

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2018

| 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 ⁶ | RPMI with 10% FCS | 50 | Yes | 1:2 | Biosera | 5 | Pooled serum of sensitised patients | 2 | 4 |
| 11 | Ficoll Hipaque + differential washes | 30 | 5x10 ⁶ | PBS/Azide | 20 | Yes | | GemCell, SIGMA | 2x2 | In-house | 2 | 2 |
| 14 | Lymphoprep | 30 | 1x10 ⁶ | PBS/BSA/Azide | 40 | Yes | | NIBSC | 2 | In-house | 2 | 2 |
| 15 | | T: 25, B:50 | 10x10 ⁶ | FACS diluent | T: 25, B: 50 | Yes | | In-house | 2 | In-house | 2 | 2 |
| 19 | | 50 | 5x10 ⁶ | PBS | 50 | Yes | | Seralab | 2 | In-house | 1 | 2 |
| 20 | Lymphoprep | 50 | 5x10 ⁶ | PBS | 50 | Yes | | In-house | 5 | NIBSC | 2 | 4 |
| 23 | Lymphoprep | 50 | 5x10 ⁶ | PBS/1%FBS | 50 | Yes | | NIBSC | 3 | In-house | 2 | 3 |
| 24 | Lymphocyte separation medium (Lympholyte) | 50 | 2.5x10 ⁶ | HBSS | 50 | Yes | | NIBSC | 2 | NIBSC and In-house | 1 | 3 |
| 25 | Miltenyi MACSPrep B/T cell isolation kits | 50 | 5M | RPMI | 50 | Yes | | NIBSC | 3 | In-House | 2 | 3 |
| 28 | Lymphocyte density gradient method | 30 | 16x10 ⁶ | PBA | 50 | Yes | | Biorad | 3 | Campath | 2 | 3 |
| 34 | Carbonyl iron lympholyte + Dextran sulphate | 50 | 6x10 ⁶ | TBS | 50 | Yes | | Local | 6 | Local | 2 | 3 |
| 35 | Lymphoprep | 50 | 5x10 ⁶ | PBS+1% FCS | 50 | Yes | | NIBSC | 3 | In-house | 3 | 3 |
| 38 | Density gradient centrifugation | 20 | 5M | PBS azide | 20 | Yes | | NIBSC | 4 | In-House | 2 | 2 |
| 39 | Lympholyte | 50 | 1x10 ⁷ | 1%FBS/PBS buffer | 50 | Yes | | NIBSC | 3 | In-House | 3 | 3 |
| 41 | Lymphoprep | 25 | 10x10 ⁶ | 2% FBS/PBS | 50 | Yes | | AB Serum | 3 | In-house | 3 | 3 |
| 42 | Ficoll-Opaque | 50 | 4x10 ⁶ | 1% FCS/PBS | 50 | Yes | | Commercial | 3 | In-house | 1 | 3 |
| 45 | | 40 | 2.5x10 ⁶ | TPM | 10 | Yes | | Local and NIBSC | 2 | 2 Local and NIBSC | 2 | 2 |
| 48 | Lympholyte | 30 | 5x10 ⁶ | Local flow diluent | 20 | Yes | | NIBSC | 2 | Local pool | 2 | 2 |
| 51 | Density gradient centrifugation | 50 | 2x10 ⁶ | PBS | 50 | Yes | | Local | 3 | Local | 2 | 2 |
| 54 | Ficoll Hypaque | 100 | 2x10 ⁶ | PBS 0.1% azide 1% normal goat + 1% normal mous serum | 50 | Yes | | LPS | 6 | In-house | 1 | 2 |
| 58 | | 50 | 5.7x10 ⁶ | PBS | 50 | Yes | | NIBSC | 4 | In-House poitive pool | 3 | 3 |
| 62 | Density gradient centrifugation | 50 | 5M | 1% NBSC 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 ⁶ | PBS | 25 | No | | Human serum AB | 2 | Pool patients | 2 | 2 |
| 114 | Density gradient separation | 100 | 2.5x10 ⁵ | 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 | 10 | 200,000 | RPMI with 20% FBS | 50 | Yes | | NIBSC | 3 | Local pool | 3 | 3 |
| 118 | | 10 | 1x10 ⁶ | PBS | 10 | No | | Local + NIBSC | 2 | Local | 2 | 2 |
| 119 | Density gradient | 100-120 | 200000 | PBS | 100 | Yes | | AB+ male | 4 | Patient pool | 3 | 3 |
| 120 | Cell wash centrifugation suspended in Hanks | 100 | 5M | PBS1x -BSA1% | 20 | Yes | | AB serum | 2 | Hyperimmunised serum | 2 | 2 |
| 122 | | 50 | 3x10 ⁵ | PBS+2% FCS | 50 | Yes | | NIBSC | 2 | Local | 1 weak, 1 strong | 2 |
| 126 | | | | | | | | | | | | |
| 130 | | | 0.25-0.3x10 ⁶ | | 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 ⁶ | RPMI | 20 | No | | AB serum | 3 | Hypersensitized sera pool | 2 | 2 |
