UK NEQAS

Histocompatibility & Immunogenetics

Annual Report
2016

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1. INTRODUCTION

Time moves on, it is already 2017, but they say some things improve with age. I hope you will agree that this applies to the service we provide at UK NEQAS for H&I. We have introduced a number of changes during the year as part of our strategic plan, following participant feedback and an ongoing commitment to service improvement.

Scheme Result Forms

One of the changes you will have noticed is our move into electronic results entry. We are trialling this using 'Google Forms' software whilst we design and procure a more comprehensive IT system. This new system will extend functionality to our participants including on-line registration and access to individual reports. We hope you will support in this trial as it will help in the development of our new system.

Correspondence

Participants' feedback is pivotal in enabling us to design schemes that are relevant, educational representative of clinical practice. Thank you to those of you who completed our recent on-line survey. As a result of your comments throughout the year we have made several changes for next year. Some of which I have briefly summarised below.

Scheme method/technique data collection

To support laboratories in assuring quality throughout the year we have looked at our distribution time table and made a few changes to provide a more even distribution of samples. We have also amended the deadlines for reporting results to make them more aligned to the requirements in the clinical setting.

The assessment for the crossmatching schemes have been amended so consensus and scoring for individual cell types will be performed. Reporting for antibody specificity will allow for DQA, DPA and DPB and participants are invited to indicate on the basis of their results which antigens they would consider unacceptable for a standard risk deceased kidney donor. These will not be assessed but will provide excellent data for comparison across laboratories.

Our two pilot schemes for HPA and KIR will become fully fledged schemes in 2017 and two new pilot schemes will be launched. One pilot is for HPA antibody for detection/specification and another for allelic level HLA genotyping.

Steering Committee

I am delighted to report that UK NEQAS for H&I successfully transitioned from CPA to ISO 17043 in 2016. A big thank you to Deb Pritchard the Scheme Manager and Felicity May seconded to UK NEQAS during Deb's maternity leave for the hard work that went into the preparation for this. I would also like to thank the Steering Committee they provide their insight and expert advice. Sadly we say goodbye to Jeanette Ayres and Leigh Keen both of who have committed their time and support for the last six years and welcome John Smith and Arthi Anand.

I am sure 2017 will be just as challenging and exciting. Thank you to all our participants for your support in enabling us to provide a quality service.

NUMBER OF PARTICIPANTS DURING 2016

The number of Schemes' participants varies slightly during the course of any one year. However, the following table shows the approximate figures for each Scheme for 2016.

Scheme	UK and ROI	RoW
Scheme 1A	7	34
Scheme 1B	54	71
Scheme 2A	21	55
Scheme 2B	23	53
Scheme 3	25	60
Scheme 4A1	29	73
Scheme 4A2	21	42
Scheme 4B	4	3
Scheme 5A	47	11
Scheme 5B	17	2
Scheme 6	24	74
Scheme 7	25	37
Scheme 8	8	31
Educational Scheme Samples	22	26
iED Scenario 1	17	33
iED Scenario 2	18	27
iED Scenario 3	13	10
Educational Crossmatch	13	9
Pilot HPA Genotyping	4	9
Pilot KIR Genotyping	3	9
Pilot MICA Genotyping	1	1

ROI – Republic of Ireland
RoW – Rest of the World
iED – Interpretive Educational

Dr Tracey Rees, UK NEQAS for H&I Director

2. OBSERVATIONS ON THE SCHEMES

SCHEME 1A: HLA PHENOTYPING

The purpose of this scheme is to assess the ability to use serological and supplementary methods to correctly identify HLA specificities. Participants can register for HLA-A, B, C, DRB1, DQB1 typing or any combination. Two random donor samples are sent 5 times in a year giving a total of 10 samples for HLA typing. Participation in the scheme in 2016 is summarised in table 1 below:

Table 1: Scheme 1A Participation

HLA	Participants (n=41)
A	41
B	41
Cw	7
DR	29
DQ	27

Assessment

Scoring of HLA type is based on 75% consensus. Each complete HLA type in agreement with the consensus phenotype is deemed acceptable. Each complete HLA type not in agreement with the consensus phenotype is deemed unacceptable. Satisfactory performance is obtaining nine or more complete HLA types in agreement with consensus in a calendar year.

Methods: Typing trays used

Participants used a range of typing trays from a variety of manufacturers: One lambda, Biotest BioRad, Innotrain and a number of labs used a combination of manufacturers (Figure 1)

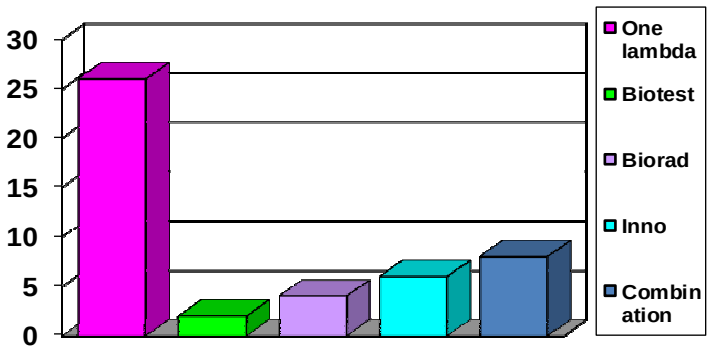


Figure 1: Manufacturers of Typing Trays

Table 2: Incorrect assignments in 2016

Sample	Report	Consensus
1A01	DQ1 DQ7	DQ5 DQ7
1A02	DQ7 DQ3	DQ7 DQ8
	DQ3 DQ8	DQ7 DQ8
1A03	B8 B71	B8 B35
1A04	DQ3 DQ2	DQ2 DQ8
1A05	A2 -	A2 A68
1A06	DRB1*11:01 DQB1* 06:02 DQB1*03:01	DR11 DQ6 DQ7
	B71 B7	B7 B35
1A09	B*4022N	B44 B60
	DR DQ NOT TESTED	DR7 DR13 DQ2 DQ6
1A10	DQ1 DQ3	DQ5 DQ9
	DR DQ NOT TESTED	DR7 DR103 DQ5 DQ9

Performance in 2016

Incorrect assignments (table 2) fell into 4 categories:

i Failure to split a broad specificity
ii Missed specificities
iii Incorrect specificities
iv Incorrect nomenclature

There were 3 unsatisfactory performers in 2016 (table 3).

Table 3: Scheme 1A Unsatisfactory Performance

	2012	2013	2014	2015	2016
Number of Participants (UK&I)	22 (10)	30 (10)	42 (9)	45 (9)	41 (7)
Number with Unsatisfactory Performance (< 90%) (UK&I)	1 (0)	0 (0)	8 (0)	4 (0)	3 (0)
% Unsatisfactory Performance	4.5%	0.0%	19%	8.9%	7.3%

Dr Leigh Keen, Histocompatibility & Immunogenetics Department, NHSBT, Filton

SCHEME 1B: HLA-B27 TESTING

The purpose of this scheme is to assess ability to correctly determine HLA-B27 status. Participants in this scheme are asked to report results as HLA-B27 positive or HLA-B27 negative. Two random donor samples are sent 5 times a year (5 cycles) giving a total of 10 samples for analysis. HLA-B27 status is determined by at least 75% agreement on the presence or absence of HLA-B27.

In 2016 there were 123 participants in the scheme (54 UK&I laboratories).

Assessment

A result in agreement with the consensus HLA-B27 status is deemed acceptable and a result not in agreement with the consensus HLA-B27 status is deemed unacceptable. Satisfactory performance is making 10 sample reports in agreement with consensus in a calendar year.

Methods: Technique

Participants used a variety of techniques for HLA-B27 typing (figure 1), with the most common techniques being PCR-SSP or flow cytometry. Some laboratories used techniques in combination.

