UKNEQAS

HISTOCOMPATIBILITY

and

IMMUNOGENETICS

Annual Report

2014

Contents:

United Kingdom National External Quality Assessment Service

CPA

Accredited EQA Scheme
Reference Number 036
Since 1999

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1. INTRODUCTION

It is easy for scheme participants to overlook the valuable work undertaken by the UK NEQAS for H&I's Steering Committee. This group busily works behind the scenes providing help, advice and professional insight to the Director and Manager. Probably the only time that most scheme participants come across Committee members is at the annual Participants' Meeting where members usually report on their particular scheme of interest.

In 2014 the group comprised 15 members. This included the Service Director and Manager and other Ordinary Members, the team that help manage Scheme 5B (Interpretative: HFE Genotype and Haemochromatosis), a clinical representative, and the British Society for Histocompatibility and Immunogenetics' representative on the UK National Quality Assurance Advisory Panel for Immunology (a non-voting Steering Committee member).

The Committee has a written Constitution and Terms of Reference that are reviewed annually and, importantly, a Chairperson - that is not the Service's Director or Manager. The secretarial work of the Committee is undertaken by the Scheme's Manager.

The Committee meets three times per year and occasionally have a 'special' meeting after the Participants' Meeting at the end of the calendar year. Agendas are invariably long and always include a comprehensive review of all the schemes including their general operation, administration and, importantly, participant's comments and suggestions - and their occasional appeals.
December 2014 saw the 'retirement' from the Committee of Dr Deborah Sage. Deb has been a long standing member of the Committee: firstly as an Ordinary Member (1997 to 2001) and then as the Committee's Chairperson (2002 to 2014).

Deb has always provided immense support and an unfailing commitment to the UK NEQAS for H&I cause over the years with her clear and practical good sense and expertise. A most sincere 'thank you' is due to Deb for all her hard work on the Committee's behalf – she will be sorely missed.
The Committee saw other changes at the end of 2014 too with the 'retirement' of Dr Mark Hathaway and Jennifer Pepperall, both Ordinary Members. Again, warm thanks are due to each of them for their noteworthy contribution to the Committee's work.

Incoming Committee members include Dr Judith Worthington – who takes over the Chair, James Kelleher (Ordinary Member) and the Deputy Service Director - Dr Tracey Rees (Ordinary Member). They are all cordially welcomed.
Finally, the team at the UK NEQAS for H&I's office at the Welsh Blood Service deserves our heartfelt gratitude for all their hard work and their valuable contribution to H&I during 2014. They are Deborah Pritchard (Scheme's Manager), Melanie Bartley (Deputy Manager), Geraint Clarke and Luke Gardner.

Chris Darke 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 2014 is summarised in table 1 below:

Table 1: Scheme 1A Participation

HLA	Number of Participants	
	Total	UK
Any	37-42	9
A	37-42	9
B	37-42	9
C	8	1
DR	26-31	9
DQ	24-28	8
A, B	8	0
A, B, C	3	0
A, B, DR	2-3	1
A, B, DR, DQ	20-24	7
A, B, C, DR, DQ	4	1

Ranges reflect changes in participant number over the distribution cycles

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 (26 labs); Biotest (2 labs); BioRad (5 labs) Innotrain (1 lab), and 6 labs using a combination of manufacturers.

Cell preparation

The majority of laboratories undertook testing using separated T cell and B cell preparations (21 labs), whilst 5 laboratories tested samples using unseparated cells. Eight laboratories used T cells only, 5 used unseparated cells plus B cells, 1 lab used unseparated cells plus T cells and 1 lab used unseparated cells plus T cells and B cells.

Table 2: Incorrect assignments in 2014

Sample	Report	Consensus	Lab Number
1A01	DQ1, blank	DQ5, blank	139, 154, 193, 262
1A02	DQ1, DQ7	A24, A29; DQ5, DQ7	139, 193
	DQ1, DQ3		154
	A23, A29		187
1A03	DR4, DR64 (14)	DR4, DR14; DQ5, DQ8	11
	DQ1, DQ3		120
	DR4, blank; DQ3, blank		159
	DQ5, DQ3		238
1A04	DQ6, blank	B7, B58; DR13, DR15; DQ6, DQ7	9
	DR13, DR2; DQ1, DQ7		120
	B7, B57		142
	DQ6, DQ3		238
1A07	DQ1, blank	DQ6, blank	294
1A08	DQ6, DQ3	DQ6, DQ8	164
	DQ1, DQ3		294
1A09	A203, blank	A2, blank; B13, B60	139
	B13, B40		139
1A10	DR4, DR1	B35, B60; DR4, DR103; DQ5, DQ8	20, 123, 164, 181, 262
	B35, B40		139
	DR4, DR*01:03; DQ5, DQ*03:02		238

Performance in 2014

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 nine incorrect assignments due to missed or incorrect specificities in 2014 involving 2 UK and Ireland (UK&I) and 6 Rest of the World (RoW) laboratories. There was no correlation with reagents or testing strategy used and the misassignments reported. Incorrect assignments due to failure to split a broad specificity occurred on 21 occasions in 2014, involving 10 laboratories (all RoW).

There were 8 unacceptable performers in 2014 (table 3).

Table 3: Scheme 1A Unacceptable Performance

Scheme 1A Unacceptable Performance	2012	2013	2014
Number of Participants	22	30	42
Number with Unacceptable Performance (< 90%)	1	0	8
% Unacceptable Performance	4.5%	0.0%	19.0%

Dr Deborah Sage, Histocompatibility & Immunogenetics Department, NHSBT, Tooting

SCHEME 1B: HLA-B27 TESTING

The purpose of this scheme is to assess ability to correctly determine HLA-B27/2708/*27 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.