| 138 | Ficoll | 100 | 10 ⁷ | PBS | 80 | Yes | | AB serum | 1 | Hypersensitized sera pool | 1 | 2 |
| 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 ⁶ | FBS | 50 | Yes | | In-house | 2 | Local pooled sera | 1 | 2 |
| 144 | Ficoll separation | 25 | 15x10 ⁶ | PBS | 25 | Yes | | Local | 1 | Local | 2 | 2 |
| 145 | Ficoll | 50 | 1x10 ⁷ | PBS | 50 | Yes | | Local | 1 | Biorad | 1 | 1 |
| 147 | Ficoll | 100 | 5000 | RPMI | 50 | Yes | | Human serum AB | 2 | Serum from polcry immunised patients | 2 | 2 |
| 149 | Ficoll | 50 | 1x10 ⁷ | PBS | 50 | Yes | | Biorad | 1 | Biorad | 1 | 1 |
| 154 | Washing and centrifugation | 20 | 15x10 ⁶ | PBS BSA 1% | 20 | Yes | | Commercial | 2 | Commercial | 2 | 2 |
| 157 | | 15 | 1.5x10 ⁵ | 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 ⁵ | RPMI+2%FBS | 50 | Yes | | Local | 2 | Biotest | 1 | 2 |
| 163 | Lymphocyte separation medium | 0 | 0.2x10 ⁶ | | 50 | Yes | | NIBSC | 2 | NIBSC | 2 | 2 |
| 167 | | | | | | | | | | | | |
| 169 | PBMC isolation (Lymphoprep) | 100 | 2E6/ml | TBS | 100 | Yes | | AB donor pool | 1 | Local serum | 1 | 1 |
| 176 | PBMC isolation | 25 | 20000 cells/µl | Lymphostabil | 25 | Yes | | BioRad | 2 | In-house | 2 | 2 |
| 186 | Ficoll | 100 | 5M | | 25 | Yes | | Pool sera for AB donors | 1 | Local sera from immunized patients | 2 | 1 |
| 189 | Density gradient | 50 | 80-200 x1000 | PBS | 50 | No | | AB serum | 1 | PRA (+) sera | 1 | 1 |
| 190 | Ficoll | 100 | 200000 | NAACL | 50 | Yes | | SAB male | 3 | Pool hyperimmunised | 2 | 3 |
| 191 | Isolated cells sent were used. Washed with PBS | 100 | 2-3x10 ⁵ | PBS | 20 | Yes | | AB Negative, PRA negative sera | 2 | Pooled PRA positive sera | 2 | 2 |
| 193 | Ficoll | 100 | 3500 | | 20 | Yes | | In-house | 1 | In-house | 1 | 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 | Ficoll | 50 | 1-1.5x10 ⁷ | 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 | Ficoll + Pronase | 25 | 10000 | PBS+FCS 2% | 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 | 10 ⁴ | 2% FCS0, 5% NaN3-PBS | 15 | Yes | | AB serum | 4 | In-house | 2 | 2 |
| 227 | STEMCELL | 50 | 6x10 ⁶ | 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 |
| 247 | | 25 | | | 25 | Yes | | AB + Healthy serum | | PRA >80% serum | | |
| 260 | Density gradient method with histopaque | 5 | 1000000 | PBS | 20 | Yes | | One Lambda | 1 | One Lambda | 1 | 1 |
| 262 | Ficoll | 50 | 500000 | RPMI + Human SAB 10% | 50 | Yes | | Human SAB | 2 | Immunised patient | 1 | 2 |
| 271 | Ficoll hypaque | Dried pellet | 250000 | PBS 2% FBS | 25 | Yes | | AB NHS Invitrogen | 2 | In-house | 1 | 2 |
| 276 | Thaw cells in suspension | 10 | 5E5 cells/mL | PBS/0.1% BSA | 20 | Yes | | AB serum | 2 | Sc101370 | 2 | 3 |
| 284 | Mononuclear cells isolated by density centrifugation over Ficoll | 50 | 5x10 ⁶ | RPMI | 50 | Yes | | NIBSC | 3 | Local + NIBSC | 2 | 2 |
| 293 | | | | | | | | | | | | |
| 297 | | 50 | 6000 | RPMI | 50 | | | NIBSC | 2 | NIBSC | 1 | 1 |
| 302 | | 25 | 1/1 | Isoflowsheath | 25 | Yes | | | | | 1 | 1 |
| 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 ⁶ | | 20 | Yes | | Local | 1 | Local | 1 | 2 |
| 358 | Density gradient centrifugation | 100 | 5x10 ⁶ | PBS +1% BSA | 50 | Yes | | Inno-Train | 1 | Inno-Train | 1 | 1 |

