ratio >3  | RatioT>1.07, Ratio B>1.17   | RatioT>1.2, RatioB>1.3  |
| 209              |   |  |   |   |   |
| 218              | Mean difference   | CTL x1.2                                 | CTL x1.7  | T-cell: CTL x1.2<br>B-cell: CTL x1.7                              | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 220              | 8000 - mean of neg control  | 8000 - mean of neg control               | 8000 - mean of neg control                                      | 8000 - mean of neg control  | Pre-transplant crossmatching  |
| 227              | Mean difference   | 1.5x neg                                 | 2x neg  | T: 1.5x neg, B: 2x neg  | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 235              | Median difference   |  |   | >2 of neg median log channel                                      | Research/Development, Pre-transplant crossmatching, Post-transplant monitoring  |
| 238              | MESF Serum/MESF neg control   |  |   | T: ratio >1.8, B: >2.2  | Pre-transplant crossmatching, Post-transplant monitoring  |
| 245              | Median difference   | 7  | 15  |   | Clinical/Diagnostic, Pre-transplant crossmatching   |
| 246              | Median log, FI ratio  | 1.75                                     | 2.5   |   | Clinical/Diagnostic   |
| 252              | Median ratio  | Median ratio ±2SD                        | Median ratio ±3SD   |   | Clinical/Diagnostic, Antibody Screening, Pre-transplant crossmatching   |
| 260              | Mean difference   | 2x neg control MFI                       | 2x neg control MFI  | 2x neg control MFI  | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring   |
| 262              | % shift   | >12%                                     | >12%  |   | Pre-transplant crossmatching, Post-transplant monitoring  |
| 271              | Median channel shift  | 64                                       | 90  | Median RFU sample/Median RFU neg >2                               | Pre-transplant crossmatching, Post-transplant monitoring  |
| 284              | Median difference   | ratio >1.2                               | ratio >1.2  | ratio >1.2  | Clinical/Diagnostic, Pre-transplant crossmatching   |

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| 10. Reporting results |                                   |  |
|-----------------------|-----------------------------------|--|
| Lab Code              | is Positive or Negative adequate? | Reason   |
| 9                     | Yes                               |  |
| 11                    | No                                | "Ok to proceed" - If positivem discussion with clinician considering patient history, sensitisation and clinical need.   |
| 14                    | Yes                               |  |
| 15                    | Yes                               |  |
| 19                    |                                   |  |
| 20                    | Yes                               |  |
| 23                    | Yes                               |  |
| 24                    | No                                | Immunological risk. Would need to know HLA mismatch and antibody levels.   |
| 25                    | Yes                               |  |
| 28                    | No                                | An equivocal range is a good idea.   |
| 34                    | Yes                               |  |
| 35                    | Yes                               |  |
| 38                    | Yes                               |  |
| 39                    | Yes                               |  |
| 41                    | Yes                               |  |
| 42                    | No                                | The positive result should be explained in relation to the prescence of antibodies.  |
| 45                    | Yes                               |  |
| 48                    | No                                | Clinically significant?  |
| 51                    |                                   |  |
| 54                    | Yes                               | We also use the term weakly positive if the test/neg MESF value is between 1.2 and 1.5.  |
| 58                    | Yes                               | As no other clinical data is available to review with the flow crossmatch results then pos/neg along with equivocal is adequate.   |
| 62                    | Yes                               |  |
| 101                   | Yes                               |  |
| 112                   | Yes                               |  |
| 114                   | Yes                               |  |
| 115                   | Yes                               |  |
| 117                   | Yes                               |  |
| 118                   | Yes                               |  |
| 119                   | Yes                               |  |
| 120                   | Yes                               |  |
| 122                   | Yes                               |  |
| 126                   |                                   |  |
| 130                   | Yes                               |  |
| 133                   | Yes                               |  |
| 136                   | Yes                               |  |
| 138                   |                                   |  |
| 139                   | Yes                               |  |
