UKNEQAS

# HISTOCOMPATIBILITY

# and

# IMMUNOGENETICS

# Annual Report

# 2012

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**April 2013**

*United Kingdom National External Quality Assessment Service*

UKNEQAS for HISTOCOMPATIBILITY and IMMUNOGENETICS
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## 1. INTRODUCTION

### Scheme 5B: Interpretative: HFE Genotype and Hereditary Haemochromatosis

This scheme has now - 2012 - been operating for five years. The Steering Committee are grateful for the work of the 'Scheme 5B Expert Assessors Group' in compiling the clinical scenarios and 'marking' the laboratory reports. We particularly thank Dr Dairena Gaffney (Glasgow) for all her hard work and enthusiasm in her role as Lead Expert Assessor during the last three years and we wish her well in her retirement later in 2013. Dr Alan Balfe (Dublin) will take over from Dairena for 2013. A warm welcome to Carol Hardy (Birmingham) who will join Alan's team - with the long standing Dr Gavin Willis (Norwich) - in 2013.

The Steering Committee have learned a great deal from the operation of this interpretative scheme and will offer a small 'pre-pilot' (NEQAS jargon for a trial) interpretative scheme involving clinical scenarios for kidney and blood stem cell transplantation in 2013.

### Overall Laboratory Performance

I am often asked the question "How well do UK NEQAS for H&I participant laboratories perform in their H&I Schemes". There is of course no simple answer – we operate 11 schemes that are assessed, each scheme is very different, laboratories might participate in just one, a selection, or all schemes and performance gently ebbs and flows from one year to another. However, below is a simple table that shows the number of laboratories that participated in each of the assessed schemes for 2012 and the percentage of laboratories that did not receive an 'Unacceptable Performance' (UP) letter in 2012. When considering these values it is vital that the assessment and performance criteria for each scheme are taken into account – This is available in our Prospectus.

| Scheme | Samples/year | Number of participants in 2012 | Number of participants sent a UP letter | % participants not receiving a UP letter |
|---|---|---|---|---|
| 1A | 10 | 22 | 1 | 96 |
| 1B | 10 | 94 | 6 | 94 |
| 2A | 40 (sera) | 49 | 7 | 86 |
| 2B | 40 (sera) | 61 | 15 | 75 |
| 3 | 10 | 51 | 6 | 88 |
| 4A1 | 10 | 95 | 6 | 94 |
| 4B | 10 | 7 | 1 | 86 |
| 4A2 | 10 | 51 | 5 | 90 |
| 5A | 10 | 58 | 3 | 95 |
| 6 | 20 | 57 | 0 | 100 |
| 7 | 10 | 42 | 1 | 98 |

**Steering Committee People**

Sincere thanks are due to Dr Leigh Keen (Bristol) for all his hard work over the past five years as the BSHI Representative to the UK National Quality Assurance Advisory Panel (NQAAP) for Immunology – and sitting on the Steering Committee as an observer. Leigh is now an Ordinary Committee Member. We warmly welcome Dr Helena Lee (Manchester) as the new BSHI Representative to the UK NQAAP for Immunology.

**The UK NEQAS for H&I Team at the Welsh Blood Service**

This small, enthusiastic and hardworking team is headed by Susan Corbin, Schemes' Manager, with Melanie Bartley, Clinical Scientist, and two MTOs, Geraint Clarke and Luke Gardner. Luke replaced our Joanne Thomas in September 2012. Joanne was a valued and long standing member of the team who has subsequently become a mother and is now training as a theatre nurse – we wish her well.

Heartfelt thanks are due to them all for their hard toil and commitment to UK NEQAS for H&I during 2012.

*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 is summarised below:

| HLA | Participants n=22 (UK 10) |
|---|---|
| A | 22 (10) |
| B | 22 10) |
| Cw | 7 (3) |
| DR | 15 (9) |
| DQ | 15 (9) |
| HLA-A, B, | 6 (1) |
| HLA-A, B, C | 1 |
| HLA-A, B, DR | 0 |
| HLA-A, B, DR, DQ | 9 (6) |
| HLA-A, B, C, DR, DQ | 6 (3) |

***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 (8 labs); Biotest (3 labs); BioRad (6 labs); other combinations (5 labs)

***Cell preparation***
Nine laboratories tested samples using unseparated cells, 9 used T&B cell preparations, 4 used T cells only and 2 used unseparated cells plus T and 2 used unseparated cells plus B cells and 1 lab used unseparated cells plus T and B cells.

***Scheme 1A samples for 2012***
The HLA types of the 10 samples distributed in 2011 are shown below:

| Sample No | A | A | B | B | Cw | Cw | DR | DR | DQ | DQ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1A01 | 2 | 68 | 44 | 62 | 3 | 7 | 11 | blank | 7 | blank |
| 1A02 | 1 | blank | 37 | 57 | 6 | blank | 1 | 7 | 5 | 9 |
| 1A03 | 2 | 30 | 7 | 18 | 5 | 7 | 9 | 17 | 2 | 9 |
| 1A04 | 30 | 68 | 44 | 65 | 8 | blank | 7 | 13 | 2 | 7 |
| 1A05 | 3 | 26 | 18 | 57 | 6 | 7 | 7 | 17 | 2 | 9 |
| 1A06 | 11 | 68 | 44 | 60 | 3 | 7 | 4 | 11 | 7 | 8 |
| 1A07 | 3 | blank | 27 | 56 | 1 | 7 | 8 | 13 | 2 | 6 |
| 1A08 | 29 | 30 | 18 | 44 | 5 | blank | 7 | 17 | 2 | blank |
| 1A09 | 2 | 29 | 44 | 62 | 3 | blank | 1 | 7 | 2 | 5 |
| 1A10 | 2 | 29 | 8 | 44 | 7 | blank | 7 | 17 | 2 | blank |