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2018

| 2. Sensitisation Stage | | 3. Detection Stage: | | | | | | | | | | | | | |
|------------------------|-------------|---------------------|------------------|----------------------------|-----------------|--------|-----------|-------------------|-----------|---|----------------------------------|---|----------|-----------------|---------------|
| Lab Code | Time (mins) | Temp (°C) | Manual Automated | 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 | PBS | 4 | 3 | 4 | Sigma | Goat | FITC/Fab2 fragment | 1+9 | PBS/Azide | 10 | 20 | 4 |
| 14 | 30 | RT | Manual | PBS | 2 | 2 | RT | Sigma | Goat | FITC/Fab2 fragment | 1/23 | PBS/BSA/Azide | 115 | 20 | 4 |
| 15 | 30 | 22 | Automated | PBS | 3 | 3 | 22 | Sigma | Goat | FITC/Fab2 fragment | 1:220 | FACS diluent | 100 | 15 | 4 |
| 19 | 30 | 37 | Manual | 0.1% FBS in PBS | 4 | 3 | 4 | Sigma | Goat | FITC/Fab2 fragment | 1:32 | PBS | 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/PBS buffer | 4 | 3 | 18-25 | Sigma | Goat | FITC/Fab2 fragment | 1:20 | 1% FBS/PBS buffer | 4 | 20 | 4-8 |
| 41 | 20 | 22 | Automated | PBS | 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 | 2 | | 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 | | 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 mouse 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 | PBS/CS | 4 | 3 | 22 | DAKO | Rabbit | FITC/Fab2 fragment | | | 14 | 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 | 3 | 4 | RT | BD | 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 | | 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 |
| 176 | 30 | 20 | Manual | TBS | 3 | 3 | 18 | DAKO | Rabbit | FITC/Fab2 fragment | | | 5 | 20 | 20 |
| 185 | 20 | RT | Automated | PBS | 4 | 2 | RT | Abcam | Goat | FITC/Fab2 fragment | 1/50 | PBS | 10 | 10 | RT |
| 186 | 30 | 4 | Manual | PBS | 2 | 2 | 4 | Beckman Coulter | Goat | FITC/Fab2 fragment | 1/200 | PBS + FBS 8% | 50 | 30 | 4 |
| 189 | 20 | RT | Manual | FACS Flow | 2 | 1 | 2-8 | DAKO | Rabbit | FITC/Fab2 fragment | 01-Oct | PBS | 50 | 30 | 2-8 |
| 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 | Phycoerythrin/Fab2 fragment | | | 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 | FITC/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 |
| 276 | 30 | 20-25 | Manual | PBS/0.1% BSA | 2.5 | 2 | 20-25 | BD | Rabbit | FITC/Fab2 fragment | 1:10 | PBS/0.1% BSA | 10 | 30 | 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 | 5 | 30 | 4 |
| 302 | 30 | 25 | Manual | Isoflowsheath | 500 | 1 | 25 | Biologend | | FITC | Jan-00 | | 5 | 30 | 25 |
| 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 |

