UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

Variation in HLA Antibody Detection and Specification Results between Kit Manufacturers in External Quality Assessment Schemes

Deborah Pritchard, Tracey Rees
UK NEQAS for H&I, Welsh Transplantation Laboratory, Welsh Blood Service, UK

Introduction

UK NEQAS for H&I Scheme 6 (HLA antibody detection) and Scheme 3 (HLA antibody specification) results from 2018 were analysed to determine any differences reported by users of Immucor (IC) kits and One Lambda (OL) kits. Labs using kits from both providers or with no kit information were excluded from the analysis.

HLA Antibody Detection

Twelve samples distributed for HLA antibody detection were analysed. Labs reported a positive/negative result for the presence of Class I and Class II antibodies. Results reported by over 74% of kit users were considered an agreed positive/negative assignment, while those reported by less than 74% was classed as no consensus.

24 labs used kits from IC and 38 from OL. The same positive/negative/no-consensus assignment between IC and OL kit users was obtained for 9/12 Class I and 11/12 Class II samples (Table 1).

The discrepancies between the kits included;
- Sample 608: All OL kit users reported Class I positive, but only 29.2% of IC kit users reported this sample as positive.
- Sample 611: 87.5% of IC users reported Class I negative, while there was no consensus reached with OL kit users (54.3% negative).
- Sample 612: 91.7% of IC users reported Class I negative, while there was no consensus reached with OL kit users (63.9% negative). Furthermore, for Class II, 87% of IC users reported negative, while 75% of OL users reported positive.

Interestingly the discrepant assignments involved 2/3 samples from non-transfused male blood donors (samples 604, 611, 612). Unfortunately Single Antigen Bead results were not reported to allow the differences to be investigated further.

Table 1: Comparison of HLA antibody detection results between kits

Sample	Class I				Class II			
	Immucor (n=24)	% Positive	One Lambda (n=38)	% Positive	Immucor (n=24)	% Positive	One Lambda (n=38)	% Positive
601	Positive	96.0%	Positive	94.7%	Positive	100.0%	Positive	97.4%
602	Negative	8.0%	Negative	0.0%	Positive	95.8%	Positive	100.0%
603	Negative	4.0%	Negative	13.5%	Negative	4.2%	Negative	8.1%
*604	No Consensus	44.0%	No Consensus	48.6%	Positive	100.0%	Positive	100.0%
605	Positive	100.0%	Positive	100.0%	Positive	100.0%	Positive	100.0%
606	Positive	96.0%	Positive	97.4%	No Consensus	45.8%	No Consensus	35.1%
607	Positive	95.8%	Positive	100.0%	Negative	0.0%	Negative	0.0%
608	No Consensus	29.2%	Positive	100.0%	Positive	100.0%	Positive	100.0%
609	Positive	100.0%	Positive	100.0%	Positive	100.0%	Positive	100.0%
610	Positive	100.0%	Positive	100.0%	Positive	100.0%	Positive	100.0%
*611	Negative	12.5%	No Consensus	45.7%	Positive	100.0%	Positive	100.0%
*612	Negative	8.3%	No Consensus	36.1%	Negative	13.0%	Positive	75.0%

* Sample sourced from non-transfused male blood donor
Red font indicates difference in assignment between Immucor and One Lambda kit users

In Summary:
- Separate analysis of kits found the same HLA antibody detection assignment in 9/12 samples (3 Class I and 1 Class II differences)
- When differences occurred, a higher proportion of OL kit users reported positive results compared to IC kit users.