***Incorrect assignments in 2012: HLA-A, B, C, DR & DQ by sample***

| Sample | Misassignment | Lab | Consensus |
|---|---|---|---|
| 1A01 | B62 called B75 | a | B44, B62 |
| 1A04 | B65 called B14 | b | B44, B65 |
| 1A06 | Missed DQ8 | c | DQ7, DQ8 |
| | B60 called B48/81 | a | B44, B60 |
| 1A08 | Missed B18 | d | B18, B44 |
| | A30 called A31 | e | A29, A30 |
| | False DQ3 | f | DQ2 |
| | Cw16 called Cw8 | g | Cw5, Cw16 |
| 1A09 | Missed A2 | h | A2, A29 |

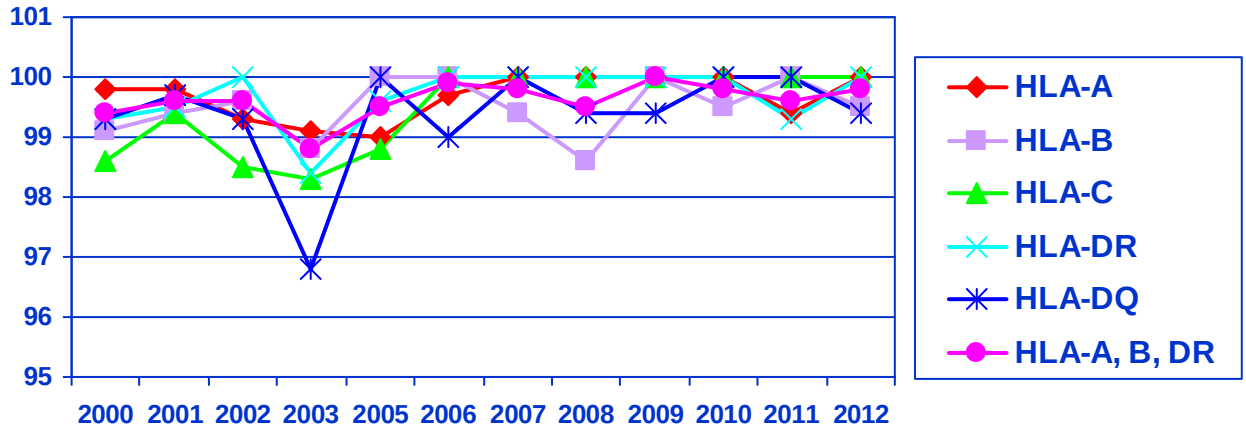
***Performance in 2012***

There were nine incorrect assignments made in 2021 involving 2 UK and 6 non-UK laboratories. Overall 21 laboratories achieved satisfactory performance with one laboratory considered to have unsatisfactory performance. There was no correlation with reagents or testing strategy used and the misassignments reported.

***Overall 2012 Accuracy Rates***

| Antigen | Error | Assignments | Accuracy Index (%) | 2010 (%) |
|---|---|---|---|---|
| HLA-A | 2 | 440 | 99.5 | 99.4 |
| HLA-B | 4 | 440 | 99.1 | 99.2 |
| HLA-Cw | 1 | 140 | 99.3 | 100 |
| HLA-DR | 0 | 300 | 100 | 99.2 |
| HLA-DQ | 2 | 300 | 99.3 | 100 |
| HLA-A,B,DR | 6 | 1180 | 99.5 | 99.3 |
| | | | | |
| UK | | | | |
| HLA-A | 0 | 200 | 100 | 99.4 |
| HLA-B | 1 | 200 | 99.5 | 100 |
| HLA-Cw | 0 | 60 | 100 | 100 |
| HLA-DR | 0 | 180 | 100 | 99.3 |
| HLA-DQ | 1 | 180 | 99.4 | 100 |
| HLA-A,B,DR | 1 | 580 | 99.8 | 99.6 |

***Overall Accuracy rates in UK***




Overall accuracy is very good for this scheme. In the UK two errors involving misassigned HLA-B and HLA-DQ types occurred in 2012.

*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.

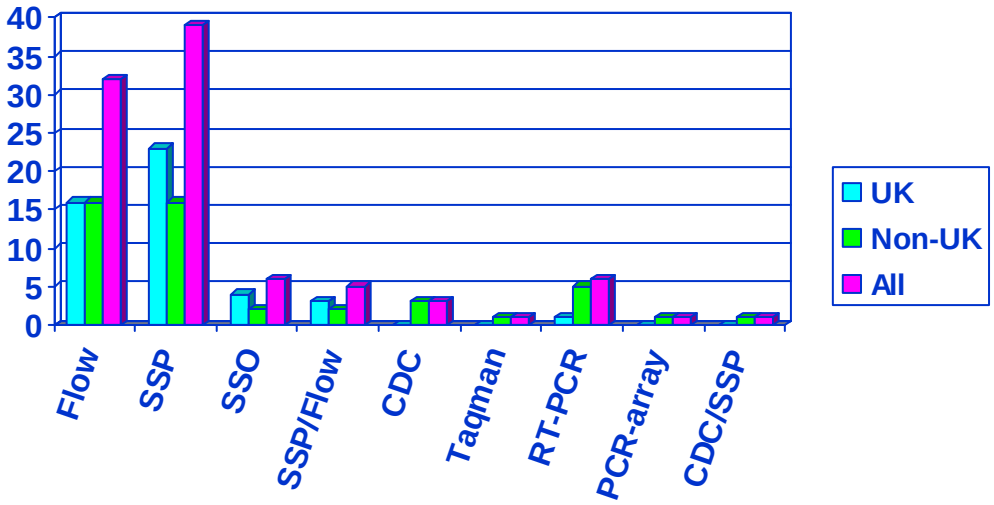
In 2012 there were 94 participants in the scheme (47 UK laboratories).