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2018

| 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 | RT | | Phycoerythrin-cyanine 5 | Yes | | Yes | PBS | 1:30 | 50 | 30 | 4 |
| 11 | BD | No | Yes | Manual | PBS/Azide | 4 | 1 | 4 | Yes | PerCp | Yes | | No | | | 3 | 20 | 4 |
| 14 | BD | Yes | | Manual | | | | | Yes | PE | Yes | | No | | 1/11.5 | 115 | 20 | 4 |
| 15 | DAKO | No | Yes | Automated | PBS | 30 | 1 | 22 | No | | Yes | | Yes | FACS diluent | 1:50 | 100 | 15 | 4 |
| 19 | Beckman Coulter | Yes | | | | | | | Yes | PE | Yes | | 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 | | PE | | No | | | 5 | 15 | 4 |
| 28 | BD | Yes | | | | | | | Yes | | Yes | | No | | | 5 | 30 | 4 |
| 34 | BD | Yes | | | | | | | Yes | 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/PBS buffer | 4 | 1 | 18-25 | Yes | RPE | Yes | | No | | | 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 | | Yes | PBA | 1/10 | 50 | 15 | ice |
| 48 | DAKO | Yes | | | | | | | Yes | | Yes | PE | | | | 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 | | | | 5 | 15 | 4 |
| 58 | | Yes | | | | | | | Yes | Phycoerythrin-cyanine 5 | Yes | | No | | | 5 | 30 | 4 |
| 62 | Beckman Coulter | Yes | | Manual | PBSCS | 4 | 1 | | Yes | | Yes | PE | No | | | 5 | 30 | 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 | | Yes | | | 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 | Yes | | | 10 | 30 | 4 |
| 122 | Immunostep | Yes | | | | | | | Yes | | Yes | PerCp | Yes | PBS+2% FCS | 1:16 | 50 | 30 | 4 |
| 126 | | | | | | | | | No | | No | | 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 | | 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 | No | | | 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 | | Yes | PC7 | Yes | | | 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 | | No | | | 5 | 30 | 4 |
| 169 | | | | | | | | | | | | | | | | | | |
| 176 | BD | No | Yes | Manual | TBS | 3 | 1 | 18 | Yes | Phycoerythrin | Yes | | | | | 10 | 20 | 20 |
| 185 | Beckman Coulter | Yes | No | | | | | | Yes | APC | Yes | | No | | | 10 | 10 | RT |
| 186 | Beckman Coulter | Yes | | Manual | PBS + FBS 8% | 2 | 1 | 4 | Yes | PE | Yes | | No | | | 10 | 30 | 4 |
| 189 | Biolegend | Yes | | Manual | PBS | 2 | 1 | RT | Yes | Phycoerythrin-cyanine 5 | Yes | | No | | | 3 | 30 | 2-8 |
| 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 | Yes | 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-cyanine 7 | Yes | | No | | | 5 | 20 | 22 |
| 245 | Beckman Coulter | Yes | | Manual | | | | | Yes | APC | Yes | | No | | | 5 | 30 | 22-24 |
| 246 | BD | Yes | | | | | | | Yes | PerCp | Yes | | No | | | 5 | 10 | 20 |
| 252 | BD | Yes | | Manual | | 1 | 3 | RT | Yes | Phycoerythrin-cyanine 5 | Yes | | Yes | | | 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 |
| 276 | BD Biosciences | No | No | | | | | | No | FITC | Yes | | Yes | PBS/0.1% BSA | 1:10 | 10 | 30 | 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 |
| 302 | Beckman Coulter | Yes | | Manual | Isoflowsheath | 500 | 1 | 25 | Yes | | Yes | | No | | | 5 | 30 | 25 |
| 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 |

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2018

| 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) | Labeled with | CD19 | CD20 | Other | Diluted | Diluent | Dilution | Vol / tube (µl) | Inc time (mins) | Inc temp (°C) |
| 9 | | | | | | | | | | | | | | | | | | |
| 11 | BD | No | Yes | Manual | PBS/Azide | 4 | 1 | 4 | Phycoerythrin | Yes | | | No | | | | 20 | 4 |
| 14 | DAKO | Yes | | | | | | | Phycoerythrin-cyanine 5 | Yes | | | Yes | PBS/BSA/Azide | 1/23 | 115 | 20 | 4 |
| 15 | | No | Yes | Automated | | 3 | 1 | 22 | Phycoerythrin | Yes | | | Yes | FACS diluent | 1:40 | 100 | 15 | 4 |
| 19 | Beckman Coulter | Yes | | | | | | | Phycoerythrin-cyanine 5 | Yes | | | No | | | 5 | 30 | 4 |
| 20 | | Yes | | | | | | | Phycoerythrin | Yes | | | Yes | Detection cocktail | 1:2 | 5 | 15 | 2-8 |
| 23 | BD | Yes | | | | | | | Phycoerythrin | Yes | | | No | | | 5 | 30 | 4 |
| 24 | BD | Yes | | | | | | | Allophycocyanin | Yes | | | No | | | 5 | 30 | 2-8 |
| 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 | 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 /PBS buffer | 4 | 1 | 18-25 | 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 | | | Yes | PBS | 1:10 | 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 | Yes | | No | | | 10 | 15 | 4 |
| 58 | | Yes | | | | | | | Phycoerythrin | Yes | | | No | | | 5 | 30 | 4 |
| 62 | Beckman Coulter | Yes | | Manual | PBSCS | 4 | 1 | | Phycoerythrin-cyanine 5 | Yes | | | No | | | 5 | 30 | 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 | | | Yes | | | 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 | | | | | | | | | | | | | | | | 5 | 30 | 4 |
| 169 | | | | | | | | | | | | | | | | | | |
| 176 | BD | No | Yes | Manual | TBS | 3 | 1 | 18 | PerCp | Yes | Yes | | No | | | 10 | 20 | 20 |
| 185 | Beckman Coulter | Yes | No | | | | | | Phycoerythrin | Yes | | | No | | | 20 | 10 | RT |
| 186 | Beckman Coulter | | | Manual | PBS + FBS 8% | 2 | 1 | 4 | Phycoerythrin | Yes | | | No | | | 10 | 30 | 4 |
| 189 | BD | Yes | | Manual | PBS | 2 | 1 | RT | Phycoerythrin | Yes | | | No | | | 5 | 30 | 2-8 |
| 190 | | Yes | | | | | | | | 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 | BD | Yes | | Manual | | 1 | 3 | RT | Phycoerythrin | Yes | | | No | | | 5 | 30 | RT |
| 260 | BD | Yes | | Manual | Cell wash BD | 1 | 1 | 22 | APC | Yes | | | 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 |
| 276 | BD Biosciences | No | No | | | | | | FITC | Yes | | | Yes | PBS/0.1% BSA | 1:10 | 10 | 30 | 4 |
| 284 | BD | Yes | | Manual | | 2 | 2 | 22 | BV421 | Yes | | | No | | | 2.5 | 30 | 4 |
| 293 | | | | | | | | | | | | | | | | | | |
| 297 | | Yes | | | | | | | APC | Yes | | | No | | | 5 | 30 | 4 |
| 302 | Beckman Coulter | Yes | No | Manual | Isoflowsheath | 500 | 1 | 25 | | Yes | | | No | | | 5 | 30 | 25 |
| 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 |