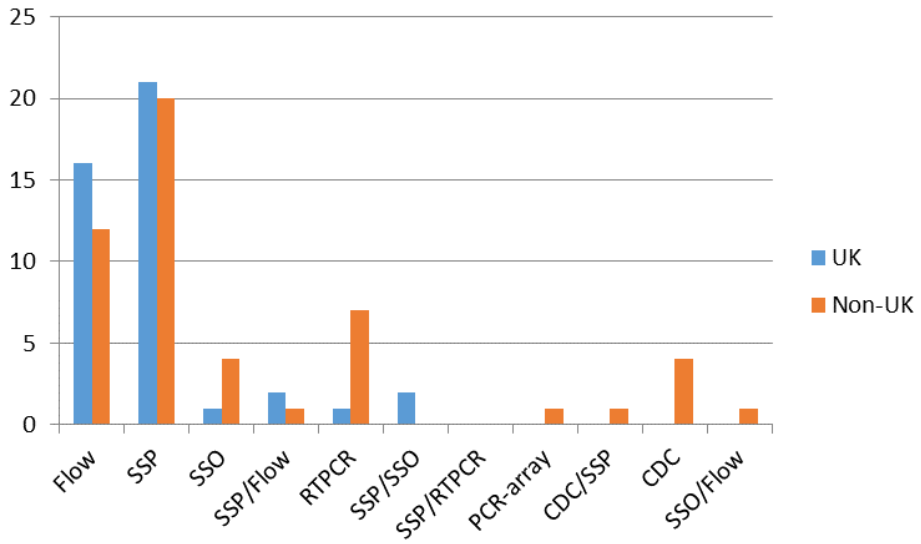


Figure 1: Techniques used for HLA-B27 testing

Methods: Monoclonal antibodies (B27 detection)

A variety of monoclonal antibodies were used for detection of HLA-B27, with 3 main suppliers (Becton Dickinson, One Lambda and Beckman Coulter)

Performance

In 2016, three samples distributed were HLA-B27 positive. Thirteen assignments were made outside of consensus (see table 1). Three misassignments were made by laboratories using flow cytometry, four by PCR-SSP and one by cytotoxicity. There were therefore 11 unacceptable performers in 2016, 9% of the participants (Table 2).

One sample (1B09) did not reach consensus in 2016, therefore only 9 samples were assessed. The sample was HLA-B*27:12, which 43/115 labs reporting the sample as negative for HLA-B27.

Table 1: Scheme 1B Incorrect Assignments

Sample	Result	Lab Number	Technique	HLA-B Type
1B03	False Pos	72	PCR-SSP	B7, B18
1B04	False Neg	71, 90	Flow	B27, B62
1B05	False Neg	93, 110, 307, 325	Flow PCR-SSP	B39, B27
1B06	False Pos	14, 307, 325	PCR-SSP	B47, B18
1B10	False Pos	112 256 292	PCR-SSP Flow Cytoxicity	B7, B61

Table 2: Scheme 1B Unsatisfactory Performance

	2013	2014	2015	2016
Number of Participants	96	107	115	123
Number with Unacceptable Performance (< 100%)	4	4	8	11
% Unacceptable Performance	4.2%	3.7%	6.9%	9%

Ruhena Sergeant, Clinical Immunology Laboratory, Hammersmith Hospital, London

SCHEME 2A: CYTOTOXIC CROSSMATCHING

Introduction: The purpose of Scheme 2A is to assess the participants' ability to correctly determine cell/serum cytotoxic crossmatch status. Participants may register to test peripheral blood lymphocytes (PBL)/T-cells and/or B-cells, with and/or without dithiothreitol (DTT) treatment of sera. A total of ten blood samples and forty serum samples were sent as five distributions. To ensure enough material is received for testing, three different blood units are distributed to groups of participants.

Table 1 Scheme 2A Participants in 2016

	Number of Participants n=77			
	UK & Ireland	Rest of the Word Whole Blood	Rest of the Word Isolated lymphocytes	Total
PBL/T & B	20	16	28	64
PBL/T	1	3	9	13
PBL/T & B +DTT	17	14	24	55
PBL/T +DTT	1	1	7	9

2PBL/T cell methodology: 45/77 (58%) of laboratories used beaded cells compared to 62% last year. There were 16 different combinations of pre- and post-complement incubation times. These ranged from 20 to 60 minutes pre-complement with the majority of UK laboratories using 45 minutes. The Post-complement incubation times varied from 30 to 120 minutes with the majority of UK laboratories using 90 minutes. Laboratories used complement from 14 different sources. EB/AO remains the most popular method for visualisation.

B cell methodology: 56/63 (89%) of laboratories used beaded cells compared to 98% last year. There were 17 different combinations of pre- and post-complement incubation times. These ranged from 20 to 60 minutes pre-complement with the majority of UK laboratories using 60 minutes. The Post-complement incubation times varied from 30 to 120 minutes with the majority of UK laboratories using 60 minutes.

Assessment and Performance: The status or result for each cell/serum combination was determined by 75% consensus between laboratories. A result in agreement with consensus was "acceptable" one not in agreement with consensus was "unacceptable". Satisfactory performance within this scheme was 85% of all reports in agreement with the consensus results within the calendar year.
The performance results without DTT are shown in Table 2. The performance results with DTT are shown in Table 3.

Table 2: Scheme 2A 2016 Performance without DTT

		UK&I	RoW WB	RoW IL
Number of Participants		21	19	37
Number XM Reports Assessed (Combined PBL/T & B Cell XM = 1 report)		35/40	38/40	38/40
PBL/T Cell	No XM Assessed	32/40	36/40	33/40
	Positive XM	9	6	1
	Negative XM	23	30	32
	% of Results Not Tested	5.4%	8.0%	14.7%
B Cell	No XM Assessed	27/40	29/40	33/40
	Positive XM	16	13	12
	Negative XM	11	16	21
	% of Results Not Tested	11.1%	13.9%	19.7
Incorrect assignments		64 (4.2%)	46 (3.7%)	99 (4.6%)
	False Positive	42	26	66
	False Negative	22	20	33
Unsatisfactory Performance		3 (71-84%)	2 (76, 82%)	9 (43-84%)

Table 3: Scheme 2A 2016 Performance With DTT

		UK&I	RoW WB	RoW IL
Number of Participants		18	15	31
Number of XM Reports Assessed (Combined PBL/T & B Cell XM = 1 report)		37 /40	38/40	38/40
PBL/T Cell	No of XM Assessed	32	36	33
	Positive XM	7	6	1
	Negative XM	35	30	32
	% of Results Not Tested	9.0%	2.5%	15.6%
B Cell	No of XM Assessed	34	33	34
	Positive XM	15	12	11
	Negative XM	19	21	23
	% of Results Not Tested	13.4%	17.0%	20.8%
Incorrect assignments		63 (5.1%)	49 (4.3%)	49 (2.7%)
	False Positive	27	17	37
	False Negative	36	32	12
Unsatisfactory Performance		6 (75-83%)	3 (71%-79%)	4 (42%-77%)

Satisfactory Performance: The overall performance of laboratories in scheme 2A is shown in table 4**.** 63/77 (82%) of laboratories achieved a satisfactory performance without DTT in 2016 compared to 61/77 (79%) in 2015. 51/64 (80%) of laboratories achieved a satisfactory performance with DTT in 2016 compared to 55/64 (86%) in 2015.
6 laboratories had unsatisfactory performance without and with DTT in 2016. 2 of these were UK laboratories.

Table 4: Scheme 2A Performance 2015-2016

(PBL/T Cells & B Cells)	Without DTT		With DTT	
	2015	2016	2015	2016
Number of Participants (UK&I)	**77 (21)**	**77 (21)**	**64 (18)**	**64 (18)**
Number with Unsatisfactory Performance (< 85%) (UK&I)	**16 (2)**	**14 (3)**	**9 (0)**	**13 (6)**
% Unsatisfactory Performance (UK&I)	**20.8% (9.5%)**	**18.2% (14.3%)**	**14.0% (0%)**	**20.3% (33.3%)**

Patrick Flynn, The Transplantation Laboratory, Manchester Royal Infirmary

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY

The purpose of this scheme was to assess participant's ability to correctly determine cell/serum flow cytometry crossmatch status.

The 2016 scheme consisted of 5 distributions of 2 blood samples plus 4 test sera per sample giving a total of 10 blood samples and 40 sera. Participants were able to register for assessment of the T cell crossmatch only or both the T cell and B cell crossmatch.

Participants were asked to assess the reactivity of a serum against a particular cell in relation to the local AB negative control serum and report the crossmatch as either positive or negative. The consensus crossmatch status of each sample was determined by at least 75% of laboratories agreeing on the positivity or negativity of each test; crossmatching tests failing to reach the 75% consensus level were not assessed. For 2016 T-cell & B-cell assessment was linked such that both results were required to be in line with the consensus results for acceptable performance.

To achieve a satisfactory performance, participants had to obtain 85% of reports on all sera in agreement with the consensus findings in the calendar year.

Performance
The majority of participants achieved a satisfactory performance, however there were 13 laboratories with unsatisfactory performance (Tables 1-3, figure 1). Please note that UK&I and RoW receive different blood samples to ensure enough cells for testing.

