UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

# H&I Laboratory Results and Clinical Interpretation for a Sample with HNA Antibodies

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## Introduction

NEQAS provide an educational exercise to enable laboratories to compare results and clinical interpretations from multiple assays. Labs are asked to perform crossmatching, HLA typing and antibody detection/specification. The exercise is offered to participants annually with the aim of mimicking a renal transplant scenario.

There was one distribution of samples for this exercise in 2021 which comprised of one 'donor' blood sample and three 'patient' serum samples.

## Methods

Laboratories were asked to perform the tests they routinely carried out in a live unrelated donor kidney transplant setting:

- HLA typing
- Antibody detection/specification
- Crossmatching

Participants were requested to list donor specific antibodies (DSAs) and to provide a clinical interpretation of the results envisaging that the three serum samples were from three different renal 'patients' who were all ABO compatible with the 'donor'.

42 labs participated in the 2021 distribution, 16 of which were based in the UK and Ireland (UK&I) and 26 from labs based in the rest of the World (RoW). Please note that not all users reported results on every aspect of the Scheme.

One serum used in the scheme contained Human Neutrophil Antigen (HNA) 3a antibodies. The female patient that supplied this sera had 3 pregnancies and multiple blood transfusions. The HNA antibody was identified after a strong positive flow cytometry crossmatch (FCXM) was noted whilst testing the patient for compatibility against a deceased donor kidney offer. At time of this offer no donor specific antibodies had been identified by Luminex Single Antigen Bead (SAB) testing. The autologous FXCM 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.

## Results

Of the labs testing this sera in the educational exercise, 94% (29/31) concluded there were Class I IgG antibodies present with 7/89 Class I antibodies reported positive by more than one lab. Two antibody specificities reached consensus (B57 91% and B58 91%). 94% of labs detected no IgG Class II antibodies, see Table 1.

A positive T cell FCXM result was reported by 93% (27/29) labs and 81% (22/27) reported B cell FCXM positive.

Complement Dependent XM (CDCXM) results were inconsistent. There was no consensus in the B cell CDCXM (64% negative pre-DTT, 50% negative post-DTT), T cell CDCXM was negative (77% pre-DTT, 92% post-DTT) and the peripheral blood lymphocyte CDCXM also had no consensus pre-DTT (71% neg) or post-DTT (50% neg). Only 1/33 labs (3%) reported HLA DSAs to HLA-Cw1 and DQ5 in this sample.

Labs were asked to provide an interpretation of this results. Comments included:

- Negative CDCXM
- Positive FCXM
- No detectable DSA but the patient does have HLA antibodies
- Possible HNA-3a antibody present
- Requires further investigation

9/33 (28%) labs classed this as a high risk/contraindication to transplant, 5/33 (16%) classed it as intermediate risk and 18/33 (56%) classed it as standard risk. Interestingly, the BSHI BTS Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation (2014) indicate that this would be classed as a standard risk transplant as reactivity is most likely due to non-HLA antibodies.

When participants were asked what their immunological advice would be they suggested performing further testing such as repeating the crossmatch and antibody screening, HNA genotyping, performing an autologous crossmatch, requesting further information on the patient such as disease status, sensitisation events and possible pharmacological interference, screening for non-HLA antibodies.

Some labs advised that if the transplant proceeded then antibody removal, enhanced immunosuppression and close post-transplant monitoring should be considered.

If labs would not advise proceeding to transplantation with this 'donor' they were asked to offer recommendations to aid future transplantation. Suggestions included considering alternative donor sources such as a kidney sharing scheme and antibody removal.

## Discussion

HNA is found 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.

There are limited published studies on the impact of HNA antibodies in transplantation although antibody mediated rejection and early graft loss were noted in a series of 7 UK case reports (Key et al., 2019). All patients were female, had an unexplained positive T and B cell FCXM with no HLA DSA defined. 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, also 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-3a antibodies are likely rare in transplant waiting list patients (Key et al., 2020 estimated 1%), but patients who develop them will be highly sensitised (approx. 95% of donors express HNA-3a).

It was interesting to note that the majority of laboratories reported a positive FCXM in the absence of HLA DSA. However, there was wide variation in the clinical risk associated with these results. It is not current practice to perform HNA typing/antibody screening; some labs suspected HNA-3a antibodies from the laboratory XM results, but they cannot be defined without additional specialist testing. HNA-3a antibodies will not be detected in a virtual XM.

Laboratories should be aware of the potential for these non-HLA antibodies to cause a strong positive XM in the absence of HLA DSAs, which may be associated with poorer transplant outcome. NEQAS distributed this serum to highlight this issue, especially for laboratories that may not have previously seen sera containing HNA-3a antibodies.

| RESULTS | CDCXM | | | | | | FCXM | |
|---|---|---|---|---|---|---|---|---|
| | PBL | | T-Cells | | B-Cells | | | |
| | Without-DTT | With-DTT | Without-DTT | With-DTT | Without-DTT | With-DTT | T-Cells | B-Cells |
| Consensus | *None* | *None* | Negative | Negative | *None* | *None* | Positive | Positive |
| Number Positive | 2 (29%) | 4 (50%) | 3 (23%) | 1 (8%) | 4 (36%) | 6 (50%) | 27 (93%) | 22 (81%) |
| Number Negative | 5 (71%) | 4 (50%) | 10 (77%) | 11 (92%) | 7 (64%) | 6 (50%) | 2 (7%) | 5 (19%) |
| HLA IgG Antibodies Present | **Class I** | Yes (94%) | | | **Class II** | No (94%) | | |
| Risk | **Contraindication/High** | 9 (28%) | | **Intermediate** | | 5 (16%) | **Standard** | 18 (56%) |

**Table 1: Summary of Crossmatch Results, HLA Antibody Detection and Interpreted Immunological Risk**

## Further Information

Full information on all UK NEQAS for H&I schemes is available at www.neqashandi.org.uk or contact **ukneqashandi@wales.nhs.uk**

**References**

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.

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.

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