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2018

| 6. Final wash | | | | | | | |
|---------------|-----------|----------------------------|-----------------|------------------|-----------|---|-----------------|
| Lab Code | Wash step | Wash medium | Vol / tube (ml) | Number of washes | Temp (°C) | Resuspension of cells in | Vol / tube (µl) |
| 9 | Manual | PBS | 100 | 3 | RT | Phosphate buffered saline | 400 |
| 11 | Manual | PBS/Azide | 4 | 1 | 4 | PBS/Azide | 400 |
| 14 | Manual | | 2 | 2 | RT | PBS/BSA/Azide | 300 |
| 15 | Automated | PBS | 3 | 1 | 22 | FACS diluent | 200 |
| 19 | Manual | 0.1% FBS in PBS | 4 | 1 | 4 | Phosphate buffered saline | 200 |
| 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/PBS buffer | 4 | 1 | 18-25 | 1% FBS/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 | Phosphate buffered saline | 500 |
| 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 | PBS 1x - 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 |
| 185 | Automated | PBS | 4 | 2 | RT | Phosphate buffered saline | 300 |
| 186 | Manual | PBS + FBS 8% | 2 | 1 | 4 | Phosphate buffered saline | 300 |
| 189 | Manual | PBS | 2 | 1 | RT | Sheath fluid | 350 |
| 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 |
| 276 | Manual | | 2.5 | 2 | 20-25 | Phosphate buffered saline, PBS/0.1% BSA | 350 |
| 284 | Manual | | 2 | 2 | 22 | Cell wash BD | 500 |
| 293 | | | | | | | |
| 297 | Manual | PBS | | 3 | 22 | Phosphate buffered saline | 500 |
| 302 | Manual | Isoflowsheath | 500 | 1 | 25 | Sheath fluid | 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 |