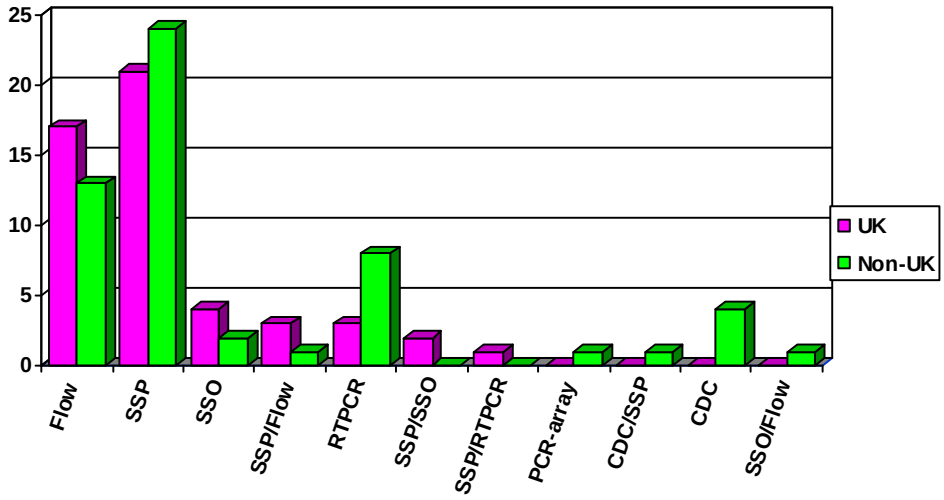
In 2014 there were 103-107 participants in the scheme (49-51 UK&I laboratories).

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, with the most common techniques being PCR-SSP or flow cytometry. Some laboratories use techniques in combination (see below).



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 2014, five samples distributed were HLA-B27 positive. Five assignments were made outside of consensus (see table 1). Three misassignments were made by laboratories using flow cytometry, one by PCR-SSP and one by PCR-SSP/SSO. There were therefore 4 unacceptable performers in 2014 (Table 2)

Table 1: Scheme 1B Incorrect Assignments

Sample	Result	Lab Number	Technique	HLA-B Type
1B01	False Pos	174	SSP/SSO	B44, B47
1B03	False Pos	300	SSP	B7, B18
1B05	False Neg	32	Flow	B27, B44
1B09	False Neg	8	Flow	B27, B44
1B10	False Neg	8	Flow	B27, B64

Table 2: Scheme 1B Unacceptable Performance

	2012	2013	2014
Number of Participants	94	96	107
Number with Unacceptable Performance (< 100%)	6	4	4
% Unacceptable Performance	6.4%	4.2%	3.7%

Dr Deborah Sage, Histocompatibility & Immunogenetics Department, NHSBT, Tooting

SCHEME 2A: CYTOTOXIC CROSSMATCHING

Introduction: The purpose of Scheme 2A is to assess the ability to correctly determine cell/serum cytotoxic crossmatch status. There were five distributions of two cells – laboratories can register for PBL/T cell only or PBL/T and B cell. Results were to be returned within 10 days and in 2014 there were 78 PBL/T cell and 61 B cell participants. UK&I and RoW laboratories receive different blood samples for testing.

PBL/T cell methodology: There was the usual variety of pre- and post-complement incubation times ranging from 20 to 60 pre-complement with the majority using 30 minutes. Post-complement the incubation varied from 30 to 120 minutes with the majority using 60 minutes. In total for all laboratories there were 14 different combinations – last year there were 15. Half of laboratories used beaded cells which is similar to last year and EB/AO remains the most popular method for visualisation. Generally cells were tested within 3 days of dispatch and generally the viability was greater than 90%.

B cell methodology: Incubation times varied between laboratories, there were 14 different combinations although the main 2 were 30+60 minutes and 60+ 60 minutes. There were a larger proportion of non-returned results for the B cells as compared with T cells: poor viability accounting for almost half (Table 6)

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 in the calendar year. PBL/T cell results were analysed separately from the B cell results.

The performance results for PBL/T cells are shown in Table 1 for UK&I laboratories and Table 2 for RoW laboratories, with a summary in Table 3. The B cell results are in Table 4 and 5, with a summary in Table 6.

Table 1: PBL/T Cell Performance Results by Cycle: UK&I laboratories

Cycle	% Range Correct	Mean % Correct	% of Labs 100% Correct	Number of Unacceptable Results	False Positives / False Negatives
1st	80-100	98.1	90.4	2	1 / 1
2nd	71.4-100	90.9	57.1	14	5 / 9
3rd	0-100	89	77.2	12	12 / 0
4th	75-100	95.4	80.9	5	5 / 0
5th	83.3-100	99.1	95.2	1	1 / 0

Table 2: PBL/T Cell Performance Results by Cycle: RoW laboratories

Cycle	% Range Correct	Mean % Correct	% of Labs 100% Correct	Number of Unacceptable Results	False Positives / False Negatives
1st	50-100	89.9	45.2	28	16 / 12
2nd	71.4-100	96.9	82.6	10	3 / 7
3rd	72.5-100	97.4	88.2	10	10 / 0
4th	66.7-100	94.4	65.9	20	20 / 0
5th	50-100	98.7	96.0	4	4 / 0

Table 3: PBL/T-Cell Summary

PBL/T Cell	UK&I	RoW
Number of participants (Number using PBLs)	23 (8)	54 (20)
Number of XM assessed (>75% consensus)	32/40	35/40
Number of Positive XM	5	5
Number of Negative XM	27	30
% of results not tested	9.8%	9.8%
Number of incorrect assignments	35 (5.3%)	72 (4.4%)
Number of False Pos	25	53
Number of False Neg	10	19
Number of Unacceptable Performers (< 85% correct)	3 (84%, 84%, 84%)	3 (77%, 82%, 82%)

Table 4: B Cell Performance Results by Cycle: UK&I laboratories

Cycle	% Range Correct	Mean % Correct	% of Labs 100% Correct	Number of Unacceptable Results	False Positives / False Negatives
1st	57.1-100	92.1	75	11	0 / 11
2nd	80-100	94.0	75	6	2 / 4
3rd	57.1-100	91.6	65	11	7 / 4
4th	50-100	90.4	79	11	9 / 2
5th	57.1-100	87.9	40	17	5 / 12

Table 5: B Cell Performance Results by Cycle: RoW laboratories

Cycle	% Range Correct	Mean % Correct	% of Labs 100% Correct	Number of Unacceptable Results	False Positives / False Negatives
1st	50-100	95	86.6	6	6 / 0
2nd	57.1-100	94	73.5	14	11 / 3
3rd	50-100	93.6	82.8	9	9 / 0
4th	50-100	93.9	75.7	12	10 / 2
5th	50-100	94.1	80	11	8 / 3

Table 6: B-Cell Summary

B-Cell	UK&I	RoW
Number of participants	21	40
Number of XM assessed (>75% consensus)	32/40	27/40
Number of Positive XM	17	6
Number of Negative XM	11	21
% of results not tested	10.9%	13.5%
Number of incorrect assignments	56 (9.2%)	52 (6.1%)
Number of False Pos	23	44
Number of False Neg	33	8
Number of Unacceptable Performers (< 85% correct)	4 (78%, 78%, 84%, 84%)	3 (63%, 70%, 84%)

Scheme 2A Unacceptable Performance

Six laboratories failed to achieve greater than 85% for PBL/T cell results over the calendar year and were therefore deemed "unsatisfactory". Seven failed to achieve greater than 85% for B cells.

