# UK NEQAS
### International Quality Expertise

## Histocompatibility & Immunogenetics

# Educational Crossmatching Scheme
Dispatched on the 20th July 2021

## Summary of Results

A total of 42 reports were received, but not all labs reported results for all tests.

Consensus HLA Type of whole blood donor sample EDXM 01/2021:

| | A* | B* | C* | DRB1* | DRB3* | DRB4* | DQA1* | DQB1* | DPA1* | DPB1* |
|---|---|---|---|---|---|---|---|---|---|---|
| | 01 | 27 | 01 | 04 | 02 | 01 | 01 | 03 (7) | 01 | 04:01 |
| | 31 | 44 | 03(9) | 14 | - | - | 03 | 05 | 02 | 14:01 |
| Number of reports | 34 | 34 | 34 | 34 | 24 | 25 | 32 | 32 | 24 | 29 |
| % Labs in consensus | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |

### EDXM 01 Serum 1/2021 Results

HLA Antibody Detection and Definition
*(Based on 75% Consensus)*

| | | HLA Class I | No of labs | % consensus | HLA Class II | No of labs | % consensus |
|---|---|---|---|---|---|---|---|
| Detection | IgG | Positive | 31/31 | 100% | Positive | 31/31 | 100% |
| | IgM | Negative | 1/1 | 100% | Negative | 1/1 | 100% |
| Definition[1] | Total Number of Specificities Reported (by ≥ 1 lab) | 84 | | | 45 | | |
| | Number of Specificities Absent (reported by < 5% labs) | 5 | | | 1 | | |
| | Number of Specificities Present (reported by ≥ 75% labs) | 31 | | | 9 | | |
| | MFI >10000 | A2 A203 A23 A24 A2403 A25 A32 B51 B52 B44 B13 B63 B77 B38 B57 B58 B49 B27 B37 B47 B53 B59 | 33 | 94-100% | DR1 DR103 DR4 DR14 DR9 DR10 DR51 | 33 | 94-100% |
| | MFI 5001 - 9999 | B2708 B67 B73 | | 82-97% | DR53 | | 97% |
| | MFI 2001 - 5000 | A69 B7 B54 B55 B56 B42 B81 | | 91-100% | DR15 | | 94% |
| | MFI <2000 | A36 | | 85% | N/A | | N/A |

[1] 75% consensus 'present' specificities are displayed within the MFI value range reported by the **majority** of participants

## Crossmatching
*(Based on 75% Consensus)*

| | CDC | | | | | | Flow Cytometry | |
|---|---|---|---|---|---|---|---|---|
| | PBL | | T Cells | | B Cells | | T Cells | B Cells |
| | Without DTT | With DTT | Without DTT | With DTT | Without DTT | With DTT | | |
| | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive |
| Number of reports | 7/7 | 7/8 | 12/13 | 10/12 | 11/11 | 12/12 | 29/29 | 27/27 |
| % Labs in consensus | 100% | 87.5% | 92% | 83% | 100% | 100% | 100% | 100% |

## Interpretation

Identification of Donor Specific Antibodies:

| Specificity | No of Participants (n=33) | MFI Range Reported |
|---|---|---|
| B27 | 33 | 5449-26983 |
| B44 | 31 | 5037-26708 |
| Cw1 | 18 | 806-3659 |
| DR4 | 29 | 5074-20567 |
| DR14 | 25 | 966-19753 |
| DR52 | 1 | 18475 |
| DR53 | 23 | 2152-18475 |
| DQ5 | 17 | 1782-10216 |
| DQA1*01 | 1 | 2853 |

Crossmatch interpretation:

| | *The most common responses included:* |
|---|---|
| Interpretation based on results | • CDC crossmatch Positive<br>• Flow cytometry crossmatch Positive<br>• Patient has multiple donor specific antibodies |
| Assigned risk | High        n = 6   (18%)<br>Contraindication    n = 27  (82%) |
| Immunological advice | • Not suitable for transplantation<br>• Seek alternative donor<br>• Consider de-sensitisation<br>• Perform additional testing e.g. autologous Flow crossmatch |
| If advice is not to transplant, recommendations for future transplants | • Possible antibody removal pre-transplant<br>• Investigate alternative donor options e.g. entering patient in a paired kidney exchange programme<br>• Review listing of unacceptable antigens<br>• Investigate whether the patient is a suitable candidate for increased immunosuppression |

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**EDXM 01 Serum 2/2021 Results**