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2018

| Lab Code | 7. Data Acquisition | | | | Type of beads | Events | | | | B-cells | | Parameter used to assess peak performance | Scale used |
|----------|--------------------------------|------------|------------------------|---|---|--------------|--------|---------|-----------------|-----------|--------|--|-----------------------------|
| | Flow cytometer | Live gated | Calibration beads used | Used for | | Total events | | T-cells | | 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 | Yes | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | CST | | | 500 | 5000 | 200 | 1000 | Median | Log channel |
| 14 | FACS Canto II | Yes | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | 7 colour set-up | | | 3000 | | 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 | Beckman Coulter Navios | | | Alignment check | Flowcheck | | | | | | | Median | Log channel |
| 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 | 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 | | 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. | |
| 39 | Beckman Coulter - FC500/Navios | Yes | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Flowcheck/Flowcheck pro, Flow set/Flow set pro, 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 |
| 46 | Beckman Coulter | Yes | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | | | | 5000 | | 1500 | | Median | Log channel |
| 51 | 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, Fluorescence check | Flow check, Flow set | 10000 | | 100 | | 100 | | Median | Log channel |
| 101 | Beckman Coulter | Yes | No | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Flowcheck, Flow set, Quantum-MESF | | | | | 1000 | | Mean | Log channel |
| 112 | Beckman Coulter FC500 | Yes | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Calibrite 3 | | | 5000 | | 1000 | | Median | Log channel |
| 114 | FACS Calibur | Yes | Yes | Alignment check, Laser check, Fluorescence check | Calibrite + Rainbow Cal. Part. | 5000 | | | 20000 or 120sec | | | Median | Linear channel |
| 115 | FACS Calibur | Yes | Yes | Alignment check, Laser check, Fluorescence check | Calibrite 3 | | | | | 350 | 1500 | Median | Log channel |
| 117 | BD | Yes | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Flowcheck, Flow set, Quick-comb, Immunobrite | | | | 5000 | | 1000 | Median | Linear channel |
| 118 | Calibur | Yes | Yes | Alignment check, Laser check, Fluorescence check | Calibrite 3 | | | | 15000-20000 | 400 | 1000 | Mean | Log channel |
| 119 | Coulter Epics-XL | No | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Calibrite 3 | | | | | 3000 | | Median | Log channel |
| 120 | FACS Canto | Yes | Yes | Alignment check, Laser check, Fluorescence check | 7 colour set-up | | | 6000 | 10000 | 800 | 1000 | Geomean | Linear channel |
| 122 | BD FACS Canto II | Yes | Yes | Alignment check, Laser check, Fluorescence check | | | | | | | | | Linear channel |
| 126 | FACS Scan | Yes | Yes | Fluorescence check, Fluorescence quantitation | Sphero rainbow calibration | 2000 | | | | | | Mean | Linear channel |
| 130 | FACS Scan | Yes | Yes | Alignment check, Laser check, Fluorescence check | Calibrite 3 beads | 20000 | 50000 | | | | | Median | Linear channel |
| 133 | FACS Calibur | No | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Calibrite | | | 5000 | 10000 | | | Geomean | Linear channel |
| 138 | BD | No | Yes | Alignment check, Laser check, Fluorescence check | CST, Rainbow | 30000 | | | | | | Kolmogorov | Log channel |
| 139 | Beckman Coulter | Yes | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | 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 | | Unlimited | | 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 | Yes | Alignment check, Laser check, Fluorescence check | Calibrite 3 | | | 12000 | 10000 | | | 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 | | 200 | | Median | Log channel |
| 185 | Navios | No | Yes | Alignment check | Flow-check | | | 2000 | | 500 | | Mean | Linear channel |
| 186 | | Yes | Yes | Alignment check, Laser check, Fluorescence check | Flowcheck, Flow set | | | 3500 | | 1500 | | Mean | Log channel |
| 189 | | Yes | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | BD Calibrate | | | | | | | Percentage | Log channel |
| 190 | Beckman Coulter Navios | Yes | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | | 12000 | | | | | | Mean | Linear channel, Log channel |
| 191 | BD FACS Calibur | Yes | Yes | Alignment check, Laser check, Fluorescence check, Fluorescence quantitation | Calibrite | 2000 | | 15000 | | 2000-5000 | | Mean | Log channel |
| 193 | FACS Calibur | No | Yes | Alignment check, Laser check, Fluorescence check | Calibrite | 10000 | | 250 | | 250 | | Median | Log channel |
| 194 | BD | 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 | | | | | 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 |
| 276 | Beckman Coulter FC500 | No | Yes | Alignment check, Fluorescence check, Fluorescence quantitation | Flowcheck, Flow set | 10000 | 50000 | 10000 | 50000 | 10000 | 50000 | 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 |
| 302 | Kaluza 1.3 | Yes | Yes | Laser check | | 50000 | 200000 | 50000 | 200000 | 50000 | 200000 | Mean | |
| 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 2018