	PBL/T Cells			B Cells		
	2012	2013	2014	2012	2013	2014
Number of Participants	49	65	78	32	50	61
Number with Unacceptable Performance (< 85%)	7	5	6	3	5	7
% Unacceptable Performance	14.3%	7.7%	7.7%	9.4%	10%	11.5%

Results with DTT

Results with DTT were not assessed in 2014.

		PBL/T-Cells		B-Cells	
		UK&I	RoW	UK&I	RoW
Number of participants		17	35	17	33
Number of XM >75% consensus		37/40	36/40	31/40	35/40
Number of Positive XM		5	3	9	5
Number of Negative XM		32	33	22	30
Number of XM same result without & with DTT (neg/neg, pos/pos, NA/NA)		34 (27 / 4 / 3)	36 (30 / 3 / 3)	24 (13 / 8 / 3)	(30) 21 / 4 / 5
Without DTT	**With DTT**				
Assessed	NA	0	1	6	0
NA	Assessed	5	2	5	8
Pos	Neg	1	1	5	2

Changes to Scheme 2A for 2015

There are a number of changes to Scheme 2A for 2015.

Results with DTT will now be assessed for those laboratories who wish to register for it (but considered independently from results without DTT).

Due to the increased number of participants, 3 different blood units will be distributed for each sample;

1) 1 unit for UK&I laboratories
2) 1 unit for RoW laboratories who receive whole blood
3) 1 unit for RoW laboratories who receive isolated lymphocytes

Finally, PBL/T-cell & B-cell assessment will be linked – both must be in line with the consensus results for acceptable performance.

Patrick Flynn, 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 2014 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. T cell and B cell results were considered independently.

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 6 laboratories with unsatisfactory T-cell performance and 12 with unsatisfactory B cell performance (Tables 1-3, figure 1)

Changes to Scheme 2B for 2015

For 2015 T-cell & B-cell assessment will be linked – both must be in line with the consensus results for acceptable performance.

Table 1: Scheme 2B T Cell Result Summary

Scheme 2B T Cell Results	UK&I	RoW
Number of participants	23	47
Number of XM assessed (>75% consensus)	31/40	328/40
Number of Positive XM	14	8
Number of Negative XM	17	20
% of results not tested	4.2%	3.7%
Number of incorrect assignments	45 (6.7%)	76 (6.0%)
Number of False Pos	17	45
Number of False Neg	28	31
Number of Unacceptable Performers (< 85% correct)	2	4

Table 2: Scheme 2B B Cell result Summary

Scheme 2B B Cell Results	UK&I	RoW
Number of participants	20	45
Number of XM assessed (>75% consensus)	34/40	25/40
Number of Positive XM	14	10
Number of Negative XM	20	15
% of results not tested	6.0%	4.6%
Number of incorrect assignments	55 (8.7%)	89 (8.5%)
Number of False Pos	34	51
Number of False Neg	21	38
Number of Unacceptable Performers (< 85% correct)	2	10

Figure 1: Scheme 2B Performance

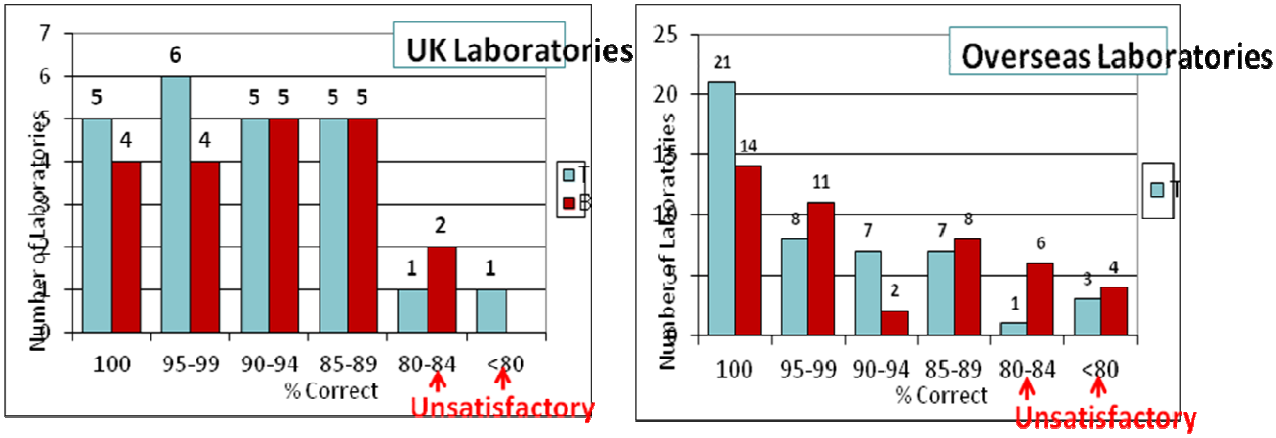


Table 3: Scheme 2B Performance

	PBL/T Cells			B Cells		
	2012	2013	2014	2012	2013	2014
Number of Participants	60	69	71	53	63	65
Number with Unacceptable Performance (< 85%)	10	6	6	8	10	12
% Unacceptable Performance	16.7%	8.7%	8.5%	15.1%	15.9%	18.5%

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 78 laboratories registered for Scheme 3, 25 from the UK&I and 40 from the RoW. For class I antibody analyses, all 78 laboratories participated. Seventy six laboratories registered for class II antibody specificity analysis.

Methods

The majority of participants tested the samples using Luminex technology (77/78 participants), only 1 participant tested using CDC only. 57 participants tested the samples using Luminex only, 18 used Luminex in combination with CDC, 1 lab used ELISA & Luminex and 1 used CDC, ELISA, Flow cytometry and Luminex.