Table 1: Scheme 2B T Cell Result Summary

Scheme 2B T Cell Results	UK&I	RoW
Number of participants	23	51-53
Number of XM assessed (>75% consensus)	34/40	35/40
Number of Positive XM	15	12
Number of Negative XM	19	23
Number of incorrect assignments	10 (1.3%)	78 (4.2%)
Number of False Pos	3	51
Number of False Neg	7	27
Number of equivocal assignments	65	170
Number of Unacceptable Performers (< 85% correct)	1	12

Table 2: Scheme 2B B Cell Result Summary

Scheme 2B B Cell Results	UK&I	RoW
Number of participants	20	48-50
Number of XM assessed (>75% consensus)	33/40	31/40
Number of Positive XM	20	15
Number of Negative XM	13	16
Number of incorrect assignments	30 (4.5%)	108 (7%)
Number of False Pos	21	53
Number of False Neg	9	55
Number of equivocal assignments	65	235

Changes to Scheme 2B for 2017

Scheme assessment will be performed separately for T-cells and B-cells. For each cell type registered for participants must achieve >85% correct for acceptable scheme performance.

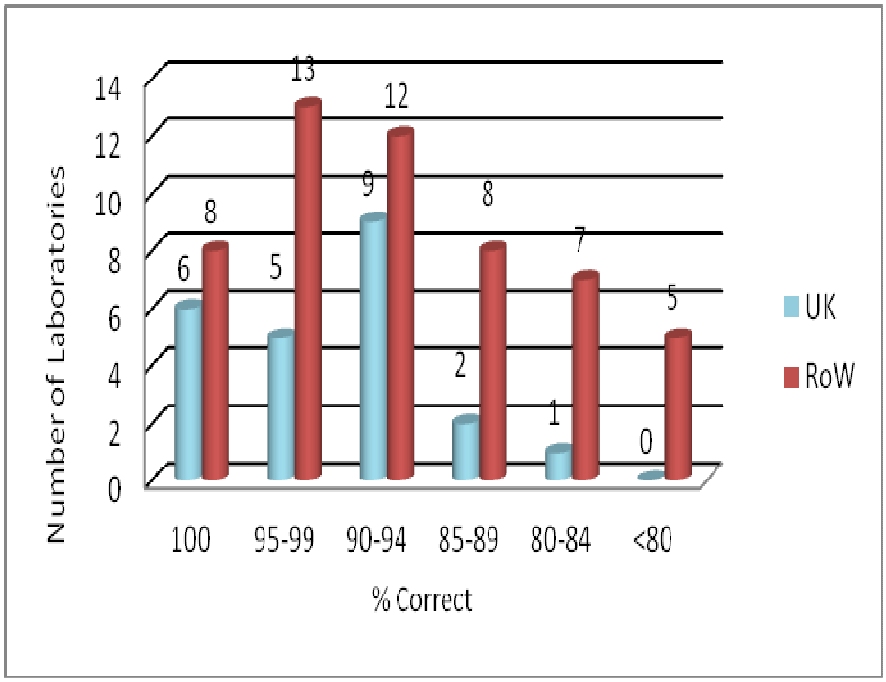


Figure 1: 2016 Scheme 2B Performance

Table 3: Scheme 2B Performance

	PBL/T Cells					B Cells				
	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016
Number of Participants	60	69	71	73	76	53	63	65	67	70
Number with Unacceptable Performance (< 85%)	10	6	6	13	13	8	10	12	n/a	n/a
% Unacceptable Performance	16.7%	8.7%	8.5%	17.8%	17.1%	15.1%	15.9%	18.5%	n/a	n/a

Please note for 2016 T-cell & B-cell assessment was linked such that both results were required to be in line with the consensus results for acceptable performance.

Jeanette Ayers, Transplant Immunology Laboratory, Churchill Hospital, Oxford

SCHEME 3: HLA ANTIBODY SPECIFICITY ANALYSIS

Purpose of scheme

Scheme 3 focuses on HLA antibody specificity analysis. Five sera are sent out on two separate occasions, and these should be tested and reported using a laboratory's routine testing method(s). Participants can register for class I and/or class II and must report results within 10 weeks of sample receipt. Scoring is via a consensus system, with 75% consensus required to score specificity positive and 95% consensus required to score specificity negative. Individual laboratory performance is assessed via agreement with consensus, being either acceptable or unacceptable. Overall, laboratories must detect 75% of the positive specificities and not detect 75% of the negative specificities for acceptable performance.

Participants

This year 85 laboratories registered for Scheme 3, 24 from the UK&I. For class I antibody analyses, 82 laboratories participated. Eighty five laboratories also registered for class II antibody specificity analysis.

Methods

The majority of participants tested the samples using Luminex technology (78/85 participants), 64 participants tested the samples using Luminex only, 14 used Luminex in combination with CDC, 1 lab used ELISA & Luminex, 1 used ELISA, Flow cytometry and Luminex and five laboratory provided no data.

There were more participants that used Luminex kits from One Lambda for testing the samples (55, 64.7%), than used Lifecodes kits (1113, 15.3%). 8 participants used a mixture of kits from both suppliers (9.4%) and 9 (10.6%) provided no information about the kits being used.

There was considerable variation noticed in the bead and serum volume used by participants in the Luminex assay, as well as the MFI cut off value used to assign positive specificities.

39 labs reported the use of EDTA to treat the serum (up from 23 the previous year), 2 labs reported the use of heat inactivation and 2 the use of DTT.

Performance

11 participants had unsatisfactory performance for Class I specificity analysis; 8 labs for antibody presence analysis, and 3 labs for antibody absence analysis (figure 1).

9 participants had unsatisfactory performance for Class II specificity analysis; 5 labs for antibody presence analysis, and 4 labs for antibody absence analysis (figure 1).

There was no correlation of unsatisfactory performance with Luminex kits.

Table 1: Class I and Class II performance

Class I		**2014**	**2015**	**2016**
Number of Participants (UK&I)		78 (24)	81 (24)	85 (24)
Number with Unsatisfactory Performance (UK&I)	Presence	15 (0)	9 (1)	8 (0)
	Absence	1 (0)	2 (0)	3 (0)
% Unsatisfactory Performance	Presence	19.2%	11.1%	9.4%
	Absence	1.3%	2.5%	3.5%
Class II		**2014**	**2015**	**2016**
Number of Participants (UK&I)		76 (24)	81 (24)	85 (24)
Number with Unsatisfactory Performance (UK&I)	Presence	5 (0)	4 (0)	5 (0)
	Absence	2 (0)	3 (0)	4 (0)
% Unsatisfactory Performance Presence/ Absence	Presence	6.6%	4.9%	5.9%
	Absence	2.6%	3.7%	4.7%

Changes to Scheme 3 for 2017

Participants can report findings of complement fixing assays, DQA, DPA and DPB antibodies, and select which HLA specificities they would list as unacceptable for kidney transplantation. These changes will not be assessed 2017.

James Kelleher, NHISSOT, Beaumont Hospital

SCHEME 4A1 - DNA HLA TYPING AT 1ST FIELD LEVEL

Format and specification

The aim is to assess participants' ability to correctly determine HLA alleles at the 1st field level of resolution.

10 samples are distributed each year comprising 2 send outs of 5 blood samples from local donors.

Participants may register for 1st field assessment of HLA-A, B, C, DRB1, DQB1, DQA1, DPA1 and for 1st field or presence of DRB3, DRB4 and DRB5. Participating laboratories are only assessed on the loci for which they have registered.

Alleles that fail to reach 75% consensus level are not assessed. Only those alleles listed in the latest full HLA nomenclature report are assessed.

Performance

There were 102 incorrect assignments reported by 15 labs in 2016 (Table 1).

- 14 incorrect assignments (e.g. A80 instead of A23)
- 11 missed assignments (e.g. reported homozygous/blank)
- 0 incorrect nomenclature (e.g. DRB1*17 instead of DRB1*03)
- 22 reported DRB3/4/5 present (lab registered for DRB3/4/5 1st Field)
- 55 not reporting when registered for locus

Satisfactory performance involves obtaining 9 or more full HLA genotypes in agreement with consensus in a calendar year. Whilst the majority of laboratories submitting results scored 100%, there were 21 laboratories classified as unsatisfactory performers in 2016 (Table 2).

The most errors were at HLA-DR3/4/5 & DQA1 (Figure 1). Of the 21 laboratories that submitted incorrect results (Table 2), 6 used Luminex, 11 used SSP and 3 used a combination of SSP and Luminex.