***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: Monoclonals 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: Assignments outside of consensus***

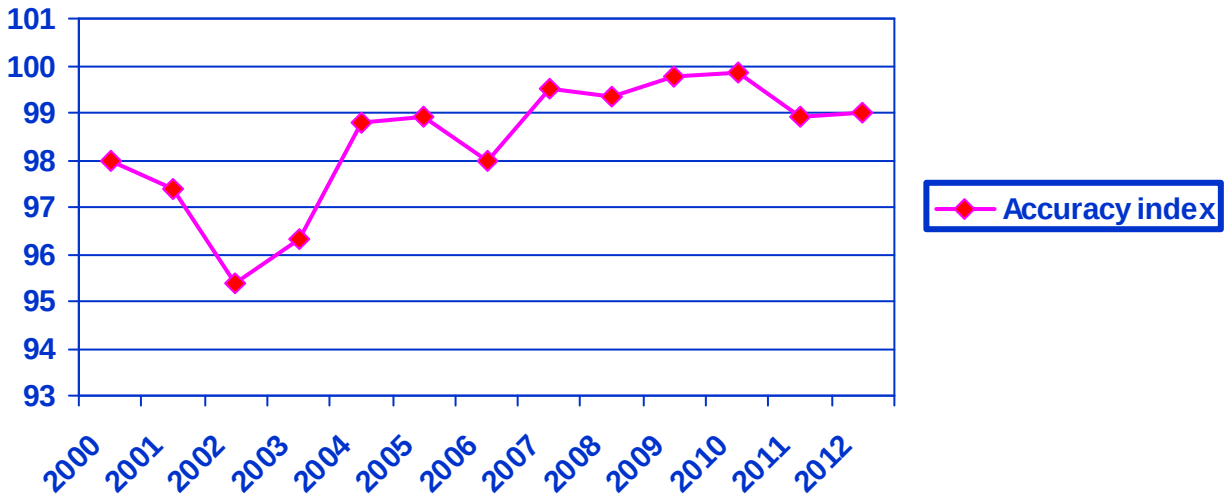
In 2012, four samples distributed were HLA-B27 positive. Nine assignments were made outside of consensus in 2012 involving 7 UK laboratories and 2 non-UK laboratories (see table below). Seven misassignments were made by laboratories using flow cytometry, one by flow plus PCR-SSP and one by PCR-SSP only.

| Sample | Result | No. of labs | Technique | HLA Type |
|---|---|---|---|---|
| 1B01 | equivocal | 1 | flow | B64, B27 |
| | false neg | 1 | flow | |
| 1B02 | equivocal | 1 | flow | B7 ,B44 |
| 1B03 | false neg | 1 | flow | B27, B65 |
| 1B05 | false pos | 1 | flow | B7, B61 |
| 1B06 | equivocal | 1 | flow | B27, B62 |
| 1B07 | false pos | 1 | flow + SSP | B7, B65 |
| 1B08 | false pos | 1 | flow | B7, B70 |
| 1B10 | false pos | 1 | SSP | B7, B57 |

***Overall performance***

Eighty one laboratories achieved acceptable performance and thirteen were considered to have unacceptable performance (5 due to not testing samples).

***Overall accuracy rates: HLA-B27 TESTING***




*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. This is the third year of the revised format where the sera were distributed at the same time as the cells as oppose to previous years where the sera were plated out at the start of the year. 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 2012 there were 48 PBL/T cell and 32 B cell participants.

### *PBL/T cell methodology*

There was the usual variety of pre- and post-complement incubation times ranging from 20 to 62 pre-complement with the majority using 60 minutes. Post-complement the incubation varied from 40 to 120 minutes with the majority using either 60 or 120 minutes. In total for the 48 laboratories there were 16 different combinations – last year there were 15. Under half of laboratories used beaded cells which is less than 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%. Result were not returned on 26 occasions (5.4%): 14 due to poor viability, 2 insufficient cells, 2 technical problems, 2 where the tubes were incorrectly labelled, 4 deliver delay and for the remaining 2 no reason was given.

### *B cell methodology*

Thirty two laboratories submitted B cell data. Again incubation times varied between laboratories, there were 9 different combinations although the main 2 were 30+60 minutes and 60+120 minutes. There were a larger proportion of non-returned results for the B cells (32 occasions; 10%) as compared with T cells: poor viability accounting for over half.

### *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 and for B cells in Table 2. Overall performance for the calendar year is shown in Figures 1 and 2. Seven laboratories failed to achieve greater than 85% for PBL/T cell results over the calendar year and were therefore deemed “unsatisfactory” and are shown in red in Figures 1. Two of these laboratories plus one other also failed to achieve greater than 85% for B cell results.

***Table 1: PBL/T Cell Performance Results by Cycle***

| Cycle | Percentage Correct Range | Percentage Correct Mean | Percentage of Labs 100% Correct | Number of Unacceptable Results (%) | False Positives / False Negatives |
|---|---|---|---|---|---|
| 1st | 57 – 100 | 91.5 | 53 | 31 (9%) | 18 / 13 |
| 2nd | 50 – 100 | 97.1 | 61 | 31 (8.4%) | 10 / 21 |
| 3rd | 57.1 – 100 | 86.1 | 73 | 20 (5.9%) | 11 / 9 |
| 4th | 42.9 – 100 | 94.7 | 87 | 7 (2.5%) | 2 / 5 |
| 5th | 66.7 - 100 | 96.3 | 82 | 10 (3.7%) | 3 / 7 |

***Table 2: B Cell Performance Results by Cycle***

| Cycle | Percentage Correct Range | Percentage Correct Mean | Percentage of Labs 100% Correct | Number of Unacceptable Results (%) | False Positives / False Negatives |
|---|---|---|---|---|---|
| 1st | 57.1 – 100 | 91.5 | 55 | 13 (8.3) | 5 / 8 |
| 2nd | 60 – 100 | 97.2 | 93 | 4 (2.8) | 4 / 0 |
| 3rd | 50 – 100 | 86.1 | 55 | 25 (14.8) | 4 / 21 |
| 4th | 66.7 – 100 | 94 | 70 | 9 (4.6) | 5 / 4 |
| 5th | 71.4 - 100 | 94.76 | 73 | 11 (5.3) | 10 / 1 |