There were more participants that used Luminex kits from One Lambda for testing the samples (53, 68.8%), than used Lifecodes kits (12, 15.6%). 9 participants used a mixture of kits from both suppliers (11.7%) and 4 (5.2%) 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. 17 labs reported the use of EDTA to treat the serum and 2 additional labs reported the use of heat inactivation.

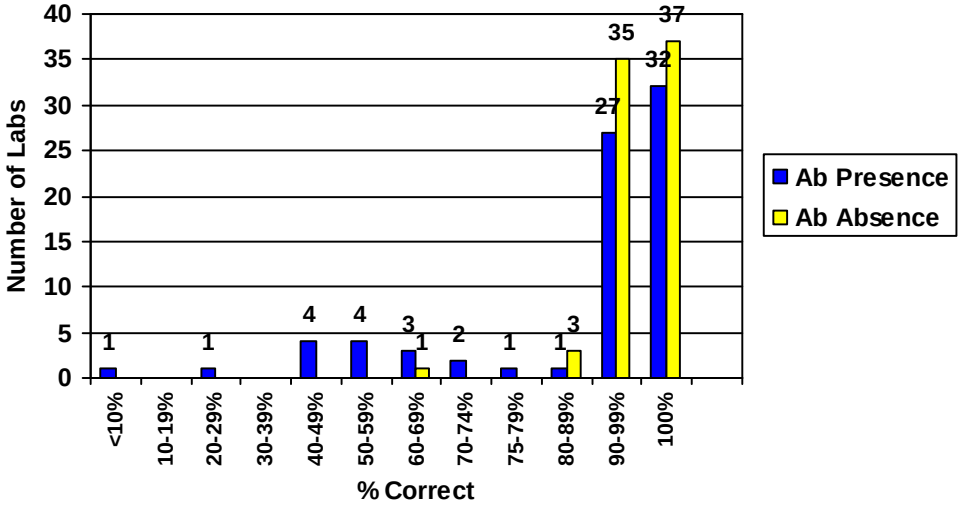
Performance

16 participants had unsatisfactory performance for Class I specificity analysis; 15 labs for antibody presence analysis, and 1 lab for antibody absence analysis (figure 1).

6 participants had unsatisfactory performance for Class II specificity analysis; 5 labs for antibody presence analysis, and 1 lab for antibody absence analysis (figure 2).

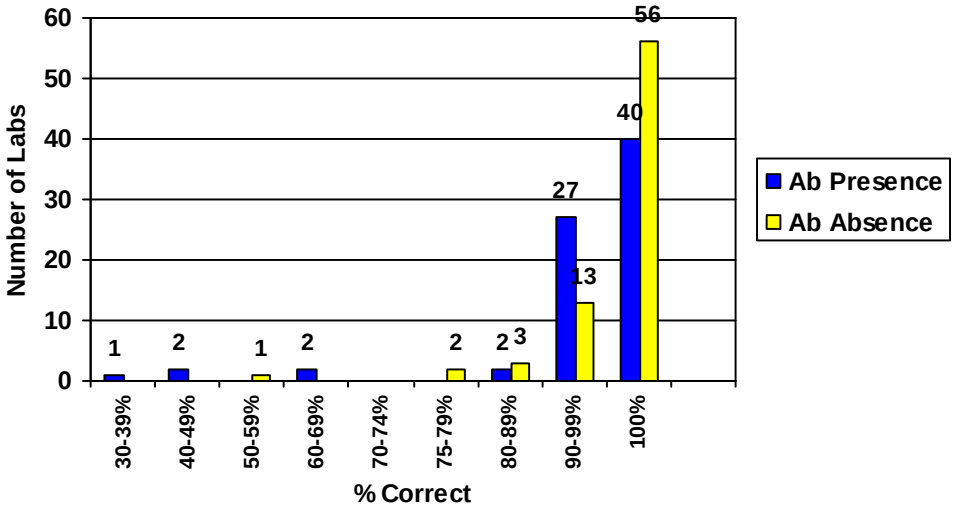
There was no clear correlation of unsatisfactory performance with Luminex kit, although 9/12 (75%) of the Lifecode kit only users had unacceptable Class I 'presence' performance (Table 1).

Figure 1: Class I Performance



Scheme 3 Class I Performance	2012	2013	2014
Number of Participants	51	66	78
Number with Unacceptable Performance (< 75%) Presence / Absence	6/2	11/2	15/1
% Unacceptable Performance Presence / Absence	11.8% 3.9%	16.7% 3.0%	19.2% 1.3%

Figure 2: Class II Performance



Scheme 3 Class II Performance	2012	2013	2014
Number of Participants	48	63	76
Number with Unacceptable Performance (< 75%) Presence / Absence	2 /1	5 / 0	5 / 2
% Unacceptable Performance Presence/ Absence	4.1% 2.1%	7.9% 0.0%	6.6% 2.6%

Table 1: Scheme 3 Unacceptable Performers 2014

Lab No	Class/ Presence (P) Absence (A)	Technique/ Kit(s) Used		%	Lab No	Class/ Presence (P) Absence (A)	Technique/ Kit(s) Used		%
112	CI P	LSA1	Lifecodes	64.7%	235	CI P CII P CII A	LSM12 LS1PRA LS2PRA	One Lamdba	68.2% 61.1% 72.2%
149	CI P	LSA1	Lifecodes	71.2%	238	CI P	LSA1	Lifecodes	47.1%
185	CI P	LM1 LSA1	Lifecodes	74.7%	262	CI P	LSA1	Lifecodes	57.1%
197	CI P CII P CII A	Information not provided		63.2% 64.1% 50.0%	267	CI P	LSM1 LSMUTR	Lifecodes & One Lambda	57.1%
216	CI P CII P	LS1PRA LS2PRA LSM12	One Lambda	48.2% 48.4%	268	CI P	CDC & LMX LM1 LSA1	Lifecodes	52.9%
218	CI P	LSA1 LM2	Lifecodes	48.2%	293	CI P CII P	LS1A04 LS2PRA	One Lambda	56.5% 36.8%
223	CI P	CDC	N/A	4.1%	297	CI P	LSA1	Lifecodes	55.9%
229	CI P CII P	LSA1 LSAII	Lifecodes	28.2% 47.4%	143	CI A	LS1A04	One Lambda	68.7%

Mark Hathaway, Tissue Typing Laboratory, NHSBT Birmingham

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.