HLA Antibody Detection and Definition
*(Based on 75% Consensus)*

| | | HLA Class I | No of labs | % consensus | HLA Class II | No of labs | % consensus |
|---|---|---|---|---|---|---|---|
| **Detection** | IgG | *No Consensus* | *22/31 Pos* | 71% | Negative | 27/31 | 94% |
| | IgM | Negative | 1/1 | 100% | Negative | 1/1 | 100% |
| **Definition [1]** | Total Number of Specificities Reported (by ≥ 1 lab) | 84 | | | 44 | | |
| | Number of Specificities Absent (reported by < 5% labs) | 5 | | | 2 | | |
| | Number of Specificities Present (reported by ≥ 75% labs) | 1 | | | 0 | | |
| | MFI >10000 | N/A | | N/A | N/A | | N/A |
| | MFI 5001 - 9999 | N/A | | N/A | N/A | | N/A |
| | MFI 2001-5000 | A25 | 33 | 79% | N/A | 33 | N/A |
| | MFI <2000 | N/A | | N/A | N/A | | N/A |

[1] 75% consensus 'present' specificities are displayed within the MFI value range reported by the **majority** of participants

Crossmatching Results
*(Based on 75% Consensus)*

| | CDC | | | | | | Flow Cytometry | |
|---|---|---|---|---|---|---|---|---|
| | **PBL** | | **T Cells** | | **B Cells** | | **T Cells** | **B Cells** |
| | **Without DTT** | **With DTT** | **Without DTT** | **With DTT** | **Without DTT** | **With DTT** | | |
| | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative |
| Number of reports | 7/7 | 8/8 | 13/13 | 12/12 | 11/11 | 12/12 | 29/29 | 27/27 |
| % Labs in consensus | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |

## Interpretation

Identification of Donor Specific Antibodies:

| Specificity | No of Participants (n=33) | MFI Range Reported |
|---|---|---|
| Cw1 | 4 (12%) | 1001-1767 |
| Cw9 | 1 (3%) | 1348 |
| DQ7 | 2 (6%) | 1186-1376 |
| DQ9▲ | 1 (3%) | 1076 |

▲Not donor specific

Crossmatch interpretation:

| | *The most common responses included:* |
|---|---|
| Interpretation based on results | • CDC crossmatch Negative<br>• Flow cytometry crossmatch Negative<br>• No donor specific antibodies present |
| Assigned risk | Low/Standard  n = 31  (94%)<br>Medium  n = 2  (6%) |
| Immunological advice | • Standard immunosuppression<br>• Regular post-transplant monitoring<br>• Ensure that no sensitising events alter the antibody status of the patient prior to transplant |
| If advice is not to transplant, recommendations for future transplants | • N/A<br>• Test if antibodies detected are capable of fixing complement<br>• Perform cellular crossmatches |

## EDXM 01 Serum 3/2021 Results

### HLA Antibody Detection and Definition
*(Based on 75% Consensus)*

| | | HLA Class I | No of labs | % consensus | HLA Class II | No of labs | % consensus |
|---|---|---|---|---|---|---|---|
| Detection | IgG | Positive | 29/31 | 94% | Negative | 29/31 | 94% |
| Detection | IgM | Negative | 1/1 | 100% | Positive | 1/1 | 100% |
| Definition[1] | Total Number of Specificities Reported (by ≥ 1 lab) | 84 | | | 44 | | |
| Definition[1] | Number of Specificities Absent (reported by < 5% labs) | 5 | | | 2 | | |
| Definition[1] | Number of Specificities Present (reported by ≥ 75% labs) | 2 | | | 0 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | MFI >10000 | N/A | | N/A | N/A | | N/A |
| | MFI 5001 - 9999 | B57 | 33 | 91% | N/A | 33 | N/A |
| | MFI 2001-5000 | B58 | | 91% | N/A | | N/A |
| | MFI <2000 | N/A | | N/A | N/A | | N/A |

[1] 75% consensus 'present' specificities are displayed within the MFI value range reported by the **majority** of participants

## Crossmatching
*(Based on 75% Consensus)*

| | CDC | | | | | | Flow Cytometry | |
|---|---|---|---|---|---|---|---|---|
| | PBL | | T Cells | | B Cells | | T Cells | B Cells |
| | Without DTT | With DTT | Without DTT | With DTT | Without DTT | With DTT | | |
| | *No Consensus* | *No Consensus* | Negative | Negative | *No Consensus* | *No Consensus* | Positive | Positive |
| Number of reports | *5/7 Neg* | *4/8* | 10/13 | 11/12 | *7/11 Neg* | *6/12* | 27/29 | 22/27 |
| % Labs in consensus | *71%* | *50%* | *77%* | *92%* | *64%* | *50%* | 93% | 81% |