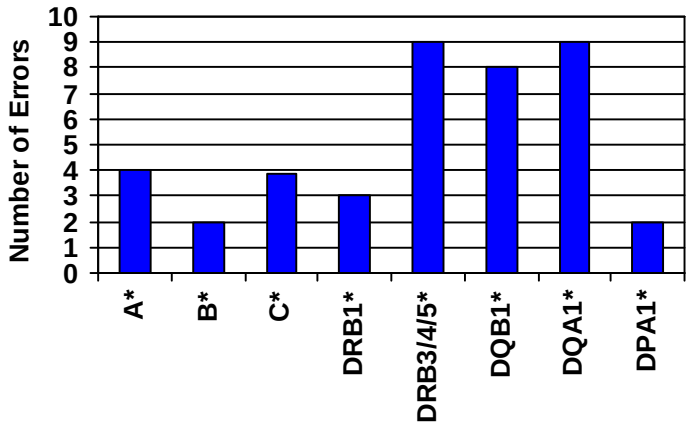


Figure 1: Scheme 4A1 errors by HLA locus

Table 1: Scheme 4A1 Incorrect Assignments

Sample	Report	Consensus	Method	Lab Number
4A1 01	DPA1*01/02, 02/03	DPA1* 01, 02	Luminex (Immucor)	100
	C*04, 04	C*03, 04	Luminex (Immucor)	189, 267
4A1 02	DRB4*01/02/03	DRB4*01	SBT/3GS (Life Technologies, Pacbio)	62
4A1 03	DQB1*06, 06	DQB1*04, 06	SSP (One Lambda)	123
4A1 04	DRB4*01/02/03	DRB4*01	SBT	62
4A1 05	DQA1*01, 06	DQA1*01, 04	SSP/LUM (One Lambda) / LUM (Immucore)	14, 267
	C*03, 03	C*03, 07	LUM (Immucore)	189
	DQB1*06, 06	DQB1*04, 06	LUM (Immucore)	302
4A1 06	DRB5*01/02	DRB5*01	SSP/LUM (One Lambda), SSP (Biorad, Olerup, In house)	14, 15
	DQA1*01, 01	DQA1*01, 02	Luminex (Immucor)	267
4A1 07	DRB3*01-03	DRB3*01	SSP/LUM (One Lambda)	14
	DQB1*06, 03	DQB1*02, 03	SSP (Olerup)	260
4A1 08	A*01, 11	A*01, 68	SSP/SSOP/LUM (One Lambda)	35
	B*44, 57	B*40, 57	SSP/SSOP/LUM (One Lambda)	35
	C*04, 06	C*03, 06	SSP/SSOP/LUM (One Lambda)	35
	DRB1*13, 07	DRB1*04, 07	SSP/SSOP/LUM (One Lambda)	35
	DRB3*02	DRB3*blank	SSP/SSOP/LUM (One Lambda)	35
	DQA1*01, 02	DQA1*02,03	SSP/SSOP/LUM (One Lambda)	35
	DQB1*03,06	DQB1*03, blank	SSP/SSOP/LUM (One Lambda), LUM (Immucor)	35, 231
	DPA1*01, 01	DPA1*01,02	LUM (Immucore, One Lambda)	351
4A1 09	DRB3*01	DRB3*02	SSP/LUM (One Lambda,	25

			Fluorogene, Innotrain)	
	A*01, 68	A*01, 11	SSP/SSOP/LUM (One Lambda)	35
	B*40, 57	B*44, 57	SSP/SSOP/LUM (One Lambda)	35
	C*03, 06	C*04, 06	SSP/SSOP/LUM (One Lambda)	35
	DRB1*04, 07	DRB1*07, 13	SSP/SSOP/LUM (One Lambda)	35
	DQA1*02, 03	DQA1*01, 02	SSP/SSOP/LUM (One Lambda)	35
	DQB1*03, 03	DQB1*03, 06	SSP/SSOP/LUM (One Lambda)/ LUM (Lifecodes)	35, 231, 240
	DQA1*01, 01	DQA1*01, 02	Luminex (Immucor)	267
	DQA1*01, 03	DQA1*01, 02	LUM (Immucore, One Lambda)	351
4A1 10	DRB1*03, 15	DRB1*07, 15	SSP/LUM (One Lambda)	14
	DRB5*01-02	DRB5*01	SSP/LUM (One Lambda)	14
	DQA1*01, 01	DQA1*01, 02	Luminex (Immucor)	267

Table 2: Scheme 4A1 Performance

Scheme 4A1 Performance	2013	2014	2015	2016
Number of Participants	96	96	100	102
Number with Unsatisfactory Performance (< 90%)	5	9	7	21
% Unsatisfactory Performance	5.2%	9.4%	7.0%	20.6%

Scheme 4A1 - Interpreted DNA Results

Participants were invited to interpret the DNA based typing results for Scheme 4A1 samples and additionally report results at the split specificity level (e.g. DQB1*03:01 allele group reported as DQ7). These 'converted' results were not formally assessed.

There were 234 incorrect 'interpreted' assignments reported by 35 labs. The majority (174) of these errors were reports of broad rather than split specificity (e.g. Cw3 not Cw9). The remaining errors could be categorised as follows:

- 32 missed assignments (e.g. reporting homozygous/blank)
- 15 reports of the wrong split (e.g. DR1 instead of DR103)
- 13 incorrect/didn't report Bw4/6

Changes to Scheme 4A1 for 2017

Participants can register for assessment of 'interpreting' Scheme 4A1 results to the appropriate specificity (e.g. DQB1*03:01 group 'interpreted' as DQ7 for donor specific antibody analysis).

Dr Leigh Keen, Histocompatibility & Immunogenetics Department, NHSBT, Filton

SCHEME 4A2: DNA HLA TYPING TO 2ND FIELD RESOLUTION

Format and specification

The aim is to assess participants' ability to correctly determine HLA alleles to the 2nd field level.

10 samples are distributed each year comprising 2 send outs of 5 blood samples from local donors.

Participants may register for 2nd field assessment of HLA-A, B, C, DRB1, DQB1, DQA1, DPA1, DPB1 and for 2nd field. Participating laboratories are only assessed on the loci for which they have registered.

Alleles that fail to reach 75% consensus level are not assessed. Allele groups may be reported, provided that participants include the consensus allele and resolve all ambiguities resulting from polymorphisms located within exons 2 and 3 for HLA class I and exon 2 for HLA class II. Only those alleles listed in the latest full HLA nomenclature report are assessed.

Performance

Satisfactory performance involves obtaining 9 or more full HLA genotypes in agreement with consensus in a calendar year. The highest number of errors were made typing for HLA Class II, in particular HLA-DQB1 (Figure 1).

There were a total of 45 incorrect assignments reported by 18 labs:

- 25 reports of alleles in a string that differ from the consensus allele in exons 2 and 3 (e.g. B*44:02/03)
- 17 reports of incorrect allele (e.g. C*07:01 not C*07:18)
- 2 reports of incorrect allele-group (e.g. B*37:01 instead of B*38:01)
- 1 missed assignments (e.g. reported homozygous/blank)

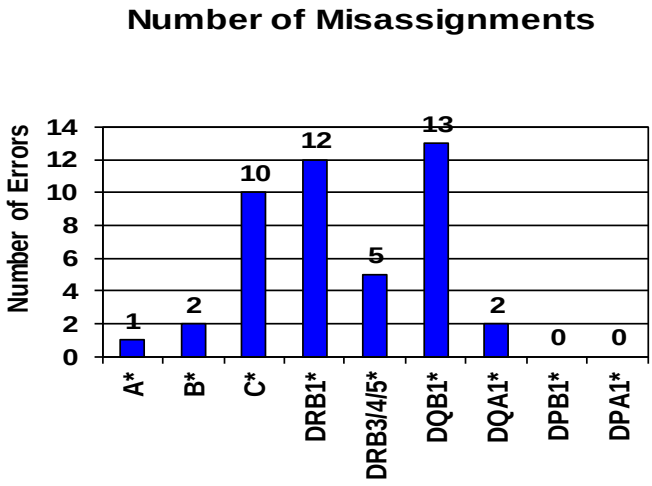


Figure 1: Scheme 4A2 errors by HLA locus

The number of participating laboratories is 63 and the number of unsatisfactory performers (21) has increased by one from 2015 (Tables 2).