| 142                   | No                                | Low positive would also be useful  |
| 143                   | Yes                               |  |
| 144                   | Yes                               |  |
| 145                   | Yes                               |  |
| 147                   | No                                | The results seen to be equivocal because they are close to the positive threshold. In general our clinical rules consist of investigating the whole historical medical patient file. |
| 149                   | Yes                               |  |
| 154                   | Yes                               |  |
| 157                   | Yes                               |  |
| 159                   | Yes                               |  |
| 160                   | No                                | A high proportion of samples sent are marginally positive. In practice such results are evaluated after consideration of other laboratory and clinical sata.                         |
| 163                   | Yes                               |  |
| 167                   |                                   |  |
| 169                   |                                   |  |
| 176                   | No                                | It would be useful to include a "Weakly reactive/Borderline" result option. In clinical terms, there is a big difference between "definitely no reacton" and "probably something"    |
| 186                   | Yes                               |  |
| 190                   | Yes                               |  |
| 191                   | Yes                               |  |
| 193                   | Yes                               |  |
| 194                   | Yes                               |  |
| 195                   | Yes                               | The option "Equivocal result" is interesting.  |
| 201                   | Yes                               |  |
| 202                   |                                   |  |
| 204                   | Yes                               |  |
| 206                   |                                   |  |
| 209                   |                                   |  |
| 218                   | No                                | Sometimes results are indeterminare or borderline. Insufficient samples is sent so they cannot be tested in duplicates as the patient samples are.                                   |
| 220                   | Yes                               |  |
| 227                   | Yes                               |  |
| 235                   | Yes                               |  |
| 238                   | Yes                               |  |
| 245                   | No                                |  |
| 246                   | Yes                               |  |
| 252                   | Yes                               |  |
| 260                   | Yes                               |  |
| 262                   | Yes                               |  |
| 271                   | Yes                               |  |
| 284                   | Yes                               |  |



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| Lab Code | Other comments   |
|----------|--|
| 9        |  |
| 11       |  |
| 14       |  |
| 15       |  |
| 19       |  |
| 20       |  |
| 23       |  |
| 24       |  |
| 25       | With the introduction of uncertainty of measurement by UKAS, how does NEQAS plan to assess potential weak positive/negative results? Will you be taking MoU into account?  |
| 28       | It might be relevant to show replication of samples and controls -> How the result was obtained as this may show the integrity of the test and the ability of the scientist.   |
| 34       |  |
| 35       |  |
| 38       |  |
| 39       |  |
| 41       |  |
| 42       |  |
| 45       | I think for the purpose of an EQA scheme you can only apply a positive/negative result to achieve meaningful results. To reflect the clinical situation is not possible. Perhaps measurement uncertainty somehow needs to be included in the future. |
| 48       |  |
| 51       |  |
| 54       |  |
| 58       |  |
| 62       |  |
| 101      |  |
| 112      |  |
| 114      |  |
| 115      |  |
| 117      |  |
| 118      |  |
| 119      |  |
| 120      | Please provide more cells for crossmatching. IS it possible to have HLA typing of cells before results?  |
| 122      |  |
| 126      |  |
| 130      |  |
| 133      |  |
| 136      |  |
| 138      |  |
| 139      |  |
| 142      |  |
| 143      |  |
| 144      |  |
| 145      |  |
| 147      |  |
| 149      |  |
| 154      |  |
| 157      |  |
| 159      |  |
| 160      | For labs that perform T and B-cell crossmatch test in separate tubes, the amount of total cell provided is often too low.  |
| 163      | Doesn't reflect a real clinical lab situation where you have knowledge of patients historic antibody status and donor/recipient HLA typing.  |
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| 218      | Samples are degenerate when they arrive. Insufficient samples is sent so they cannot be tested in duplicates as the patient samples are.   |
| 220      |  |
| 227      |  |
| 235      | Add the possibility of equivocal   |
| 238      |  |
| 245      |  |
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