UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

UK NEQAS for H&I's Educational Scheme for combined HLA Typing, Antibody - Detection/Specification and Crossmatching: A Summary of Results from 2017-2018

Amy De'Ath & Deborah Pritchard

Introduction

To enable laboratories to compare results and clinical interpretation from multiple assays, an educational exercise is offered to participants annually with the aim of mimicking a renal transplant scenario.

There was 1 distribution of samples in 2017 and 2018. Each distribution comprised of one 'donor' blood sample and three 'patient' serum samples.

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

- HLA typing (PCR-SSP, PCR-SSO, SBT)
- Antibody detection/specification (Luminex, CDC, ELISA)
- Crossmatching (CDC with/without DTT, FCXM)

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'.

35 labs participated in the 2017 distribution and 32 in 2018, although not all users reported on every aspect.

2017 Results

***Serum 1**: sourced from a moderately sensitised parous female.*
Of the 32 labs that reported on antibody detection, 100% agreed that Class I and Class II IgG specific antibodies were present in this sample. One lab (3%) detected DSAs with an MFI of 480 (see Table 1).
The CDCXM and FCXM results were negative reflecting this lack of DSAs.
25/33 (76%) of labs classed this as a 'low risk' transplant based on the negative crossmatch results and lack of DSAs. The remaining 8/33 (24%) classed it as 'medium risk' as the patient is sensitised.

2017		Serum 1		Serum 2		Serum 3	
DSA Defined by Luminex		Class I	Class II	Class I	Class II	Class I	Class II
MFI >10,000							DQ2 (97%) DQA1*05 (18%)
MFI 5,000 - 9,999						B8 (58%)	
MFI 3,000 - 4,999							
MFI 1,500 - 2,999						A3 (49%) B7 (21%)	DQA1*01 (3%)
MFI <1,499		A32 (3%)				A32 (3%)	DR17 (15%)
CDCXM	No DTT	Negative		Negative		Positive	
	DTT	Negative		Negative		Negative	
FCXM	T Cell	Negative		Negative		Negative	
	B Cell	Negative		Negative		Positive	

Table 1: Summary of Crossmatch and Donor Specific Antibody Detection Results from 2017

***Serum 2**: sourced from a non-transfused female.*
Of the 32 labs 97% agreed there was no Class I IgG antibodies and 88% agreed that there was no IgG Class II antibodies in this sample (see Table 1).
The CDCXM and FCXM results were negative reflecting this lack of DSAs.
32/33 (97%) of labs classed this as a 'low risk' transplant as the patient is unsensitised.

***Serum 3**: sourced from a highly sensitised multi-parous female.*
Of the 32 labs 73% concluded there was Class I IgG antibodies and 100% detected IgG Class II antibodies in this sample.
The CDCXM results without DTT only reached consensus for B cells with 71% of labs agreeing the result was positive. With DTT the consensus result was negative for PBLs (75%) and T cells (93%) but no consensus was reached for B cells. The FCXM results were T cell negative (77% consensus) and B cell positive (77% consensus).
Labs detected multiple DSAs in this sample and 32/33 (97%) of participants detected a HLA-DQ2 DSA with the highest reported MFI at 19,337 (see Table 1).
18/32 (56%) of labs classed this as a contraindication to transplant based on the positive crossmatch results and presence of DSAs. The remaining 14/32 (44%) classed it as 'high or medium risk'.
In this case multiple DSAs were detected, some with an MFI of >10,000 resulting in a positive B cell FCXM but a negative CDCXM with DTT (in PBL and T cell only).

2018 Results

***Serum 1**: sourced from a sensitised parous female.*
Of the 31 labs 84% concluded there was Class I IgG antibodies and 100% detected Class II IgG antibodies in this sample.
CDCXM results were negative. The FCXM results did not reach consensus for T cells (65%) but was B cell positive (86% consensus).
Labs detected multiple DSAs in this sample and 28/29 (97%) of participants detected a HLA-DR4 DSA with the highest reported MFI at 17,567 (see Table 2).
7/29 (24%) of labs classed this as a contraindication to transplant based on the positive FCXM crossmatch result and presence of DSAs. The remaining 22/29 (76%) classed it as 'high or medium risk'.