Scheme 4A2 Performance	2013	2014	2015	2016
Number of Participants	58	59	59	63
Number with Unsatisfactory Performance (< 90%)	5	5	7	8
% Unsatisfactory Performance	8.6%	8.5%	11.9%	12.7%

Table 2: Scheme 4A2 Performance

Allele resolution typing

Participants performing HLA typing at the allelic resolution (e.g. using next generation sequencing) were able to report these results using scheme 4A2 samples. 13 participants reported results above the 2nd field level. These results were not formally assessed in 2016 but summarised to allow comparison with other laboratories performing allelic level typing.

Dr Leigh Keen, Histocompatibility & Immunogenetics Department, NHSBT, Filton

SCHEME 4B: ABO GROUPING BY DNA METHODS

This scheme is designed to assess participants' ability to correctly determine ABO blood groups using DNA-based methodology. The scheme utilises scheme 4A1 samples

In 2016 there were 7 participants. All participants used PCR-SSP technology with 5 using commercial kits and the remainder using 'in house' developed primer sets.

There was no unsatisfactory performers in 2016.

Scheme 4B Performance	2013	2014	2015	2016
Number of Participants	7	10	7	7
Number with Unsatisfactory Performance (< 100%)	1	0	0	0
% Unsatisfactory Performance	14.3%	0%	0%	0%

Dr Leigh Keen, Histocompatibility & Immunogenetics Department, NHSBT, Filton

SCHEME 5A: HFE TYPING

This scheme is designed to assess participants' ability to correctly determine HFE mutations, to do so the participants were required to report on codon 63 and 282 and could also report codon 65.

Satisfactory performance is correctly assigning all 10 samples.

Table 1: Scheme 5A samples distributed in 2016

5A Samples Distributed			
Codon 63	**Codon 282**	**Codon 65**	**Number Of Samples**
HH	CC	SS	3
HD	CC	SS	2
HD	CY	SS	1
HH	YY	SS	2
HH	CY	SS	1
DD	CC	SS	1

Performance

In 2016 there were 58 participants; 22 - 23 reported codon 65 results, 58 reported codon 282 and 58 reported on codon 63. A variety of techniques were used including RT-PCR, PCR-SSP and PCR melt curve analysis.

There were two laboratories with unacceptable performance

Scheme 5A Performance	2013	2014	2015	2016
Number of Participants	58	59	60	58
Number with Unacceptable Performance (< 100%)	2	2	0	2
% Unacceptable Performance	3.9%	3.4%	0%	3.4%

Ruhena Sergeant, Clinical Immunology Laboratory, Hammersmith Hospital, London

SCHEME 5B: INTERPRETATIVE: HFE GENOTYPE AND HEREDITARY HAEMOCHROMATOSIS

Introduction

The purpose of this scheme is to assess participants' ability to make an accurate, clear, concise and timely clinical report, appropriate for the range of clinical staff involved in a patient's care and treatment, given HFE genotype and other relevant clinical information.

Format

Two fictitious patient scenarios with HFE genotype (for C282Y and H63D mutations) and clinical information were provided in each of two distributions during the year, coinciding with the scheme 5A sample distributions. The scenarios were devised based on real cases and typical experience. Participants were expected to return a report within 4 weeks, in a format identical to that used for routine clinical reporting in their laboratories, providing appropriate interpretation and suggested actions.

Assessment

While designing the scenarios, the three expert assessors agreed on aspects of the report that were considered essential and what specific errors should attract penalty points, depending on their importance.

Criteria were classed under five general headings (describe the result, advise actions to be taken, state disease risk for patient, advise on risk to family members and on testing, and an open heading of common sense/other error). Allowing for one penalty award under each heading makes a maximum of five possible penalties.

Performance by a laboratory was classified "unacceptable" for any scenario where more than 50% of the possible penalty points were allocated, *i.e.* three penalties. Satisfactory performance for 2016 was achieved by obtaining four 'acceptable' classifications in the year.

Participation and results

Returns were received from 19 participants for the both distributions of scenarios 1-4. The maximum possible penalty was 5 points per scenario, 20 points in total.

0	labs got	0 penalty points
3	labs got	1 penalty point
1	lab got	2 penalty points
8	labs got	3 penalty points
2	labs got	4 penalty points
2	labs got	5 penalty points
1	lab got	6 penalty points
1	lab got	7 penalty points
1	lab got	8 penalty points

Average Annual total points was 3.6, higher than the average of 2.28 for 2015.

Satisfactory performance was achieved by all participants in 2016 with none receiving >3 penalty points for any of the four scenarios.

Table 1. Performance Summary

	Scenario 1	Scenario 2	Scenario 3	Scenario 4
No. of labs with 0 penalty points	**3**	**5**	**12**	**5**
No. of labs with 1 penalty point	**12**	**11**	**3**	**7**
Labs with 0 or 1 penalty points	**83.3 %**	**88.9%**	**78.9 %**	**63 %**
Average penalty	**1**	**0.83**	**0.68**	**1.2**
No. of labs with classification "unacceptable" (3+ pts)	**0**	**0**	**0**	**0**

Recurrent reasons for penalty points being awarded by the assessor across all four scenarios are as follows:

Scenario 1

- Ignoring information on current ferritin level within the normal range
- Absence of advice for immediate tfsat measuring and monitoring intervals
- Weak or inappropriate advice for follow up of at risk adult first degree relatives

Scenario 2

- Incorrect interpretation of tfsat as indicative of iron overload
- Not advising further investigation of raised ferritin.
- Not suggesting that secondary causes of iron overload should be considered

Scenario 3

- Ignoring current tfsat/ferritin levels
- Over interpretation of tfsat/ferritin levels as indicative of iron overload
- No advice on the need for life long monitoring

Scenario 4

- Stating that patient is at low risk of developing HH
- No suggestion of immediate tfsat measurement
- No information on risk to offspring provided

Carol Hardy, West Midlands Regional Genetic Laboratory, Birmingham, UK.

SCHEME 6: HLA ANTIBODY DETECTION

Overview

The purpose of Scheme 6 is to assess participant's ability to correctly determine the likely presence of HLA specific antibodies. A total of 20 serum samples are sent each year as two distributions of ten serum samples. At registration participants may opt for class I only or class I and class II antibody assessment, results are to be reported within 8 weeks. In 2016 there were 98 participants. Consensus Class I/Class II positivity or negativity of each sample is determined by at least 75% of laboratories agreeing, samples failing to reach 75% consensus will not be assessed. Each report in agreement with the consensus is considered Acceptable and each non agreement Unacceptable. Satisfactory performance is making 80% of reports on all sera in agreement with the consensus in a calendar year.

Methodology

Details of methodology used are requested as part of the reporting process. The methodology used to test the samples is shown in Table 1.

Table 1: Methodology

Technique	1st cycle	2nd cycle
CDC only	1	2
FLOW only	3	4
Luminex only	89	89
Luminex + CDC	20	21
Luminex + ELISA	1	1
Luminex + Flow	3	4
Luminex + CDC + ELISA	0	0

Performance: Satisfactory performance is making 80% of reports on all sera in agreement with consensus in a calendar year. In 2016 18 laboratories failed to reach 80% these are compared to the preceeding 2 years in Figure 1.
The breakdown in % correct can be seen Figure 2.
Overall there were more false positive results (67%) than false negative results (33%) which follows the same trend as 2015.

Table 1: Unsatisfactory Performance Scheme 6

	2014	2015	2016
Number of Participants (UK&I)	83 (25)	97 (24)	98 (24)
Number with Unsatisfactory Performance (< 80%) (UK&I)	5 (1)	6 (3)	18 (4)
% Unsatisfactory Performance	6.0%	6.2%	18.4%

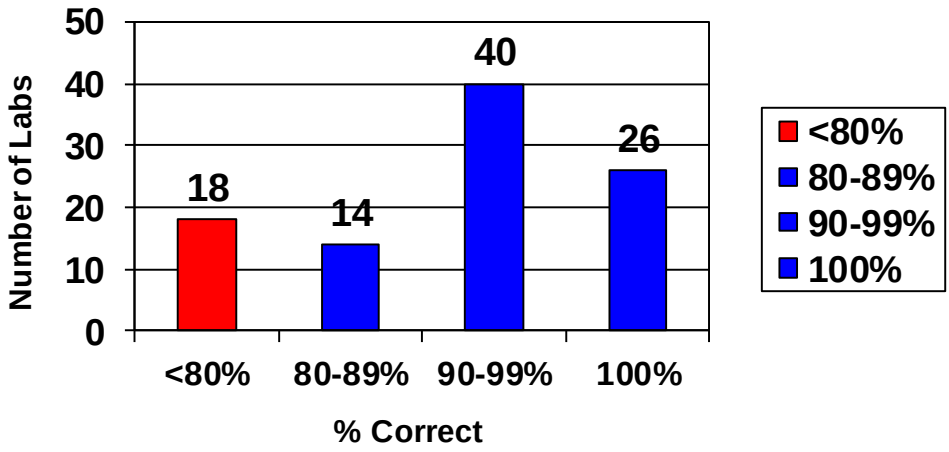


Figure 2: Laboratory results by % correct.