***Figure 1: Calendar Year PBL/T Performance***

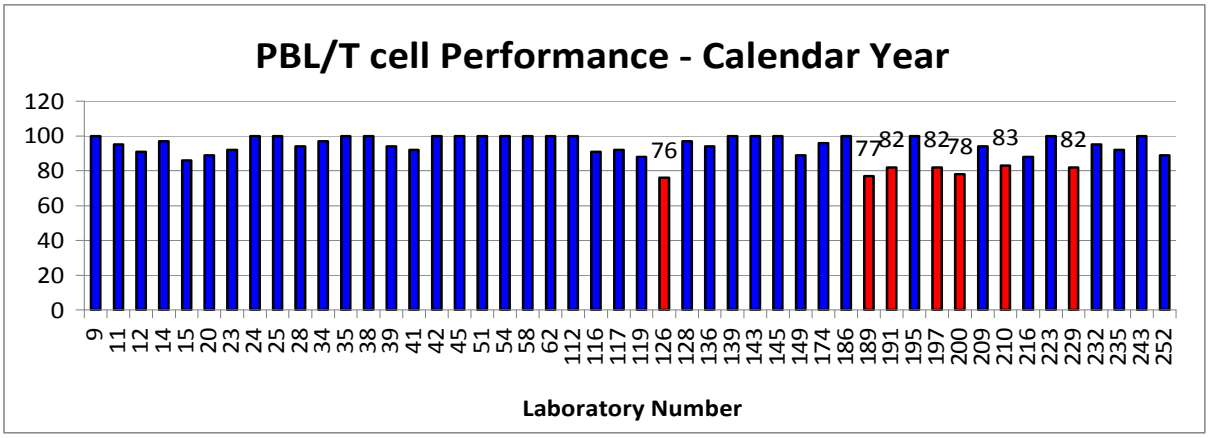
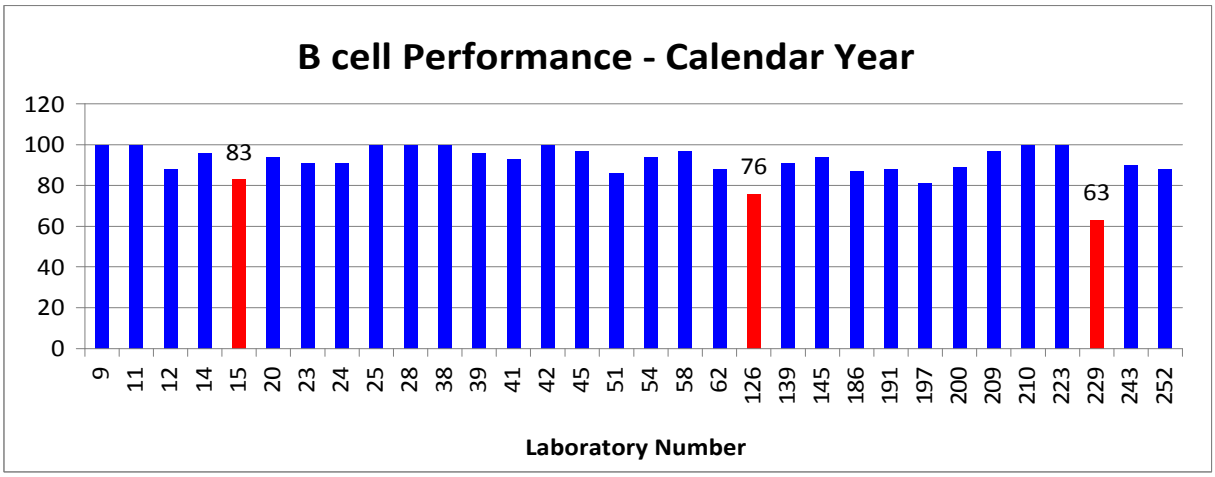



***Figure 2: Calendar Year B cell performance***




There are no planned changes to Scheme 2A for 2013.

*Judith Worthington, 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 2012 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.

The majority of participants achieved a satisfactory performance; the accompanying slide set presented at the AGM can be found at http://www.wtail.org.uk/neqas/presentations.asp

There are no planned changes to Scheme 2B for 2013.

*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. This year, in order to fulfil EFI requirements, five sera were 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. Negative specificities are not assessed for EFI purposes. Individual laboratory performance is assessed via agreement with consensus, being either acceptable or unacceptable. Overall, laboratories have to detect 75% of the positive specificities for performance to be acceptable.

### *Participants*

This year 51 laboratories registered with the scheme, 26 UK and 25 non-UK labs. This represents an increase of 1 since 2011. For class I antibody analyses, all UK and overseas laboratories participated. This is the same as 2011. Fifty laboratories registered for class II antibody specificity analysis, all UK labs and 24 from overseas. This represents an increase of 2 UK laboratories and 1 non-UK compared to 2011. There was no difference in the number of participating laboratories between cycles 1 and 2.

### *Methods*

The Luminex platform continues its domination for antibody analysis with 25/26 UK labs using this technology. This is the same as 2011. It is the sole technique in 18 laboratories, the same as in 2011. CDC remains in use in 5 labs (-1 cf 2011), all in combination with Luminex 2 laboratories use Luminex plus ELISA (+1 cf 2011). A single laboratory used flow as a stand alone technique.

The number of overseas laboratories using Luminex now stands at 23 (+1 cf 2011). Sixteen labs now use Luminex as a stand alone technique (+2 cf 2011). Seven laboratories continue with CDC (no change cf 2011), 6 in conjunction with Luminex (no change cf 2011), 1 in conjunction with ELISA, and 1 in conjunction with ELISA and flow (identical to 2010). Use of ELISA in overseas laboratories remains at 2 in 2012.

The choice of methods for analysis was less compared with 2011. For the second cycle (307-12), 1 UK laboratory dropped CDC. There was no change of techniques between cycles in the overseas laboratories. There was no addition of differing techniques between the 2 cycles, either in UK or overseas laboratories.

### *Performance*

Overall performance for UK laboratories was excellent for both class I and class II analyses across both cycles. For class I analysis all (-4 cf 2011) UK lab's received a satisfactory performance in cycle 1 (75% present). All laboratories were also satisfactory for 95% absence across both sample cycles.

This was repeated for class II antibody analysis where all UK laboratories achieved satisfactory performance for 75% consensus positive and 95% consensus negative over both cycles.

The situation in the overseas laboratories shows marginal improvement since 2011 and remains of concern since unsatisfactory performance appears to persist since 2010. For class I antibody analysis, 6 were unsatisfactory for 75% present in cycle 1 (-1 cf 2011). Only 2 of these went on to achieve a satisfactory performance in cycle 2.