2018		Serum 1		Serum 2		Serum 3	
DSA Defined by Luminex		Class I	Class II	Class I	Class II	Class I	Class II
MFI >10,000		Cw6 (90%)			DR7 (28%) DQ2 (28%) DQ8 (22%) DR53 (19%) DQA1*02:01 (8%) DQA1*03:01 (2%)		DQ8 (25%)
MFI 5,000 - 9,999			DR4 (97%)			A26 (9%)	
MFI 3,000-4,999				B45 (14%)	DPB1*01:01 (10%) DPB1*04:02 (16%)	B60 (15%) B45 (16%)	DPB1*04:02 (17%)
MFI 1,500 - 2,999			DR7 (14%) DQ8 (14%)		DPA1*01:03 (1%) DPA1*02:01 (1%)	A24 (14%)	DPA1*01:03 (1%) DPA1*02:01 (1%) DQA1*03:01 (1%) DQA1*02:01 (1%) DQ2 (1%)
MFI <1,499		A24 (14%)	DR53 (3%) DQA1*03 (3%)			Cw6 (2%) Cw10 (1%)	DR7 (2%) DR4 (1%)
CDCXM	No DTT	Negative		Positive		Negative	
	DTT	Negative		Negative (T cell) / Positive (B cell)		Negative	
FCXM	T Cell	Not Assessed		Negative		Not Assessed	
	B Cell	Positive		Positive		Not Assessed	

Table 2: Summary of Crossmatch and Donor Specific Antibody Detection Results from 2018

***Serum 2**: sourced from a highly sensitised multi-parous female.*
Of the 31 labs 100% concluded there was Class I and Class II IgG antibodies in this sample.
The CDCXM results without DTT were positive for PBLs (80%) and B cells (100%) but negative for T cells (93%). With DTT the consensus result was negative for T cells (92%) and positive for B cells (100%) but no consensus was reached for PBLs. The FCXM results were T cell negative (85% consensus) and B cell positive (100% consensus).
Labs detected multiple DSAs in this sample with 28/29 (97%) of participants detecting HLA-DR7 and DQ2 DSA with the highest reported MFI at 41,634 and 34,845 respectively (see Table 2).
20/29 (69%) of labs classed this as a contraindication to transplant based on the positive FCXM crossmatch result and presence of DSAs. The remaining 9/29 (31%) classed it as 'high or medium risk'.

***Serum 3**: sourced from a sensitised multi-parous female.*
Of the 31 labs 94% concluded there was Class I IgG antibodies and 100% detected IgG Class II antibodies in this sample.
CDCXM results were negative. The FCXM results did not reach consensus for T or B cells.
Labs detected multiple DSAs in this sample and 25/29 (86%) of participants detected an HLA-DQ8 DSA with the highest reported MFI at 16,477.
5/29 (17.5%) of labs classed this as a contraindication to transplant based on the FCXM crossmatch result and presence of DSAs. The remaining 19/29 (65%) classed it as 'high or medium risk' and 5/29 (17.5%) classed it as low risk.

Comment

This exercise has highlighted both concordances but also important variations between different laboratories.

DSAs with high MFI values have a noticeable affect on FCXM crossmatch results but seem to affect labs differently in terms of detection in the CDCXM which is evident by the lack of consensus achieved in some of the samples.

Laboratories must have a robust validation procedure so they can be confident the MFI values they produce offer an indication of both cytotoxic and flow cytometry crossmatch outcome.

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**.

GIG CYMRU NHS WALES
Ymddiriedolaeth GIG Felindre Velindre NHS Trust

WELSH TRANSPLANTATION AND IMMUNOGENETICS LABORATORY

Welsh Blood Service
Gwasanaeth Gwaed Cymru