Changes to Scheme 6 for 2017
The number of samples distributed for Scheme 6 will be reduced from 20 to 12 in 2017.

James Kelleher, NHISSOT, Beaumont Hospital

SCHEME 7- HLA-B*57:01 TYPING FOR DRUG HYPERSENSITIVITY

The requirement for B*57:01 typing is founded on the finding that hypersensitivity to abacavir - a nucleoside analogue reverse transcriptase inhibitor used to treat HIV and AIDS patients - is strongly associated with possession of B*57:01. HIV/AIDS treatment guidelines firmly recommend B*57:01 testing prior to abacavir treatment and withholding the drug from B*57:01 'positive' patients.

The purpose of this scheme, therefore, is to assess participants' ability to correctly determine HLA-B*57:01 status. Accordingly, participants were required to report on the samples' B*57:01 'positive' or 'negative' status and, for information only, to specify any B*57 positive non-B*57:01 alleles identified.

Scheme assessment was based on the usual 75% consensus level and satisfactory performance was achieving all 10 reports in accord with the consensus findings.
In 2016 two distributions were made of 5 blood samples each and there were 62 participants. The methods used to test the samples are shown in figure 1.

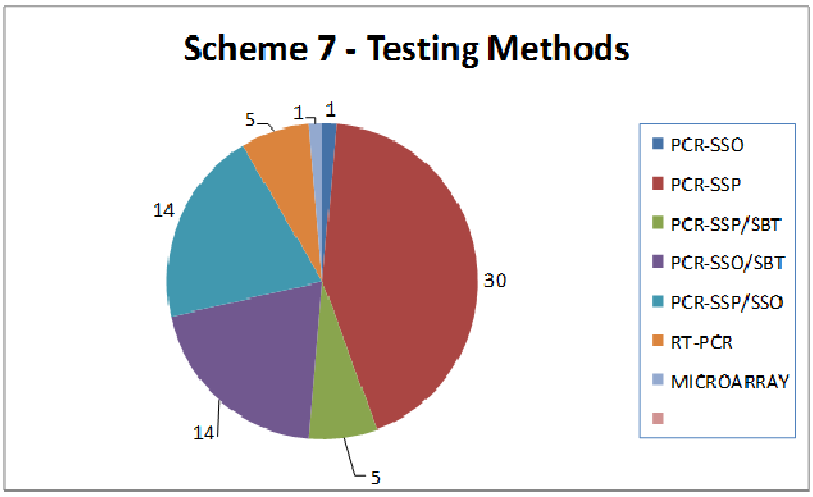


Figure 1: Scheme 7 testing methods

Of the 10 samples supplied 4 were from B*57:01 positive donors and 6 were from B*57:01 negative donors. There was one laboratory with unsatisfactory performance in 2016.

Table 1: Scheme 7 number participants

Scheme 7 Performance	2013	2014	2015	2016
Number of Participants	47	56	62	62
Number with Unacceptable Performance (< 100%)	0	1	0	1
% Unacceptable Performance	0%	1.8%	0%	1.6%

Ruhena Sergeant, Clinical Immunology Laboratory, Hammersmith Hospital, London

SCHEME 8 – HLA AND DISEASE TYPING FOR HLA-DR/DQ/DP ONLY

This scheme is aimed at enabling participants to external quality assess their HLA typing for conditions associated with HLA Class II alleles. The diseases commonly typed include: coeliac disease, narcolepsy, rheumatoid arthritis and type I diabetes.

The purpose of the scheme, therefore, is to assess participants' ability to correctly determine HLA-DR/DQ/DP allele families/alleles.

In 2016 there were 2 sample distributions – each of 5 DNA preparations. Importantly, all of these samples had previously been tested in Scheme 4A2 – DNA HLA Typing to the 2nd Field - so possessed well documented HLA types.

Laboratories were required to report their Class II findings at a resolution as required or as a HLA type or presence/absence.
Assessment was made for the loci reported and for the presence or absence.

Table 1: Scheme 8 incorrect assignments

Sample	Lab No	Reported	Method
801	86, 319	DQA1*03:01	SSP, qPCR
802	333	DQB1*03:02 Present	SSP
804	317	DQA1*02/03:01 Absent, DQA1*05 Absent	Euroimmun
805	333	DQB1*03:02 Present	SSP
808	17	DQA1*05 Present	SSP
809	201	DQA1*05 Present, DQB1*02 Present	SSP

Satisfactory performance was achieved by obtaining at least 10 genotypes in accord with the consensus type.

There were 38 participants in 2016. 4 laboratories reported both presence/absence and class 2 type, 23 reported presence/absence only and 12 reported class 2 type only. There were 12 misassignments made in 2016. 6 of the 10 samples had reporting errors (Table 1). 8 laboratories had unsatisfactory performance (Table 2).

Table 2: Scheme 8 Unsatisfactory Performance

Scheme 8 Unacceptable Performance	2013	2014	2015	2016
Number of Participants	19	21	30	38
Number with Unsatisfactory Performance (< 90%)	2	3	8	8
% Unsatisfactory Performance	10.5%	14.3%	26.7%	21.0%

Melanie Bartley, UK NEQAS for H&I Deputy Manager

EDUCATIONAL SCHEME SAMPLES

The purpose of this Scheme is to provide a variety of interesting HLA alleles/specificities that offer an educational element. The material is normally acquired from the Welsh Bone Marrow Donor Panel of some 87,000 HLA typed donors.

There were 48 participants in 2016, of which 22 were from the UK & Ireland.

In 2016 all 4 Educational Scheme samples were sent as DNA extracts. The suggested procedure is to perform "low resolution" typing by DNA- based method of HLA-A, B, C, DR, DQ and focus on the area of interest for further typing, if required.

The alleles of interest were: ED01/16 – A*01:01:65, ED02/16 – A*02:24, ED03/16 – C*02:22, ED04/16 – B*40:10. 46 laboratories reported on the samples – the findings were:

- **ED01/16 – HLA-A*01:01:65**

46 DNA-based HLA-A findings:

27 reports of A*01:01:65 – thus 58% of labs identified this allele
12 reports of A*01
4 reports of A*01:01
1 report of A*01:01/01:01L-01:168
1 report of A*36
1 report of A*01:01/99/102/167

- **ED02/16 – HLA-A*02:24**

46 DNA-based HLA-A findings:

30 reports of A*02:24 - thus 65.2% of labs identified this allele
15 reports of A*02
1 report of A*02:01/02:012-02:554

- **ED03/16 – HLA-C*02:22**

44 DNA-based HLA-C findings:

34 reports of C*02:22 – thus 77.3% of labs identified this allele
10 reports of C*02

- **ED04/16 – HLA-B*40:10**

33 DNA-based HLA-B findings:

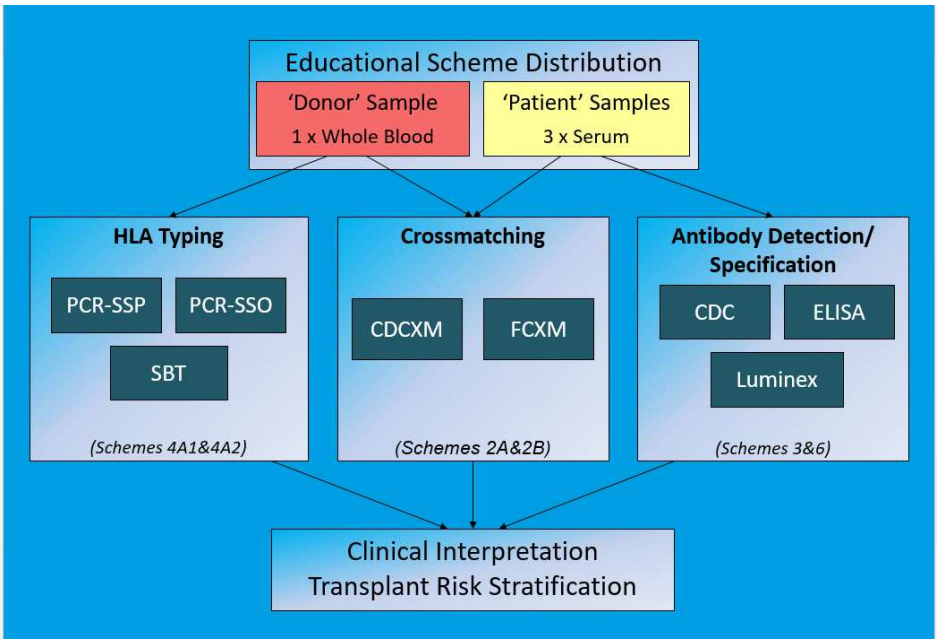
20 reports of B*40:10 – thus 60.6% of labs identified this allele
6 reports of B*40:10:01
1 report of B*40:10:01:02
1 report of B*40:10/282
5 reports of B*40