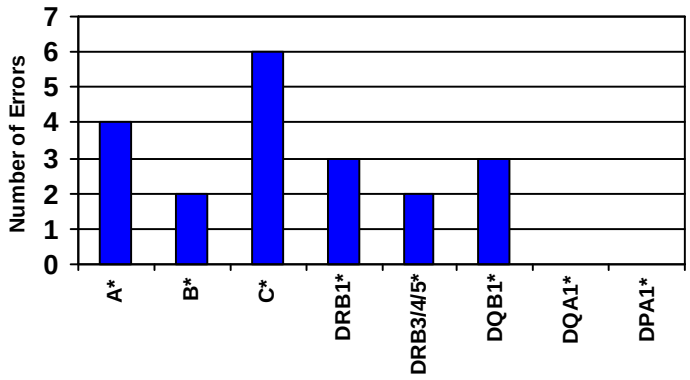
Participants may register for 1st field assessment of HLA-A, B, C, DRB1, DQB1, DQA1, DPA1 and for 1st field or presence of DRB3, DRB4 and DRB5. Participating laboratories are only assessed on the loci for which they have registered.

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

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 9 laboratories classified as unacceptable performers in 2014. 4 of these were due to laboratories not submitting results for loci for which they had registered. The most errors were made typing for HLA-C (figure 1). Laboratories submitting incorrect results used a mixture of SSP and SSOP approaches (Table 1).

Figure 1: Scheme 4A1 errors by HLA locus



Scheme 4A1 Performance	2012	2013	2014
Number of Participants	91	96	96
Number with Unacceptable Performance (< 90%)	6	5	9
% Unacceptable Performance	6.6%	5.2%	9.4%

Table 1: Scheme 4A1 Incorrect Assignments

Sample	Report	Consensus	Method	Lab Number (s)
4A1 01	A*02, 24	A*02, 23	Luminex SSP (kit)	230 266
4A1 02	A*02, 32	A*02, 25	SSP (CTS)	112
	C*08, 07	C*05, 07	SSOP (One Lambda) Luminex (One Lambda)	191 215
4A01 03	C*08, 07	C*05, 07	SSOP (One Lambda) Luminex (One Lambda)	191 197
4A01 04	A*24, 23 C*07, blank	A*24, 32 C*07, 08	SSP(Histotype/BAG Healthcare)	300
	DQB1*02, 03	DQB1*02, 05	SSP(Olerup/Invitrogen)	244
4A01 05	A*23, 32	A*02, 29	Luminex (One Lambda)	215
	B*48, 40	B*39, 40	SSP (CTS)	112
	C*07, blank	C*03, 07	SSP (Invitrogen)	190
	DRB1*07, 13	DRB1*04, 13	Luminex (One Lambda)	215
	DRB1*04, 14		SSP(Olerup/Invitrogen)	244
	DRB1*04, 03		SSP(Histotype/BAG Healthcare)	300
	DRB3*01	DRB3*03	Luminex (Biorad/Olerup)	15
	DRB4*blank	DRB4*present	Luminex (Immucor)	231
	DQB1*blank, 06	DQB1*03, 06	SSP(Histotype/BAG Healthcare)	300
4A1 07	DQB1*blank, 06	DQB1*03, 06	SSP/Luminex (kits)	242
4A1 09	B*07/ 42, 08	B*07, 08	Luminex (One Lambda)	103
4A1 10	C*05, blank	C*04, 05	SSP (One Lambda)	123
	C*07, 05	C*04, 05	SSP (Olerup)	300
4A01 06-10	C not reported	Registered for HLA-C	SSP (Olerup)	252
4A01 06-10	DQA1 not reported	Registered for DQA1	Luminex (Olerup)	159
	DQB1 not reported	Registered for DQB1	SSP (Olerup)	252
	DRB3/4/5 not reported	Registered for DRB3/4/5	Luminex (kit)	301

Leigh Keen, H&I Laboratory, 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 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.

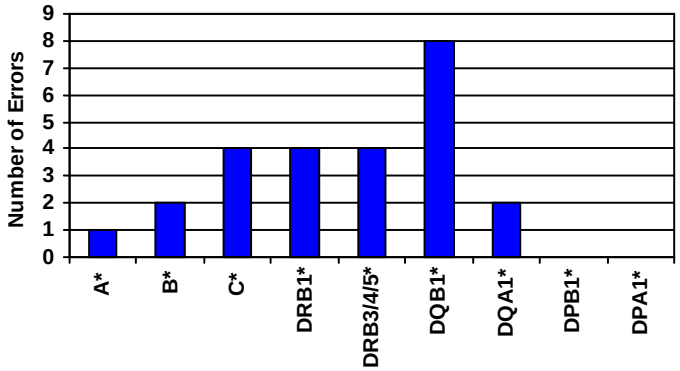
Performance

Satisfactory performance involves obtaining 9 or more full HLA genotypes in agreement with consensus in a calendar year.

59 laboratories participated in 2014, and there were 5 laboratories with unacceptable performance.

The most errors were made typing for HLA-DQB1 (figure 1). Laboratories submitting incorrect results used a mixture of SSP and SSOP approaches (Table 1).

Figure 1: Scheme 4A2 errors by HLA locus



	2012	2013	2014
Number of Participants	50	58	59
Number with Unacceptable Performance (< 90%)	5	5	5
% Unacceptable Performance	10%	8.6%	8.5%