| 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 | |
| 9 | Median difference | T-cell pos/neg trimmed mean value >3 | B-cell >1.5 x trimmed mean | | Pre-transplant crossmatching |
| 11 | Mean difference | 1.2x RMF | 1.2x RMF | 1.2x RMF | Pre-transplant crossmatching |
| 14 | Median difference | 4x neg | 1.5x mean neg | T-cells: 2.4x mean neg B-cells: 2.45x mean neg OTX RMF >4 Prev. Tx RMF >2.3 | Clinical/Diagnostic, Pre-transplant crossmatching |
| 15 | Median difference | 20x neg | 10x neg | NEQAS RMF >1.5 | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching |
| 19 | Log median X | 1.48x Av Neg | 1.88x Av Neg | 40 | Pre-transplant crossmatching |
| 20 | Median difference | 60 | 40 | 40 | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching |
| 23 | Median difference | +3SD for mean of AB | +2SD | T: +3SD for mean of AB, 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 RMF >1.3 | Clinical/Diagnostic, Pre-transplant crossmatching |
| 25 | Median difference | | | T-cell ≥1.6 B-cell ≥2.0 | 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 | +2SD Mean +3SD | 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 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 | >1.5 RMF / >2.0 RMF | Pre-transplant crossmatching |
| 41 | Mean difference | 10x neg | 10x neg | T: 2SD, B: 3SD | Pre-transplant crossmatching |
| 42 | Mean difference | MCS ~30, RMF >1.3 | MCS ~45, RMF >1.5 | T: RMF <1.3, B: RMF <1.5 | Pre-transplant crossmatching |
| 45 | Relative median fluorescence | 40 channel shift | 4x neg | 40 channel shift 1.3x neg | Pre-transplant crossmatching |
| 51 | Test of neg control MESF ratio | a test/neg MESF ratio of >10 | ≥1.2 MESF ratio | ≥1.5 MESF ratio | Pre-transplant crossmatching |
| 54 | Median difference | 1.3x mean | 1.3x mean | 1.3x mean | Pre-transplant crossmatching |
| 58 | Median difference | RMF >2 | RMF >5 | T-cell RMF >1.5, B-cell RMF >3 | Pre-transplant crossmatching |
| 62 | Mean difference | | | | Pre-transplant crossmatching |
| 101 | Ratio | >1.5 ratio | >2.0 ratio | T-cell: >1.5 ratio B-cell: >2.0 ratio | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 112 | Median difference | 40 channel | 70 channel | 50 | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 114 | Median difference | >40 | >40 channel shift | >40 channel shift | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 115 | Median difference | | RMF >4 | T: RMF >1.3, B: RMF >1.5 | Clinical/Diagnostic, Post-transplant monitoring |
| 117 | Median difference | | T: Med neg +66 ch, B: Med neg +25.5 ch | T: Med neg +66 ch, B: Med neg +25.5 ch | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring |
| 118 | Mean difference | ratio >1.285 | ratio >1.7 | T-cells: ratio >1.285 B-cells: ratio >1.7 | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 119 | Median difference | ratio >13 | ratio >12 | T-cells: ratio >1.6 B-cells: ratio >2 | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 120 | Fluorescence index | FI >1.5 | FI >2.0 | 3SD | Clinical/Diagnostic, Research/Development, Antibody screening, Pre-transplant crossmatching, Post-transplant monitoring |
| 122 | Mean difference | | | T: >40 channels, B: >80 channels | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 126 | Peak shift, Median difference, Mode difference | >x4 of NC | >x4 of NC | >+50% of neg, >+100% of neg NR >200 | Pre-transplant crossmatching Pre-transplant crossmatching, Post-transplant monitoring |
| 130 | Mean difference | | | 1.5 or 2 (T or B cells) of neg mean log channel | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching |
| 133 | Peak shift, Median difference, Mode difference | | | Mean +3SD | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 136 | Mean difference | | | >500 | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring |
| 138 | Mean difference | | | Ratio | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 139 | Peak shift | | | Mean ratio +2SD | Clinical/Diagnostic, Pre-transplant crossmatching |
| 142 | Mean difference, Median difference | 40 | 2.5 | t: >40 channel, B: >80 channel | Pre-transplant crossmatching, Post-transplant monitoring |
| 143 | Mean difference, Median difference | 1.76 | 2.5 | Mean ratio +2SD | Clinical/Diagnostic, Pre-transplant crossmatching |
| 144 | Ratio median log channel test/Median log channel neg | Mean ratio +2SD | Mean ratio +2SD | None | Pre-transplant crossmatching, Post-transplant monitoring |
| 145 | % shift | Tpos-Tneg=200 | Mean ratio +2SD | 150% of neg control and shape of the curve | Clinical/Diagnostic, Pre-transplant crossmatching |
| 147 | Ratio median log channel /median log channel neg control | Mean ratio +2SD | Mean ratio +2SD | 2SD from neg control | Pre-transplant crossmatching, Post-transplant monitoring |
| 149 | Mean difference | 150 channel shift | 250 channel shift | T: 40 channel shift, B: 100 channel shift | Pre-transplant crossmatching, Post-transplant monitoring |
| 154 | Mean difference | 100 channel shift | 100 channel shift | >Mean of neg sera +2SD | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 157 | Mean difference | 100 mean log channel | 170 mean log channel | T: 2.3, B: 1.7 | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 159 | Mean difference | Ratio of geometric mean | 2.3 | | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 160 | Ratio of geometric mean | | | | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 163 | 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 |
| 176 | Mean difference/Median difference | Undefined | Undefined | T: 2SD, B: 1.5SD | Clinical/Diagnostic |
| 185 | Mean difference | | | T-cells: ratio >1.5 x the local neg B-cells: ratio >2 x the local neg | Pre-transplant crossmatching |
| 186 | % shift | 40 | 40 | 40 | Clinical/Diagnostic |
| 189 | Mean difference | | | | Clinical/Diagnostic, Pre-transplant crossmatching |
| 190 | Mean difference | | | T: <1.2Neg >1.5Pos B: <1.2Neg >2.5Pos T: >30, B: >100 | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 191 | Geometric mean | ratio >0.7 | ratio >1 | Neg geometric mean x2.5 | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 193 | Median difference | | | ratio sample/neg control, T:1.3, B: 1.6 | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 194 | % shift | 2 | 2.5 | NA | Clinical/Diagnostic, Pre-transplant crossmatching |
| 195 | Ratio x-mean sample/NC | | | | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 201 | Mean difference | 1.5x | 2x | T-cells: 1.5x the local neg B-cells: 2x the local neg Ratio >1.07, Ratio B >1.17 | Pre-transplant crossmatching, Post-transplant monitoring |
| 202 | Mean difference | Ratio >2 | Ratio >3 | Ratio >1.2, Ratio B >1.3 | Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring |
| 204 | Mean difference | CTL x1.2 | CTL x1.7 | T-cell: CTL x1.2 B-cell: CTL x1.7 | Clinical/Diagnostic, Pre-transplant crossmatching |
| 206 | 8000 - mean of neg control | 8000 - mean of neg control | 8000 - mean of neg control | 8000 - mean of neg control | Pre-transplant crossmatching |
| 209 | Median difference | 1.5x neg | 2x neg | T: 1.5x neg, B: 2x neg | Clinical/Diagnostic, Pre-transplant crossmatching |
| 218 | Median difference | | | >2 of neg median log channel T: ratio >1.8, B: >2.2 | Research/Development, Pre-transplant crossmatching, Post-transplant monitoring |
| 220 | Median difference | 7 | 15 | | Pre-transplant crossmatching, Post-transplant monitoring |
| 227 | MESF Serum/MESF negative control | 7 | 15 | | Clinical/Diagnostic, Pre-transplant crossmatching |
| 235 | Median difference | 1.75 | 2.5 | | Clinical/Diagnostic |
| 238 | Median log, FI ratio | Median ratio ±2SD | Median ratio ±3SD | | Pre-transplant crossmatching Research/development |
| 245 | Median ratio | 2x neg control MFI | 2x neg control MFI | 2x neg control MFI | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 260 | % shift | >12% | >12% | Median RFU sample/Median RFU neg >2 | Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring |
| 262 | Median channel shift | 64 | 90 | | Pre-transplant crossmatching, Post-transplant monitoring |
| 271 | Median difference | 4SD | 4SD | 2SD from neg control | Research/Development |
| 276 | Median difference | ratio >1.2 | ratio >1.2 | ratio >1.2 | Clinical/Diagnostic, Pre-transplant crossmatching |
| 284 | Median difference | >1.5x neg control | >1.5x neg control | | Clinical/Diagnostic, Post-transplant monitoring |
| 293 | % shift | | | | Human reproduction |
| 302 | Geomean | 1.47x geomean of NC | 1.7x geomean of NC | 3SD | Pre-transplant crossmatching, Post-transplant monitoring |
| 341 | Peak shift, Median difference | 40 | 20 | T: 500+neg results B: 2500+ Neg results 3x median neg | |
| 351 | Ratio of median fluorescence of test sample to negative control | 5x neg control | 5x neg control | | |