Melanie Bartley, UK NEQAS for H&I Deputy Manager

EDUCATIONAL CROSSMATCH SCHEME SAMPLES

An educational crossmatch exercise was introduced in 2016. Participants could register to receive samples to perform combined crossmatching, HLA typing and antibody detection/specification to mimic testing performed for solid organ transplantation. Participants were asked to report the results of all the individual test components as performed in their laboratory, and also provide an interpretation of the results if they were obtained in a clinical kidney transplant setting.

There were two distribution of samples in 2016. Each distribution consisted of one 'donor' blood sample and three 'patient' serum samples.



20 laboratories participated in the first distribution and 22 in the second, although not every laboratory reported on every aspect. A summary of the results in shown in table 1 and table 2.

There was good agreement on same aspects of the results for some sera (e.g. presence and absence of DSA for serum 1 & 2, distribution 2), but other results were more variable (e.g. assigned risk for serum 3 distribution 1).

The scheme is not formally assessed, but allows participants to monitor the performance of multiple techniques with a single scheme, make clinical interpretations based on their own results and review variations in practice.

Table 1: Summary of results for EDXM distribution 1

Test/Interpretation			Report	No. of Labs
Serum 1	DSAs Present?		Yes	12/20 (60.0%)
	CDCXM	No DTT	Negative	15/16 (93.8%)
		DTT	Negative	16/16 (100.0%)
	FCXM		Negative	13/14 (92.9%)
	Assigned Risk		Low	14/20 (70.0%)
Serum 2	DSAs Present?		Yes	20/20 (100%)
	CDCXM	No DTT	Positive	15/16 (93.8%)
		DTT	Positive	11/16 (78.6%)
	FCXM		Positive	14/14 (100.0%)
	Assigned Risk		Veto	19/20 (95.0%)
Serum 3	DSAs Present?		Yes	19/19 (100.0%)
	CDCXM	No DTT	Negative	14/16 (87.5%)
		DTT	Negative	14/14 (100.0%)
	FCXM	T Cell	Positive	8/12 (66.7%)
		B Cell	Negative	8/11 (72.7%)
	Assigned Risk		Medium	12/20 (60.0%)

Table 2: Summary of results for EDXM distribution 2

Test/Interpretation			Report	No. of Labs
Serum 1	DSAs Present?		Yes	22/22 (100%)
	PBL/T Cell CDCXM	No DTT	Negative	14/15 (93.3%)
		DTT	Negative	11/12 (91.7%)
	B Cell CDCXM	No DTT	Positive	15/15 (100%)
		DTT	Positive	11/11 (100.0%)
	FCXM		Positive	17/17 (100%)
	Assigned Risk		Veto	15/20 (75.0%)
Serum 2	DSAs Present?		No	22/22 (100%)
	CDCXM	No DTT	Negative	15/15 (100%)
		DTT	Negative	12/12 (100%)
	FCXM		Negative	16/17 (94.1%)
	Assigned Risk		Low	22/22 (100%)
Serum 3	DSAs Present?		No	14/22 (63.6%)
	CDCXM	No DTT	Negative	15/15 (100%)
		DTT	Negative	12/12 (100%)
	FCXM	T Cell	Negative	16/17 (94.1%)
		B Cell	Negative	13/16 (81.3%)
	Assigned Risk		Low	16/22 (72.7%)

Deborah Pritchard, UK NEQAS for H&I Manager

INTERPRETATIVE EDUCATIONAL SCHEME CLINICAL SCENARIOS

The introduction of clinical interpretative scenarios (iED) has been a very popular addition to UK NEQAS for H&I repertoire.

These scenarios are based around a case study, with set questions regarding the testing and clinical advice participants would provide given the information presented in the scenario. Three scenarios were distributed in 2016 and again this year the participants were given the option to register for the different scenarios independent of whether they participated in the corresponding EQA scheme. This change was made in recognition of the educational value to our participants irrespective of whether they perform the task routinely.

Participants were given 6 weeks to return the results. These scenarios are not formally assessed and result summaries are returned to each laboratory that submits a response.

Clinical Scenario 1: Solid Organ Transplantation

The first scenario was based on a sensitised renal patient case, Results were received from 50 participants.

The HLA typing information of the recipient, previous liver transplant HLA type and details of pregnancies and transfusions were presented along with HLA antibody testing results. Details of potential live and deceased donors were provided with details of crossmatch results. Participants were asked to complete questions based on the results provided. This included questing regarding virtual crossmatching (figure 1) and the risk level associated with different donors for this sensitised patient.

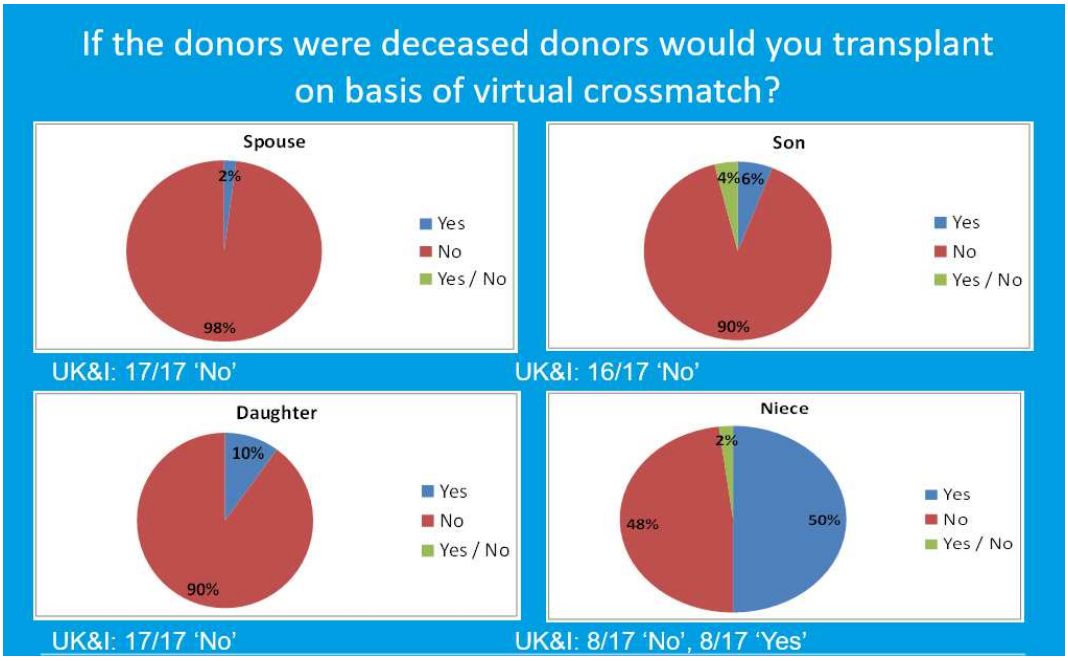


Figure 1: Results of Virtual Crossmatch Question.

Clinical Scenario 2: Haematopoietic Stem Cell Transplantation

The second scenario was based on a based on a patient with AML. Results were received from 45 participants.

Information was provided on the HLA type, CMV status, weight and blood group of the patient. The results of an unrelated donor, cord blood unit (CBU) search and HLA antibody testing results were provided.

Participants were asked to complete questions based on the results provided. This included the selection of suitable donors from the unrelated donor search results (figure 1), CBU search results and the clinical advice that would be given based on the HLA antibody results.