Table 1: Scheme 4A2 Incorrect Assignments

Sample	Report	Consensus	Lab Number
4A2 01	A*02:01,11:02	A*02:01, 11:01	123
4A2 02	B*15:16, 44:02/03	B*15:16, 44:03	112
4A2 06	B*07:02/05/06/29/61, 08:01/07	B*07:02, 08:01	25
	C*07:01/02/06/18/19/50, 07:02/06/18/27/50	C*07:01, 07:02	
4A2 07	B*50:01, 40:02	B*40:01, 40:02	123
4A2 09	C*03:03/04 , 03:04/20N	C*03:03, *03:04	25
	C*03:03, blank		123, 242
	C*03:04, blank		133
4A2 01	DQB1*03:01, 03:01	DQB1*03:01, 03:02	165
	DQB1*03:01/03:02, 03:02/03:19		267
4A2 02	DRB4*01:01, 01:02	DRB4*01:03, 01:03	113
	DRB4*01:01/03:01N , 01:03		158
	DQA1*02:01, 03:01	DQA1*02:01, 03:03	165
	DQB1*02:01, 05:01	DQB1*02:02, 03:02	248
	DRB1*04:05, 07:01/05/11/13	DRB1*04:05, 07:01	267
4A2 03	DRB3*01:01/02	DRB3*01:01	267
4A2 04	DRB1*01:01/07, 15:01/20	DRB1*01:01, 15:01	267
4A2 05	DRB4*01:01/03:01N, 01:03	DRB4*01:01, 01:03	158
	DQA1*02:01, 03:01	DQA1*02:01, 03:03	165
	DRB1*04:01, 07:01/05/11/13	DRB1*04:01, 07:01	267
4A2 06	DQB1*02:01, 06:01	DQB1*02:01, 06:02	147
4A2 07	DQB1*06:03/26N, 06:04/34/36/38/39	DQB1*06:03, 06:04	103
	DQB1*06:03/39, 06:04		156
	DQB1*06:04, blank		259
	DRB1*1302, blank	DRB1*1301, 1302	112
4A2 08	DQB1*02:01, 03:01	DQB1*02:01, 03:02	128

Fotini Partheniou, H&I Dept, NHSBT Newcastle.

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 2014 there were 10 participants. All participants used PCR-SSP technology with 8 using commercial kits and the remainder using 'in house' developed primer sets.

There were no unsatisfactory performers in 2014.

Scheme 4B Performance	2012	2013	2014
Number of Participants	7	7	10
Number with Unacceptable Performance (< 100%)	1	1	0
% Unacceptable Performance	14.3%	14.3%	0%

Jennifer Pepperall, Welsh Transplantation and Immunogenetics Laboratory, Cardiff

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 2014

5A Samples Distributed			
Codon 63	**Codon 282**	**Codon 65**	**Number Of Samples**
HD	CY	SS	2
HH	CC	SS	6
HH	YY	SS	1
HH	CY	SS	1

Performance

In 2014 there were 59 participants; 49% (n=29) reported codon 65 results, and 1 lab reported codon 168. The most common technique used was RT-PCR.

There were 2 laboratories with unacceptable performance (table 2).

Table 2: Scheme 5A Incorrect Assignments

Scheme 5A Misassignments				
Sample	**Codon**	**Report**	**Consensus**	**Lab Number**
5A01	282	CC	CY	13
5A07	63	DD	HD	96

Scheme 5A Performance	2012	2013	2014
Number of Participants	58	58	59
Number with Unacceptable Performance (< 100%)	3	2	2
% Unacceptable Performance	5.2%	3.9%	3.4%

Jennifer Pepperall, Welsh Transplantation and Immunogenetics Laboratory, Cardiff

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 2014 was achieved by obtaining four 'acceptable' classifications in the year.

Participation and results

Returns were received from 20 participants for the first distribution (scenarios 1 and 2) and 19 participants made returns for the second distribution (scenarios 3 and 4).
Maximum possible penalty was 5 points per scenario, 20 points in total.

1	lab got	2 penalty points (0 points on 2 scenarios)
5	labs got	3 penalty points
6	labs got	4 penalty points
1	lab got	5 penalty points
2	labs got	6 penalty points
2	labs got	7 penalty points
1	lab got	8 penalty points
1	lab got	10 penalty points

Average Annual total points was 4.7.

Satisfactory performance was achieved by all but four participants.

Table 1. Performance Summary

	Scenario 1	Scenario 2	Scenario 3	Scenario 4
No. of labs with 0 penalty points	**3**	**3**	**9**	**2**
No. of labs with 1 penalty point	**11**	**6**	**7**	**12**
Labs with 0 or 1 penalty points	**70 %**	**45 %**	**84 %**	**74 %**
Average penalty	**1.15**	**1.6**	**0.74**	**1.32**
No. of labs with classification "unacceptable" (3+ pts)	**0**	**2**	**1**	**2**

General deficiencies identified in reports included the following:

- Not relating genotype to phenotype
- Mis-interpreting the biochemical iron indices – in particular the significance of a normal serum Tfsat with raised ferritin
- Not suggesting that secondary causes of iron overload or of raised iron indices should be considered
- Not stating that other rarer mutations or types of haemochromatosis were not ruled out
- Advising "regular" monitoring of iron indices without saying how often
- Failing to advise testing of first degree relatives
- Advising family testing but not advising against genetic testing of minors for this adult-onset condition

Alan Balfe, Molecular Diagnostics Laboratory, Dept. of Clinical Biochemistry, St. James's Hospital, Dublin.

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 2014 there were 83 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	0	0
ELISA only	1	0
FLOW only	1	1
LUMINEX only	57	56
Luminex + CDC	16	20
Luminex + ELISA	1	3
Luminex + Flow	1	0
Luminex + CDC + ELISA	1	1

Sensitivity and Specificity: Of the 20 sera distributed 14 had provisional specificities assigned to them based on historic testing which was predominantly, although not exclusively, CDC testing (Table 2). The remaining 6 sera were AB serum or "hidden negatives". A number of sera distributed this year were sera which had been previously distributed as either Scheme 3 or Scheme 6 sera. Table 2 shows their provisional specificity and the percentage of laboratories reporting class I and class II positivity. Only one Class I result was not assessed as it did not reach the 75% consensus level (sample 609/14), which was reported as positive by 74% of participants.

Table 2: Provisional Specificities and Concordance

Serum ID	Provisional Specificity	% of labs detecting Ab	% class I positive	% class II positive
601	CDC DQ3; LUM B7creg, A66, A34, DQ3, DR4	100	88.3	100
602	CDC DQ2	100	100	100
603	LUM A9, Bw4, DR7, DR2+	98.7	98.7	98.7
605	LUM B5+, DQ1+	100	100	100
607	CDC DR17 LUM BW6+, DR3+	100	100	100
608	LUM A2, B17, DR11, DQ3, DP	81.8	81.8	76
609	CDC DQ2; LUM A3+, DQ2, DQ3, DP+	100	74	100
611	CDC A10, B37, DR52	100	100	100
612	CDC A1, A29,	100	100	100
613	LUM B62+	97.5	97.5	22
616	CDC DQ3	100	78.8	100
617	CDC DR17; LUM B8, (DQ2)	95	95	7.7
618	CDC DQ2 LUM A1, A9, A11, A25, A36, A43, A80; B8, B44, B76, B82; Cw 7; DQ2; DR4, DR7, DR9, DR13:03	100	100	100
620	CDC DQ7; LUM A34, A66; B7, B27, B60, B61, B81; DQ3, DQ4	100	95	100

Performance: Satisfactory performance is making 80% of reports on all sera in agreement with consensus in a calendar year. In 2014 5 laboratories failed to reach 80% these are summarised in Figure 1 and Table 3. Overall there were more false negative results (57%) than false positive results.