For 95% consensus negative, 2 laboratories were unsatisfactory in cycle 1 (-1 cf 2011) and both failed to achieve satisfactory status in cycle 2. These trends were less apparent for Class II antibody analysis. Five (2) laboratories were unsatisfactory for 75% presence in cycle 1 (-3 cf 2011). Both failed to achieve a satisfactory performance in cycle 2 and 1 laboratory went from satisfactory in cycle 1 to unsatisfactory in cycle 2.

Performance for 95% negative was better. One laboratory failed to achieve satisfactory performance cycle 1, but had corrected this by cycle A second laboratory went in the reverse direction, achieving a satisfactory performance in cycle 1 and unsatisfactory in cycle 2.

***Developments***
Numbers of samples tested per cycle has dropped to 5 each to comply with EFI regulations.

*Mark Hathaway, Tissue Typing Laboratory, NHSBT Birmingham*

**SCHEMES 4A1 and 4A2**

**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 1st field level.

Samples comprise 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 and for 1st field or presence of DRB3, DRB4 and DRB5.

Results should be returned within 4 weeks of despatch.

Participating laboratories will only be assessed on the loci that they have registered for.

Alleles failing to reach the 75% consensus level will not be assessed.

A 'blank' forms part of the assessment if at least 75% of laboratories report a single allele at a locus.

Only those alleles appearing in the latest full HLA nomenclature report will be assessed.

***Class I – 1st field***

| Misassignment | Consensus |
|---|---|
| A*11, A*66 | A*11, A*25 |
| A*11, A*25 | A*02, A*29 |
| A*01, A*29 | A*29, A*32 |
| A*68 | A*02, A*68 |
| | |
| B*39, B*44 | B*14, B*44 |
| B*15, B*38 | B*15, B*37 |
| B*08, B*14 | B*08, B*39 |
| B*14, B*40 | B*39, B*40 |
| B*14 | B*07, B*14 |
| | |
| C*03 | C*03, C*16 |
| C*03 | C*03, C*16 |
| C*01, C*04 | C*02, C*12 |
| C*06 | C*06, C*07 |
| C*07 | C*07, C*08 |
| C*06 | C*06, C*07 |

***Class II – 1st field***

| Misassignment | Consensus |
|---|---|
| DRB1*04 | DRB1*01, DRB1*04 |
| DRB1*07 | DRB1*01, DRB1*07 |
| DRB1*04 | DRB1*04, DRB1*15 |
| DRB1*04, DRB1*11 | DRB1*01, DRB1*07 |
| DRB1*07, DRB1*13 | DRB1*07 |
| | |
| DRB3, DRB4 | DRB4 |
| DRB3, DRB4 | DRB4 |
| | |
| DQB1*03 | DQB1*02, DQB1*03 |
| DQB1*03, DQB1*06 | DQB1*06 |
| DQB1*06 | DQB1*03, DQB1*06 |

## SCHEME 4A2 - DNA HLA TYPING TO 2ND FIELD LEVEL

***Format and specification***

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

Samples comprise 2 send outs of 5 blood samples from 'local' donors.

Participants may register for 2nd field assessment of HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQB1, DQA1, and DPB1 for 1st and 2nd field assignment.

HLA alleles should be assigned on the basis of differences in exon 2 and 3 for HLA class I and exon 2 for HLA class II.

Participants registered for 2nd field assessment should define all ambiguities that encompass a null allele wherever the polymorphism is located.

Results are expected within 6 weeks of despatch of samples.

***Class I – 1st field***

| Misassignment | Consensus |
|---|---|
| A*02:01 | A*02:01, A*11:01 |
| A*01:01/**09**, A*68:01/11N | A*01:01, A*68:01 |
| A*02:01 | A*02:01, A*32:01 |
| A*02:01 | A*02:01, A*32:01 |

***Class II – 1st field***

| Misassignment | Consensus |
|---|---|
| DRB1*13:02, DRB1*14:**39**/54 | DRB1*13:02, DRB1*14:01/54 |
| DRB1*04:01, DRB1*14:**39**/54 | DRB1*04:01, DRB1*14:01/54 |
| DRB1*03:01/**42/50**, DRB1*07:01 | DRB1*03:01, DRB1*07:01 |
| DRB1*01:01/**07/22**, DRB1*03:01/**42/50** | DRB1*01:01, DRB1*03:01 |
| DRB1*03:01/**37**, DRB1*07:01 | DRB1*03:01, DRB1*07:01 |
| | |
| DRB3*02:02 | DRB3*02:02, DRB3*03:01 |
| DRB3*02:02/**09** | DRB3*02:02, DRB3*03:01 |
| DRB4*01:01/**03:01N**, DRB4*01:03 | DRB4*01:01, DRB4*01:03 |
| DRB4*01:01/**03:01N**, DRB4*01:03 | DRB4*01:01, DRB4*01:03 |
| DRB3*02:02, DRB4*01:01/**03:01N** | DRB3*02:02, DRB4*01:01 |
| DRB3*02:02/**14**, DRB5*01:01 | DRB3*02:02, DRB5*01:01 |
| | |
| DQB1*05:03, DQB1***06:04/34/36/38/39** | DQB1*05:03, DQB1*06:09 |
| DQB1*02:02 | DQB1*02:01/02 |
| DQB1*05:01, DQB1***16:03** | DQB1*05:01, DQB1*06:03 |
| | |
| DPB1***04:02**, DPB1*06:01 | DPB1*01:01, DPB1*06:01 |
| DPB1*02:01, DPB1*04:01/126/**134** | DPB1*02:01, DPB1*04:01 |

Overall there were less class I discrepancies than in the 2011 submission. However the number of class II discrepancies rose from 7 to 16 (one laboratory was responsible for four of these).

One sample of interest was 4A2 09/2012. In this sample 12 laboratories reported an insertion in exon 4 of the HLA-A*03 gene. This was characterised as a 4 base insertion (CATG). The insertion led to a premature stop codon and therefore the description of a novel A*03 null allele.

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

## SCHEME 4B - ABO GROUPING BY DNA-BASED METHODS

***Purpose***
To assess participants' ability to correctly determine ABO blood groups using DNA-based methodology.

Uses scheme 4A1 samples – 5 blood samples, 2 times a year from 'local' blood donors.