HLA Antibody Specification

Nine samples were analysed for HLA antibody specification results. Users were asked to report HLA-A, B, Cw, DR, DQB antibodies. Users of IC kits only (n=16) and OL kits only (n=40) were separated for analysis. Each reported antibody specificity was assigned according to the following criteria:
- If ≥75% of kit users reported a specificity = Present
- If 0-4% of kit users reported a specificity = Absent
- If 5-74% of kit users reported the specificity = No Consensus

Figure 1: Comparison of HLA antibody specification results between kits
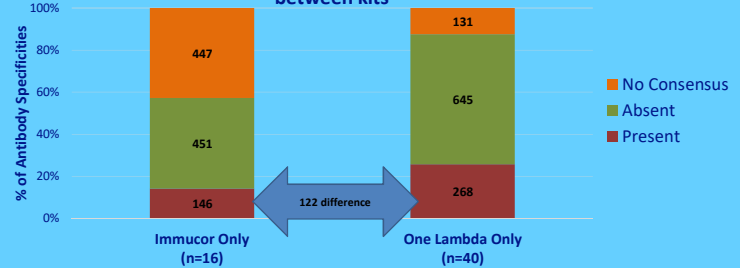
From the 9 samples analysed, 146 specificities were assigned as present by IC users, compared to 268 specificities by OL users (Figure 1). OL kit users also agreed more frequently on the absence of a specificity (645 v 451). The majority of differences in antibodies assigned as 'present' between the 2 kits (117/122) were due to Class I antibodies. The trend for OL users to agree on the presence of HLA antibodies more frequently than IC kit users was apparent across all 9 samples (range 5-29).
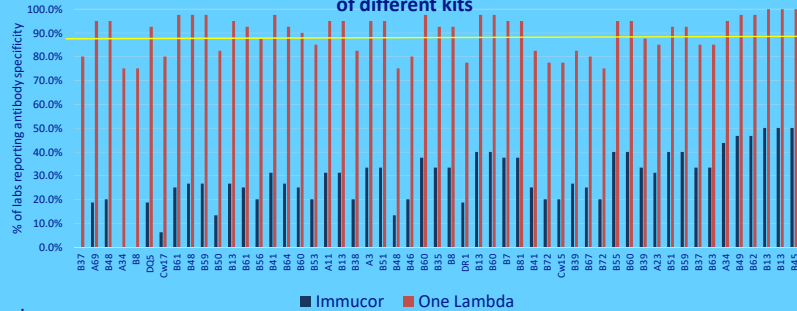
Some specificities were reported as 'present' in multiple samples by OL kit users, but not IC kit users (Table 2).

Table 2: Specificities assigned as 'present' in multiple samples by OL kit users, but not IC kit users

Number of Samples	Antibody Specificities
5	B13, B41
4	B60, B61
3	A43, B39, B44, B45, B48, B51, B59, B67, B7, B71, B72, B8, B81
2	A23, A34, B18, B35, B37, B38, B42, B47, B49, B50, B52, B54, B55, B56, B58, B62, B63, B64, B75, B76, B78

53/122 specificities had over 50% detection difference between OL & IC kit users (Figure 2), with 3 of these reported by >74% of OL labs, but by 0 IC users (B37, A34, B8); however reported OL MFI values for these 3 antibodies were generally under 2000 MFI.

Figure 2: Specificities with >50% difference in detection between users of different kits

In summary;
- OL kit users had more specificities classed as 'present' and 'absent' compared to IC kit users. This could indicate more consistent reporting between OL kit users.
- There were a high number of 'no consensus' specificities for IC kit users. This could be linked to the smaller number of labs reporting using IC kits only.
- Immucor kits users reported antibodies classed as 'present' by OL kits in all but 3 cases.

Comment

This analysis shows differences in EQA reporting dependant on the kit provider. OL users reported more positive detection results/specificities than IC users. The majority of differences were Class I. OL user reports were more consistent and reached an agreed result more often than IC user results; however there were fewer IC users. Only 3 specificities were exclusively detected by OL users, indicating IC kits are capable of producing the same antibody profile as OL kits.

Ymddiriedolaeth GIG Prifysgol Felindre
Velindre University NHS Trust

WELSH TRANSPLANTATION AND IMMUNOGENETICS LABORATORY

Gwasanaeth Gwaed Cymru
Welsh Blood Service