Figure 1: Scheme 6 Performance

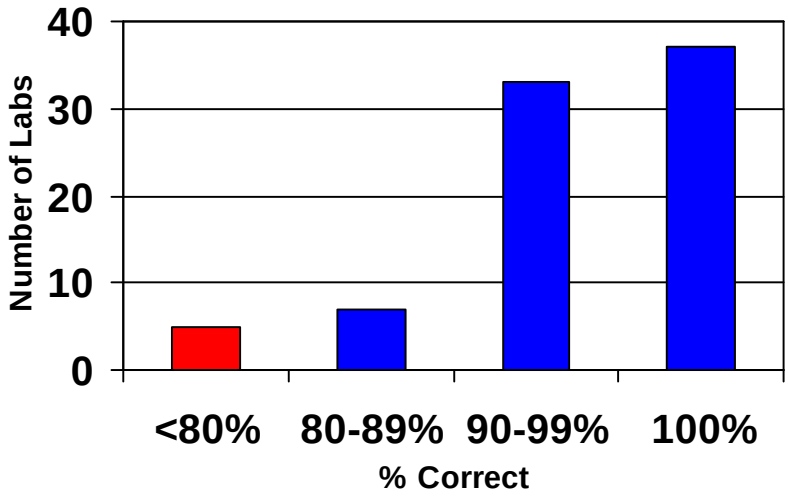


Table 3: Scheme 6 incorrect assignments

Lab No	Techniques Used	% Correct
20	CDC & LSM12 (One Lambda)	75.0%
117	ELISA (LATM10) & LSM12 (One Lambda)	78.9%
191	LSM12 (One Lambda)	75.0%
229	LMX (Immucor)	70.0%
303	LSM12 (One Lambda)	65.0%

Scheme 6 Performance	2012	2013	2014
Number of Participants	57	68	83
Number with Unacceptable Performance (< 80%)	0	3	5
% Unacceptable Performance	0%	4.4%	6.0%

Patrick Flynn, Transplantation Laboratory, Manchester Royal Infirmary

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

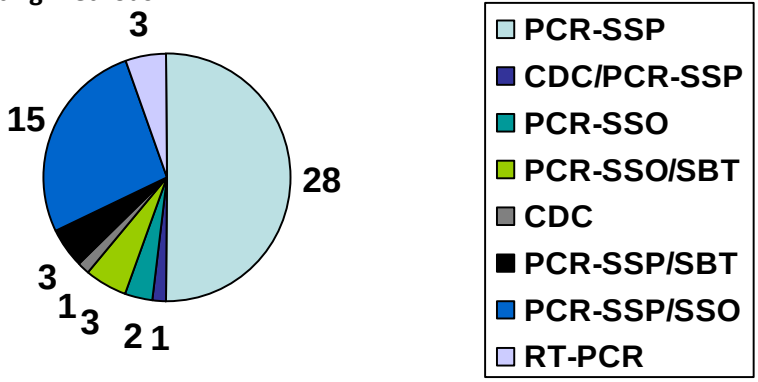
The requirement for B*57:01 typing is founded on the finding that hypersensitivity to abacavir - a nucleoside analogue reverse transcriptase inhibitor used to treat HIV and AIDS patients - is strongly associated with possession of B*57:01. HIV/AIDS treatment guidelines firmly recommend B*57:01 testing 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 2014 two distributions were made of 5 blood samples each and there were 56 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 1 laboratory with unsatisfactory performance in 2014, with a false positive result (Table 1)

Table 1: Scheme 7 unsatisfactory performance

Sample	Result	Lab Number	Technique	HLA Type
707	False Pos	181	SSP	B44, -

Scheme 7 Performance	2012	2013	2014
Number of Participants	42	47	56
Number with Unacceptable Performance (< 100%)	1	0	1
% Unacceptable Performance	2.4%	0.0%	1.8%

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 2014 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 for the loci they tested in a clinical setting and at the resolution level they normally reported.

Assessment was made for the loci reported and at the allele family level or the allele to the 2nd field, in agreement with the Scheme 4A2's consensus type.

Satisfactory performance was achieved by obtaining at least 9 genotypes in accord with the consensus type.

There were 21 participants in 2014, and three laboratories with unacceptable performance (Table 1). A large number of 'reporting' errors were made by several participants who were new to the scheme for 2014 (Table 2).

Table 1: Scheme 8 Unacceptable Performance

Scheme 8 Unacceptable Performance	2013	2014
Number of Participants	19	21
Number with Unacceptable Performance (< 90%)	2	3
% Unacceptable Performance	10.5%	14.3%

Changes for 2015

The minimum satisfactory performance criteria is changing from 9/10 samples correct to 10/10 samples correct to bring this scheme in line with the other UK NEQAS for H&I disease association schemes.