Should be reported as fully as possible, using the appropriate nomenclature.

7 participants in 2012.

All using PCR-SSP methodology for testing. 4 labs used commercial kits and the other 3 using 'in house' primer designs.

One unacceptable performance in 2012.

*Jennifer Pepperall, Welsh Transplantation and Immunogenetics Laboratory*

**SCHEME 5A – HFE TYPING**

***Overview***
To assess participants' ability to correctly determine HFE mutations

2012: 5 blood samples - 2 times a year

Must report on codon 63 and 282 - may also report codon 65

**2012** – 56 participants; 43% (n=24) reported codon 65 results

**2012** – in the first distribution, 7 errors were reported by 3 laboratories – 2 at codon 63 and 5 at codon 65 (4 of these 5 errors were reported by the same laboratory and appear to be due to a reporting error rather than a technical issue. No errors were reported at codon 282. There were no errors reported for the second distribution.

*Jennifer Pepperall, Welsh Transplantation and Immunogenetics Laboratory*

## PILOT 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.

In 2012 Scheme 5B became a full scheme, thus reports were assessed and scored.

In line with many other UK NEQAS for H&I interpretative schemes unacceptable performance was not be reported to the NQAAP for Immunology but the report to the participating laboratories will provide suggestions for improvement.

### *Format*

Patient scenarios with HFE genotype (for C282Y and H63D mutations) and clinical information were provided for two fictitious patients twice during the year and were distributed at the same time as scheme 5A samples. Participants were expected to return a report, within 4 weeks, in their own format that provided appropriate interpretation and suggested actions.

### *Assessors*

The Scenarios were devised and the reports assessed by three expert assessors. These were Alan Balfe, and Gavin Willis with Dairena Gaffney being the lead assessor in 2012. Alan Balfe will take over this role in 2013. Carol Hardy from Birmingham will join the assessors' team in 2013 to replace Dairena Gaffney who is stepping down.

### *Assessment*

While designing the scenarios, the assessors agreed on aspects of the report that were considered essential and whether one or two penalty points should be given for specific errors, depending on their importance. Each scenario had up to five possible penalty points based round six categories:

Description and Nomenclature
Action
Risk
Family
Rationality
Other

The assessors recorded the penalty points obtained and, importantly, provided explanations for these. In this way the scheme offered both an educational element and suggestions for improvement.

Each Scenario was scored and indicated as 'acceptable' or 'unacceptable'. Each scenario where more than 50% of the possible penalty points were allocated was deemed unacceptable.

Satisfactory performance for 2012 was achieved by obtaining four 'acceptable' classifications in the year.

### *Participation and results*

There were 21 participants for both distributions of 2 scenarios each. Maximum points per scenario was 5, i.e. 20 points altogether in the year.

Total penalty points obtained were:
0 penalty points - 1 laboratory
1 penalty point - 5 laboratories
2 penalty points - 6 laboratories
3 penalty points - 5 laboratories
4 penalty points - 2 laboratories
5 penalty points - 1 laboratory
6 penalty points - 1 laboratory

The average total number of points obtained in 2012 was 2.5 (this was 3.4 in 2012).

In 2012
3 out of 21 participants received an “unsatisfactory” rating but each gained its unacceptable classification from a different single scenario.

In 2011
1 out of 17 participants received an “unsatisfactory” rating.

There are no planned changes to Scheme 5B for 2013.

*Dairena Gaffney, DNA Laboratory, Biochemistry, Glasgow Royal Infirmary*

## SCHEME 6 – 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 2012 there were 54 participants in the first cycle and 57 in the second cycle. Consensus 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 consensus is considered Acceptable and each not agreement Unacceptable. Satisfactory performance is making 85% of reports on all sera in agreement with consensus in a calendar year.

### *Methodology*

Details of methodology used are requested as part of the reporting process. The number of laboratories using ELISA and flow had declined over the last few years and now appears to have stabilised. Around 15% are using CDC, 10% ELISA, 5% flow and the vast majority (90%) are using luminex (Table 1). In terms of specific kit usage; until 2009 ELISA was split fairly equally between GTI and One Lambda; for the last two years the majority of ELISA usage is the GTI kits. Whilst for luminex the One Lambda kits are favoured although there is increased use of the GenProbe kits particularly in combination with the LabScreen.

***Table 1: Methodology***

| Technique | 1st Cycle (2011 figures) | 2nd Cycle (2011 figures) |
|---|---|---|
| CDC | 8 (2) | 7 (8) |
| ELISA | 5 (7) | 7 (5) |
| FLOW | 3 (4) | 5 (4) |
| LUMINEX | 49 (46) | 51 (45) |

| | | | | | |
|---|---|---|---|---|---|
| ELISA | Quikscreen / B-Screen | = 4 | LUMINEX | LABScreen | = 28 |
| LATM | | = 2 | | Lifecodes | = 17 |
| Biotest | | = 1 | | both | = 6 |

78% of the laboratories are using a single technique for this scheme; the most popular single technique is luminex (Table 2). The other 22% of the participants used various combinations of different techniques and all of these incorporate the use of luminex as shown in Table 3.

***Table 2:*** **Participants using a single technique**

| Technique | 1st Cycle (2011 figures) | 2nd Cycle (2011 figures) |
|---|---|---|
| CDC only | 0 (1) | 0 (1) |
| ELISA only | 3 (3) | 3 (2) |
| FLOW only | 2 (2) | 3 (2) |
| LUMINEX only | 38 (31) | 38 (35) |

***Table 3:*** **Combinations of Techniques Used**

| Technique | 1st Cycle | 2nd Cycle |
|---|---|---|
| CDC + LS | 8 | 7 |
| ELISA + LS | 2 | 4 |
| FLOW + LS | 1 | 2 |

### *Sensitivity and Specificity:*

Of the 20 sera distributed 12 had provisional specificities assigned to them based on historic testing which was predominantly, although not exclusively, CDC testing (Table 4). A number of sera distributed this year were sera which had been previously distributed as either Scheme 3 or Scheme 6 sera. Table 4 shows the provisional specificity of and the percentage of laboratories reporting class I and class II positivity. Overall there were very few problems and there were no particular patterns or trends in terms of missing / extra reactions and methodology.