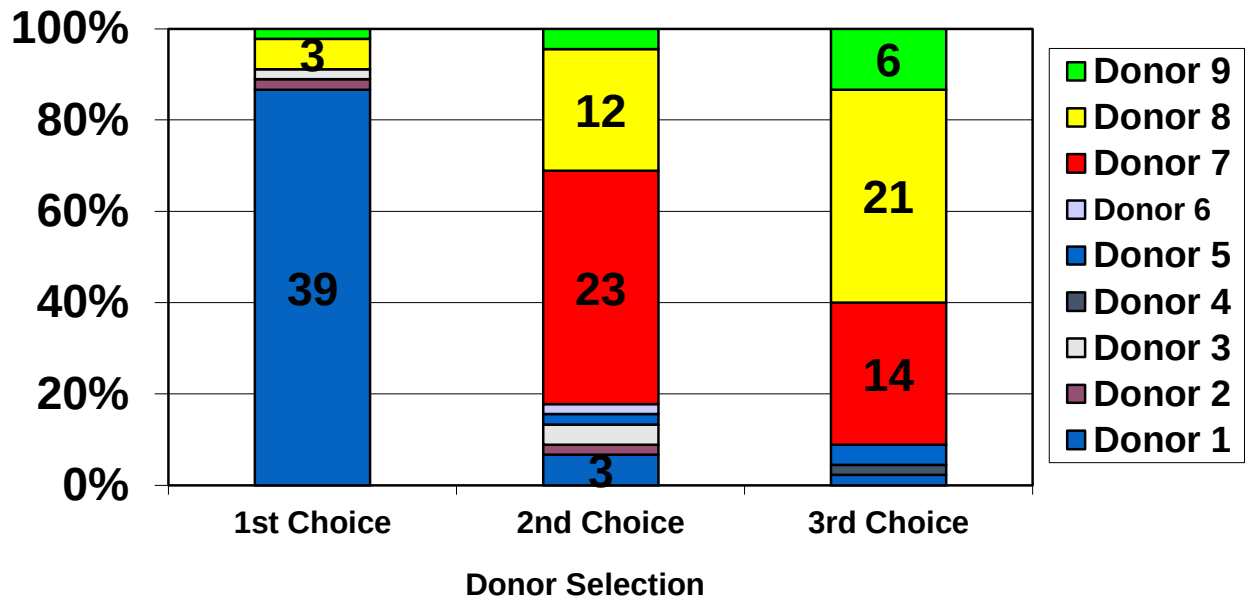


Figure 2: Unrelated donor selection results

Clinical Scenario 3: Platelet Transfusion Case

The final scenario for 2016 was a patient with AML requiring platelet support. Results were received from 23 participants.

Information was provided on the patient's ABO, CMV status, HLA type and Luminex Single Antigen bead data. In addition a platelet unit search was included and post infusion increment data.

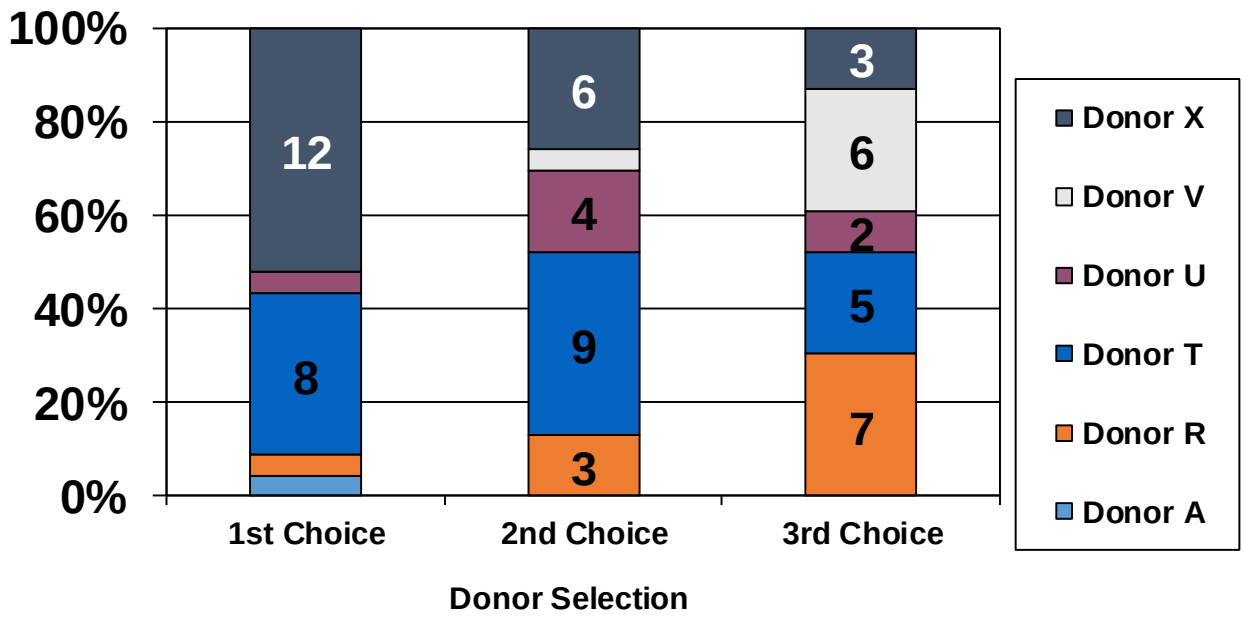


Figure 3: Platelet donor selection results

Deborah Pritchard, UK NEQAS for H&I Manager

PILOT GENOTYPING SCHEMES

Three Pilot Schemes were continued in 2016 for Genotyping of the following; HPA (Human Platelet Antigen), KIR (Killer-cell immunoglobulin-like receptor) and MICA (MHC class I polypeptide-related sequence A)

Participants were provided with 5 blood samples to use for all three schemes. The results were not formally assessed. 2017 will see two of the three schemes becoming full schemes (Scheme 9 will be KIR Genotyping; Scheme 10 will be HPA Genotyping). MICA will be discontinued due to low participant numbers.

Pilot HPA Genotyping

Participants able to report any of the following: HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-6, HPA-15 as well as any other HPA polymorphisms detected, for information. There were 12 participants in 2016. All 12 labs reported HPA-1, 2, 3, 4, 5 and 15. 10 labs also reported HPA -6 and 1 lab also reported HPA-9

There was no report out of consensus,

Pilot KIR Genotyping

Participants able to report any of the following: KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR2DP1, KIR3DP1. Participants were also able to report any other KIR polymorphisms they detected for information.

There were12 participants in 2016 and there were 4 reports out of consensus.

Table 1: KIR Genotyping Reports Out of Consensus

Sample	Report	Consensus	Method	Lab Number
4A1 06/2016	3DS1 negative	Positive	SSO	164
4A1 07/2016	3DS1 negative	Positive	SSO	164
4A1 08/2016	3DS1 negative	Positive	SSO	164
4A1 10/2016	3DS1 negative	Positive	SSO	164

Pilot MICA Genotyping

Participant asked to report the MICA alleles as fully as possible using the appropriate nomenclature. There were 2 participants in 2016, both reporting in varying levels, therefore this scheme will be discontinued in 2017.

Deborah Pritchard, UK NEQAS for H&I Manager

3. 2016 ANNUAL PARTICIPANT MEETING – BIRMINGHAM

The UK NEQAS for H&I Annual Participant Meeting was held on the 5th December 2016 at The Aston Conference Centre in Birmingham. 59 delegates representing 31 laboratories attended. The new venue was again well received and will be used for the 2017 Annual Participant Meeting.

Slides from all of the annual meeting presentations may be downloaded from: http://www.wtail.org.uk/neqas/presentations.asp or requested from Deborah Pritchard, UK NEQAS for H&I Manager.

The 2016 UK NEQAS for H&I participant meeting is valid for 3 Royal College of Pathologists' CPD points and 0.3 Credits for the Institute of Biomedical Science's CPD Scheme. It constitutes an appropriate meeting to attract BSHI CPD scheme points and should be documented by attendees who are BSHI Diploma Trainees.

4. FOR 2017 PLEASE NOTE

Laboratories will retain their code numbers for 2017. Laboratory code information is known only to the Scheme Manager and UK NEQAS for H&I staff.

Two new pilot schemes have been introduced for 2017: A HPA Antibody detection/specification scheme and an allelic level HLA genotyping scheme. Participants can register to participate in these schemes free of charge.

An up-to-date list of contact names is provided in the Participant Manual

Important UK NEQAS for H&I dates for distributions, result deadlines, reporting and meetings are provided in the Participant Manual. Please see the 2017 Participant Manual for full details of the assessment system which is available to download from the website:

http://www.wtail.org.uk/neqas/participantmanual.asp

Deborah Pritchard, UK NEQAS for H&I Manager