UK NEQAS
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Histocompatibility & Immunogenetics

Are HLA antibodies present? An analysis of results not meeting consensus in UK NEQAS for H&I's HLA Antibody Detection Scheme

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Introduction

Testing for the presence of HLA Class I or Class II antibodies is crucial for the support of solid organ, haematopoietic stem cell transplantation, provision of blood products or the investigation of certain clinical conditions, e.g. thrombocytopaenia.

Many laboratories undertake these services using a two-tier system. The first stage – antibody detection determines which sera should be selected for antibody specificity assignment. The correct antibody detection is therefore important since failure to detect the presence of antibodies during "screening" will deny the sample the benefit of the more comprehensive testing associated with antibody specification.

The UK NEQAS for H&I's Scheme 6 assesses participants' ability to detect HLA Class I/Class II antibodies. 20 (2012-2016) or 12 (2017) undisclosed serum samples were distributed to participants each year. Sera were assigned as positive/negative for Class I/Class II antibodies if ≥ 75% of reports were in agreement.

UK NEQAS for H&I have operated an antibody detection EQA scheme since 2001. Here we report the results of testing from 2012-2017.

EQA Scheme Findings

Between 2012-2017 there were 56-98 participants (24-25 UK & Ireland). All but 4 labs tested using Luminex.

From the 112 samples distributed between 2012-2017, 14 results did not reach consensus (Table 1); 11 Class I and 3 Class II. 5 of these occurred in 2017 compared to a maximum of 2 for other years. The percentage agreement on the non-consensus results varied from 63.5%-74% for Class I, with 10/11 results favouring positive. For Class II this was 55.4%-70.7% all favouring negative.

Table 1: Samples not reaching 75% consensus 2012-2017

Sample	Class	% consensus All Labs	% consensus UK&I Labs	Sample Origin
619/2012	Class I	73.2% Neg	64.0% Neg	Multiparous female
601/2013	Class I	71.2% Pos	88.0% Pos	Multiparous female
607/2013	Class I	72.7% Pos	96.0% Pos	Multiparous female
615/2013	Class I	73.5% Pos	96.0% Pos	Non-transfused Male
609/2014	Class I	74.0% Pos	92.0% Pos	Multiparous female
602/2015	Class I	72.0% Pos	95.8% Pos	Multiparous female
617/2015	Class I	66.0% Pos	95.8% Pos	Multiparous female
609/2016	Class I	72.0% Pos	100% Pos	Multiparous female
616/2016	Class I	72.3% Pos	100% Pos	Multiparous female
602/2017	Class II	55.4% Neg	62.5% Neg	Multiparous female
605/2017	Class I	70.1% Pos	95.8% Pos	Multiparous female
606/2017	Class II	70.7% Neg	91.7% Neg	Non-transfused Male
608/2017	Class I	63.5% Neg	79.2% Neg	Non-transfused Male
612/2017	Class II	70.2% Neg	87.5% Neg	Non-transfused Male

Affected samples were from multiparous female donors and non-transfused male blood donors. For 12/14 non-consensus results, consensus would have been reached when just considering results from UK&I labs.

Non-Consensus Results

The five 2017 non-consensus results were re-analysed by kit manufacturer (Table 2). For labs reporting using One Lambda kits only (n=52), four out of the five results reached consensus. For Immucor only users (n=31), two out of the five results reached consensus. However, for all five results the consensus/majority result differed between the manufacturers (Table 2). For example, the Class I consensus result for sample 608/2017 for One Lambda was 88.9% negative and for Immuncor was 80% positive.

Table 2: Difference in HLA antibody detection results when considering results by kit manufacturer

Sample	% Consensus		
	All Labs (n=98)	One Lambda (n=52)	Immucor (n=31)
602/2017 Class II	55.4% (Neg)	60.4% (Pos)	75.9% (Neg)
605/2017 Class I	70.1% (Pos)	96.3% (Pos)	66.7% (Neg)
606/2017 Class II	70.7% (Neg)	94.3% (Neg)	64.5% (Pos)
608/2017 Class I	63.5% (Pos)	88.9% (Neg)	80.0% (Pos)
612/2017 Class II	70.2% (Neg)	87.7% (Neg)	69.0% (Pos)

Mixed kits have an 'undetermined' region but the Scheme requires a positive or negative result. Therefore labs may have tested using additional kits.

Indeed, when the types of Luminex kit used by labs to test the 2017 samples were analysed, 25% used ID/SAB kits which may be considered 'more sensitive' than Mixed bead kits and could have contributed to differences in positive/negative reporting.

	Class I		Class II	
	False Pos	False Neg	False Pos	False Neg
2014	6	5	4	2
2015	20	2	17	3
2016	29	3	10	8
2017	5	0	2	2

Table 3: False Pos/Neg Reporting by UK&I Labs

Table 3 shows a comparison of false negative and positive reporting by labs. It is interesting to note a higher proportion of false positive than negative results are reported annually.

Table 4 shows unsatisfactory performance within UK&I labs between 2012-2017, after a period of concern in 2015/16 unacceptable performance has returned to 0% in 2017.

Table 4: Unsatisfactory Performance	2012	2013	2014	2015	2016	2017
Number of Participants (UK&I)	25	25	25	24	24	24
Number with UP (<80%)	0	0	1	3	4	0
% Unsatisfactory Performance	**0**	**0**	**4**	**12.5**	**16.6**	**0**

Comment

The reason consensus is not reached may be due to samples containing marginal antibodies, differences between manufacturer kits, kit sensitivity, or differences in lab interpretation of results into a positive/negative report.

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