***Table 3:*** **Provisional Specificities and Concordance**

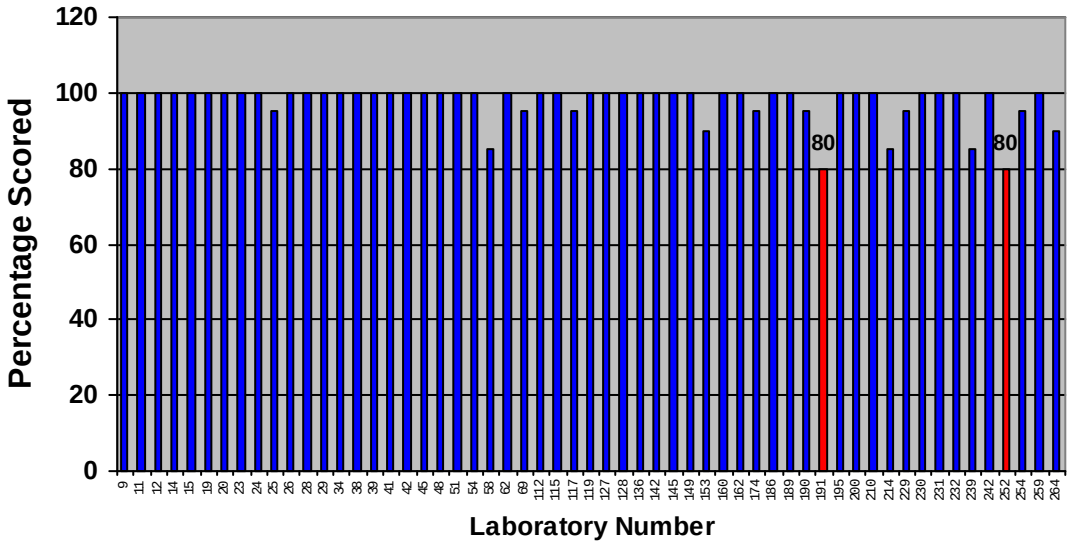
| Sera ID | Provisional Specificity | % of labs detecting antibody | Class I positive | Class II positive |
|---|---|---|---|---|
| 601/12 | B7 CREG;DR4;DQ3 | 100 | 94 | 100 |
| 603/12 | B5;DR3;53 | 91 | 87 | 88 |
| 604/12 | A2,28;DR4 | 98 | 93 | 100 |
| 606/12 | A3;B8;DR7;53;DQ2,3 | 96 | 77 | 98 |
| 608/12 | Bw4;DR7,9;53;DQ2 | 100 | 96 | 100 |
| 609/12 | A1,29 (CDC only) | 100 | 98 | 100 |
| 611/12 | A10;B37,55;52 (CDC only) | 100 | 100 | 100 |
| 612/12 | DR;DQ2 | 100 | 100 | 100 |
| 614/12 | B5;DQ1 | 100 | 100 | 98 |
| 616/12 | A2;B17;DR11;DQ3 | 89 | 86 | 81 |
| 609/12 | DR3 | 96 | 27 | 96 |
| 620/12 | DR11;52;DQ3,4 | 100 | 88 | 100 |

The remaining 8 sera were AB serum or “hidden negative” – in 2009 and 2010 the percentage of false positives had been around the 10% level last year was an all time low of 2.5 this year it was 5.7% and these were reported by only 8 laboratories.

***Performance***

Satisfactory performance is making 85% of reports on all sera in agreement with consensus in a calendar year. In 2012 only 2 laboratories failed to reach 85% these are summarised in Figure 1.

***Figure 1: Performance Chart for Scheme***




There are no planned changes to Scheme 6 for 2013.

*Judith Worthington, Transplantation Laboratory, Manchester Royal Infirmary*

**SCHEME 7 – HLA-B*57:01 TYPING**

The purpose of this Scheme is to assess participants' ability to correctly determine HLA-B*57:01 status.

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.

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. The assessment was based on the usual 75% consensus level and satisfactory performance was achieving all 10 reports in accord with that of the consensus findings.

There were 42 participants in 2012 – 22 from UK laboratories and 20 from non-UK labs. Of the 10 samples supplied 2 were B*57:01 positive donors and 8 were B*57:01 negative – 1 of these possessed the B*57:03 allele.

The 42 findings for the B*57:'03 were: 54.8% of laboratories (n=23) reported B*57:03, 12 labs reported a B*57 allele string - containing B*57:03 but not B*57:01, 6 laboratories reported "not B*57:01 and 1 laboratory assigned B*57:39.

The performance in this Scheme over the last 3 years has been:
2010 - UK labs -1 error – a false B*57:01 negative; non-UK labs - no errors
2011 – no errors
2012 - UK labs - no errors; non-UK labs -1 error a false B*57:01 negative.

There are no planned changes to Scheme 7 for 2013.

*Chris Darke, UK NEQAS for H&I Director*

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

Participants had requested a scheme - for their HLA-Class II HLA and disease typing - that was flexible so they could test what HLA loci and at what resolution they required. The diseases commonly HLA typed for were: Coeliac disease (associated with DQA1*05, DQB1*02), narcolepsy (DQB1*06:02), rheumatoid arthritis (DRB1*04, DPB1*02:01, 04:01, 09:01) and type I diabetes (DRB3/4, DPB1).

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

In 2012 there were 2 sample distributions – each of 3 DNA preparations. Importantly, all these samples had previously been tested in Scheme 4 – DNA HLA Typing - so had well documented HLA types.

Laboratories were required to report their Class II findings for the loci they tested for and at the resolution level they reported.

Assessment was made for the loci reported and at the allele family level or the allele to the 2$^{nd}$ field in agreement with the consensus type. There were no performance requirements as this was a pilot scheme during 2012.

In 2012 there were 19 participants – 9 were UK laboratories and 10 were non-UK laboratories.

For the 19 participants the HLA loci typed ranked as follows:
DQB1 – 19
DRB1 – 13
DQA1 - 8
DRB3/4/5 – 5
DPB1 – 2.