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2018

| 10. Reporting results | | |
|-----------------------|-----------------------------------|--|
| Lab Code | Is Positive or Negative adequate? | Reason |
| 9 | Yes | |
| 11 | Yes | |
| 14 | Yes | |
| 15 | Yes | |
| 19 | No | Ability to qualify a positive result. E.g. Due to non-HLA antibodies |
| 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 | No | |
| 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" |
| 185 | | |
| 186 | Yes | |
| 189 | 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 | |
| 276 | Yes | |
| 284 | Yes | |
| 293 | | |
| 297 | Yes | |
| 302 | Yes | |
| 341 | Yes | |
| 351 | Yes | |
| 358 | | |

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2018

| | Other comments |
|----------|--|
| Lab Code | |
| 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. |
| 167 | |
| 169 | |
| 176 | |
| 185 | Method in validation |
| 186 | |
| 189 | |
| 190 | |
| 191 | |
| 193 | |
| 194 | |
| 195 | |
| 201 | |
| 202 | |
| 204 | |
| 206 | |
| 209 | |
| 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 | |
| 238 | Add the possibility of equivocal |
| 245 | |
| 246 | |
| 252 | |
| 260 | |
| 262 | |
| 271 | |
| 276 | |
| 284 | |
| 293 | |
| 297 | |
| 302 | |
| 341 | |
| 351 | |
| 358 | |