Table 2: Scheme 8 incorrect assignments

Sample	Report	Consensus	Lab Number
801	DQA1*02:01, 03:01	DQA1*02:01, 03:03	26
	DQA1*05 present		173
802	DQA1*01:02, 05:01	DQA1*01:02, 05:09	26
	DQA1*05:01/05 present		16
	DQ2 present	DQB1*03:01, 06:02	272
803	DQA1*02:01, 05:01	DQA1*02:02, 05:05	26
	DQB1*03:02, blank	DQB1*03:01, 03:03	85
	DQB1*03:01, 03:02		272
	DQB1*03:01, 03:02		274
805	DQA1*05:01, 05:01	DQA1*05:01, 05:05	26
806	DQA1*03:01, 05:01	DQA1*03:03, 05:01	15
	DQA1*02		278
	DQB1*02, 03:02	DQB1*02:01, 03:01	278
807	DRB3*01	DRB4*01:01, 01:03	24
	DQB1*03:02 present	DQB1*02:02, 03:01	278
808	DRB3*01	DRB4*01:03, DRB5*01:01	24
	DQA1*03, 03:01	DQA1*01:02, 03:01	278
810	DQB1*03:02 present	DQB1*02:01, 05:01	278

Ruhena Sergeant, Clinical Immunology Laboratory, Hammersmith Hospital, London

EDUCATIONAL SCHEME

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

Some 40 labs participated in 2014 - about 20 UK and 20 non-UK labs

In 2014 all 4 Educational Scheme samples were sent as DNA extracts. The alleles of interest were: ED01/14 – C*04:03, ED02/14 – A*24:21, ED03/14 – A*24:17, ED04/14 – A*32:04. 44 laboratories reported on the samples – the findings were:

- **ED01/14 – HLA-C*04:03**

HLA-C*04:03 is most similar to C*04:01, differing by 10 nucleotides, 9 of which are located in the region from nucleotide 98 to 218. This region of C*04:03 is identical to both C*02:01 and C*02:02:02.

The 9 nucleotide differences between C*04:01 and C*04:03 results in 6 amino acid differences in the alpha 1 domain. C*04:01 and C*04:03 also differ in a coding substitution at nucleotide 979 in exon 5.

The guanine found in C*04:03 is identical to all HLA-C alleles except HLA-C*04:01, which has an adenine. Thus, C*04:03 was most likely formed by a gene conversion event between C*02 and C*04, involving a minimum of 121 to a maximum of 215 nucleotides (*Tissue Antigens 1996*, **48**, 113).

38 DNA-based HLA-C findings:

26 reports of C*04:03 and 4 of C*04:03:01 – thus 80.0% of labs identified this allele
1 report of C*04:03/06-107
7 reports of C*04

- **ED02/14 – HLA-A*24:21**

A*24:21 differs from A*24:02:01:01 at codon 127 (AAA>AAC) which results in a single amino acid change of lysine (K) to asparagine (N) in the alpha 2 domain connecting loop *(Eur J Immunogenet* 2004, **31**, 234).

40 DNA-based HLA-A findings:

19 reports of A*24:21and 8 of A*24:21:01 - thus 67.5% of labs identified this allele
11 reports of A*24
1 report of A*24:02 and 1 report of A*24:02/02L-226

- **ED03/14 – HLA-A*24:17**

A*24:17 differs from A*24:02:01:01 at nucleotide positions 413, 414 and 418 in exon 3. This results in amino acid differences at codons 114 and 116 (*Int J Immunogenet* 2008, **35,** 481).

41 DNA-based HLA-A findings:

27 reports of A*24:17, 2 of A*24:17/41 and 1 of A*24:17/90N – thus 73.2% of labs identified, or came close to identifying, this allele
10 reports of A*24
1 report of A*24:02/17-208

- **ED04/14 – HLA-A*32:04**

A*32:04 is essentially a 'hybrid' of A*03:01 and A*32:01. Thus, A*32:04 is almost identical to A*32:01:01 (except position 180) in exon 2 but identical to A*03:01:01:01 in exon 3 (*Tissue Antigens* 2000, **55**, 369; *Eur J Immunogenet*. 2002, **29,** 355).

40 DNA-based HLA-A findings:

33 reports of A*32:04 – thus 82.5% of labs identified this allele
7 reports of A*32

A*32:04 distribution in 2002

A*32:04 was also distributed in 2002 as a whole blood sample – there were 34 DNA-based reports. A*32:04 was assigned by 17 labs (50.0%), while 9 reported A*03 or an A*03 group of alleles, 5 assigned A*32, 2 missed A*32:04 and 1 reported A*03:08.

Chris Darke, UK NEQAS for H&I Director

INTERPRETATIVE EDUCATIONAL SCHEME CLINICAL SCENARIOS

A new element of the Educational Scheme was launched in 2013 with the introduction of clinical interpretative scenarios (iED).

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. Two scenarios were distributed in 2014 to participants registered for other relevant UK NEQAS for H&I schemes. 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 deceased donor kidney transplant case.

The HLA typing information of the recipient and potential deceased donor were presented along with HLA antibody testing and crossmatch results. Participants were asked to complete questions based on the results provided. This included what specificities they would list as unacceptable for transplantation, if they thought the crossmatch results were a contraindication to transplantation, and the clinical advice they would give on expected outcome if the transplant proceeded.

Results were received from 50 participants.

Clinical Scenario 2: Haematopoietic Stem Cell Transplantation

The second scenario was based on a based on a patient who was referred to the laboratory for a haematopoietic stem cell transplant (HSCT).

Information was provided on the HLA type of the patient, siblings along with unrelated donor search results. Participants were asked to complete questions based on the results provided. This included the selection of suitable donors form the unrelated donor search and the clinical advice that would be given if transplantation was to proceed with a mismatched donor.

Results were received from 42 participants.

For 2015

Three clinical scenarios are to be distributed in 2015. Participants of other UK NEQAS fro H&I schemes are able to register to receive the solid organ transplant scenario and/or a HSCT scenario and/or platelet transfusion support cases.

Deborah Pritchard, UK NEQAS for H&I Manager

3. NUMBER OF PARTICIPANTS DURING 2014

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 2014.

Scheme	UK and ROI	RoW
Scheme 1A	9	33
Scheme 1B	51	56
Scheme 2A	23	53
Scheme 2B	23	47
Scheme 3	26	53
Scheme 4A1	31	65
Scheme 4B	5	5
Scheme 4A2	21	38
Scheme 5A	49	9
Scheme 5B	19	1
Scheme 6	26	58
Scheme 7	24	32
Scheme 8	10	12
Educational Scheme Samples	10	22
Educational Scheme Clinical Scenarios	21/23	29/28

ROI – Republic of Ireland
RoW – Rest of the World

4. 2014 ANNUAL PARTICIPANT MEETING – BRISTOL

64 participants representing 22 laboratories attended the UK NEQAS for H&I annual participant meeting in Bristol on the 3rd December 2014.

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, Schemes' Manager.

The 2014 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.

5. FOR 2015 PLEASE NOTE

Laboratories will retain their code numbers for 2015. Laboratory code information is known only to the Scheme Manager and UK NEQAS for H&I staff.

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 2014 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