Success in Scheme 8 in 2012 for the 19 participants was:

15 labs had all typings correct (2 labs only tested the last 3 samples)
2 labs made 1 error each – these were both non-UK laboratories
2 labs made 2 errors each – these were 1 UK and 1 non-UK lab – and under the 2013 'rules' (see below) would have been unacceptable performers.

In 2013 Scheme 8 will be a fully assessed Scheme, 10 DNA samples will be distributed and satisfactory performance will be obtaining at least 9 out of the 10 samples tested – for whatever loci and resolution – in accordance with the consensus findings.

*Chris Darke, UK NEQAS for H&I Director*

## EDUCATIONAL SCHEME

In 2012 all 4 Educational Scheme samples were sent as DNA preparations.
The alleles of interest in were: ED01/12 - B*40:92, ED02/12 - A*03:01:01:02N, ED03/12 - A*02:24, ED04/12 - A*03:01:03.
Between 26 and 30 laboratories participated in the Scheme – the findings were:

### *ED01/12 - B*40:92*

B*40:92 differs from B*40:01:01 by 6 nucleotides in exon 3. It has a substitution motif of at least 60 nucleotides found in many alleles including B*15 and B*35 families. It is likely that B*40:92 occurred following an interallelic recombination event involving B*40:01:01 and a B*35 allele. It encodes a 'short' B40/B60 specificity which displays some HLA-B35 reactivity.
There were 26 DNA-based HLA-B findings:
24 (92.3%) laboratories reported HLA-B*40:92
1 reported B*40 and 1 assigned B*40:101/63.

### *ED02/12 - A*03:01:01:02N*

This HLA-A*03 null allele was created by a point mutation in intron 4 - g1846G>A – which causes incorrect splicing and a premature stop codon.
There were 26 HLA-A DNA-based findings:
12 assigned A*03:01:01:02N, 1 reported A*03:01N, 1 reported A*03:01:01:02N/01:18, 8 reported A*03 only, 3 reported A*03:01, 1 reported A*03:01/02:01/05. Thus, 46.2% of participants failed to recognise the 'null' allele.

### *ED03/12 - A*02:24*

A*02:24 differs from A*02:01 by a single base (C/A) at position 453.
Interestingly, this substitution corresponds to the annealing site of a primer commonly used in A*02-amplifying PCR-SSP mixtures. Thus, in 2002's Educational Scheme 29.2% (7/24) of labs missed A*02:24 using DNA-based methods.
There were 30 HLA DNA-based findings – all participants identified A*02 - additionally:
17 reported A*02:24 (11 A*02:24 and 6 A*02:24:01) and 10 assigned A*02 only.
3 participants assigned various A*02 allele groups but 1 of these lacked A*02:24.

### *ED04/12 - A*03:01:03*

A*03:01:03 differs from A*03:01:01 by a single non-coding substitution of G to T at position 167 in exon 2.
There were 30 DNA-based findings:
13 (43.3%) laboratories reported A*03:01:03, 16 reported A*03 only – 11 as A*03 and 5 as large A*03 allele groups and 1 reported A*03:01.

### *An Educational Interpretative Scheme will be tested in 2013.*

In this Scheme participants will be given various clinical scenarios - involving solid organ or HSC transplants and asked to address various questions, for example:

- Is this a contraindication to transplantation?
- What further tests would you perform, if any?
- What clinical advice would you give regarding the expected post-operative course if the transplant proceeds?

Each answer will be restricted to 40 words.
The replies will be gathered and circulated to all participating laboratories – using the normal laboratory codes.
In 2013 – where 2 scenarios will be provided - this scheme will be gratis and open to all except those participating in schemes 1B, 4B, 5A/B, 7, or 8 ONLY.
As with the current Educational Scheme participation will be at the discretion of the Head of Laboratory.
The scheme will be debated at the 2013 AGM.

*Chris Darke, UK NEQAS for H&I Director*

## 3. NUMBER OF PARTICIPANTS DURING 2012

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 2012.

| Scheme | UK + ROI | Overseas |
|---|---|---|
| 1A | 10 | 12 |
| 1B | 48 | 46 |
| 2A | 22 | 27 |
| 2B | 23 | 37 |
| 3 | 26 | 25 |
| 4A1 | 31 | 60 |
| 4A2 | 21 | 29 |
| 4B | 4 | 3 |
| 5A | 51 | 8 |
| 5B | 21 | 0 |
| 6 | 25 | 32 |
| 7 | 23 | 19 |
| 8 | 8 | 10 |
| Educational | 24 | 7 |

ROI – Republic of Ireland

## 4. 2012 ANNUAL GENERAL MEETING – BRISTOL

60 participants representing 28 laboratories attended the UK NEQAS for H&I AGM in Bristol on the 4th December, 2012.

There were two scientific presentations at the AGM:
Epitope matching in platelet transfusion in alloimmunised patients presented by Delordson Kallon, Histocompatibility & Immunogenetics Department, NHSBT, Tooting.

Exploiting the HIF pathway in kidney transplantation; current prospective and emerging avenues presented by Zeeshan Akhtar, Nuffield Department of Surgical Sciences, University of Oxford.

Slides from all of the annual meeting presentations may be downloaded from: http://www.wtail.org.uk/NEQASHI.htm or may be requested from Susan Corbin, Schemes' Manager, UK NEQAS for H&I, Welsh Blood Service, Ely Valley Road, Talbot Green, Pontyclun CF72 9WB. Tel: 01443 622185; Fax: 01443 622001; e-mail: ukneqashandi@wales.nhs.uk).

The 2012 UK NEQAS for H&I AGM 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 2013 - PLEASE NOTE

Laboratories will retain their code numbers for 2013. Laboratory code information is known only to the Scheme Organiser, Manager and UK NEQAS for H&I staff.

An up-to-date list of contact names is provided in the Prospectus.

Important UK NEQAS for H&I dates for distributions, result deadlines, reporting and meetings are provided in the Prospectus.

Additional "Essential Scheme Information" is provided in the 2013 Prospectus, further copies are available from the Schemes' Manager.

Please see the 2013 Prospectus for full details of the assessment system.