## Interpretation

Identification of Donor Specific Antibodies:

| Specificity | No of Participants (n=33) | MFI Range Reported |
|---|---|---|
| Cw1 | 1 (3%) | 1048 |
| DQ5 | 1 (3%) | 14724 |

Crossmatch interpretation:

| | *The most common responses included:* |
|---|---|
| Interpretation based on results | • Negative CDC crossmatch<br>• Positive Flow cytometry crossmatch<br>• No DSAs detected but patient has detectable HLA antibodies<br>• Perform further testing e.g. autologous crossmatch, non-HLA, possible HLA antibody present not detected by single antigen bead panel<br>• HNA-3a antibody detected |
| Assigned risk | Low/standard    n = 18    (56%)<br>Medium    n = 5    (16%)<br>High    n = 7    (22%)<br>Contraindication    n = 2    (6%) |

| Immunological advice | • Further investigation of unexplained positive crossmatch result required including pharmacological interference, autologous antibodies and non-HLA antibodies<br>• Suitable for transplant with increased immunosuppression<br>• Discuss with clinical team |
|---|---|
| If advice is not to transplant, recommendations for future transplants | • Repeat testing<br>• Perform additional testing<br>• Investigate other transplant options<br>• Desensitisation<br>• Investigate patient disease |

## UK NEQAS Comments – Serum 3

This serum contained Human Neutrophil Antigen (HNA) 3a antibodies.

The female patient that supplied this sera has had 3 pregnancies and multiple blood transfusions. The HNA antibodies were identified through a strong positive flow cytometry crossmatch with a deceased donor kidney offer. At time of this offer no donor specific antibodies had been identified by Luminex Single Antigen Bead testing. The autologous flow cytometry crossmatch was also negative. The transplant did not proceed and a further two crossmatches with third party 'donor' cells were performed, which were also strong T & B cell positive in the absence of donor specific antibodies.

Samples were sent to the specialist reference laboratory in the UK for granulocyte immunology testing. The reference lab confirmed the patient's HNA type as HNA-3b/3b and the presence of HNA-3a antibodies.

HNA-3 is expressed on platelets, lymphocytes, endothelial, kidney, spleen and placental cells. Approximately 5% of our local population are HNA-3b/3b and can become sensitised through exposure to HNA-3a (95% of individuals).

There are limited published studies on the impact of HNA antibodies in transplantation.

A UK case series report of 7 patients found a high rate of antibody mediated rejection (AMR) (Key *et al.,* 2019). All patients were female, had an unexplained positive T and B cell flow cytometry crossmatch with no HLA donor specific antibodies. Retrospective testing identified they were all HNA-3b/3b with HNA3a antibodies. Two of the patients had graft failures at 10 and 12 months.

A further two cases from the UK were reported in an abstract for the EFI/BSHI conference held in 2021 (McConnell *et al.,* 2020). In these cases one of the patients who received a live donor transplant has a functioning graft and no AMR, but the other patient who was the recipient of a deceased donor kidney had antibody mediated rejection and poor graft function despite treatment with ATG and rituximab.

HNA antibodies are likely to be rare in transplant waiting list patients (Key *et al*, 2020 estimated to be approx. 1%), however, laboratories should be aware of the potential for these non-HLA antibodies to cause strong T and B cell flow cytometry crossmatches. UK NEQAS for H&I distributed this serum to highlight this, especially for laboratories that may not have previously seen sera containing HNA-3a antibodies. It was interesting to note that the majority of laboratories (but not all) reported a positive flow cytometry crossmatch in the absence of donor specific antibodies. However, there was wide variation in the clinical risk associated with these results.

Key T, Carter V, Day S, Goodwin J,  Goodwin P,  Knight A, Mather F, Poles A, Shaw O, Rigg K, McKane W Human Neutrophil Antibodies are Associated with Early and Chronic Antibody Mediated Rejection in Kidney Transplant Recipients.2019, J Renal Transplant Sci, 2(2), 81.  https://www.scitcentral.com/documents/16f32d2225ef8c1ca869bf29397171b4.pdf

McConnell S, Battle R, Henderson L, Calvert C, Sellers F, Poles A, Turner D. Unexpected positive flow crossmatches due to the presence of HNA-3a antibodies. 2020. HLA, 95(4), 346.  https://onlinelibrary.wiley.com/doi/10.1111/tan.13844