UKNEQAS

# HISTOCOMPATIBILITY

# and

# IMMUNOGENETICS

# Annual Report

# 2013

Contents:



*United Kingdom National External Quality Assessment Service*

CPA

Accredited EQA Scheme
Reference Number 036
Since 1999

UKNEQAS for HISTOCOMPATIBILITY and IMMUNOGENETICS
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## 1. INTRODUCTION

### Retirement of Susan Corbin after 22 years as UK NEQAS for H&I Manager

I was first acquainted with Susan Corbin when we worked together at the National Tissue Typing Reference Laboratory at the Blood Transfusion Centre in Bristol in the early 1970ies. The NTTRL was later to become the UK Transplant Service.

Susan established the UK NEQAS for H&I Manager role in the early 1990ies. Originally at UKTS, and then, from January 2000, at the Welsh Blood Service. I have learned a great deal from Susan over the years; she has always bestowed immense energy, wisdom and natural good judgement to the operation of our EQA Service.

Susan has also been instrumental in shaping the nature of external quality assessment in Europe and beyond by her long, some 6 years, membership of the UK NEQAS Organisation's Executive Committee.

For the past 15 years Susan was an active member of the European Federation for Immunogenetics' External Proficiency Testing Committee and was co-chair for the last 5 years. Susan played a significant role in devising the EFI EPT Standards for Laboratories and the Standards for EQA/EPT Providers.

There is no doubt that Susan will be sadly missed from the EQA scene and we all wish her well for a long and happy retirement and the immense enjoyment of her other passion – which is gardening.

### UK NEQAS for H&I Steering Committee News for 2013

Retirements were also a feature of the Steering Committee's membership for 2013. Thus, Derwood Pamphilon (Clinical Representative) and Dairena Gaffney (Lead Assessor for Scheme 5B) have both retired. They have been replaced by Edwin Massey and Alan Balfe, respectively. Also Terry Horsburgh and Judith Worthington (both long serving Ordinary Members) stood down at the Annual Participants' Meeting in December 2013.

New members of the Committee are Carol Hardy (Ordinary Member and Scheme 5B Assessor) and we also welcome, for 2014, Patrick Flynn and Ruhena Sergeant as Ordinary Members.

Finally, and importantly, UK NEQAS for H&I welcomed Debbie Singleton in June 2013 as the new UK NEQAS for H&I Manager.

A sincere ‘thank you’ is owed to all our outgoing Committee Members for all their loyal and hard work over the years. Similarly, a warm welcome is due to our new Committee Members – which will be important conduits of fresh ideas for the future of UK NEQAS for H&I.

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

Our small, energetic and hardworking team is now head by Debbie Singleton - Schemes’ Manager, with Melanie Bartley, Geraint Clarke and Luke Gardner.
Wholehearted thanks are due to them all for their hard toil and untiring commitment to UK NEQAS for H&I during 2013.

*Chris Darke UK NEQAS for H&I Organiser*

## 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 2013 is summarised below:

| HLA | Number of Participants | |
|---|---|---|
| | Total | UK |
| Any | 23-30 | 10 |
| A | 23-30 | 10 |
| B | 23-30 | 10 |
| C | 6-8 | 2-4 |
| DR | 15-18 | 9 |
| DQ | 15-18 | 9 |
| A, B | 7-10 | 1 |
| A, B, C | 1-2 | 1-2 |
| A, B, DR | 0 | 0 |
| A, B, DR, DQ | 7-12 | 5-7 |
| A, B, C, DR, DQ | 5-7 | 3-4 |

*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 (18 labs); Innotrain (4 labs); BioRad (12 labs).

#### Cell preparation

Six laboratories tested samples using unseparated cells, 12 used T&B cell preparations, 7 used T cells only, 2 used unseparated cells plus B cells and 1 lab used unseparated cells plus T cells.

**Scheme 1A samples for 2013**

The HLA types of the 10 samples distributed in 2013 are shown below:

| Sample No | A | A | B | B | C | C | DR | DR | DQ | DQ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1A01 | 2 | blank | 35 | 60 | 3 | 4 | 8 | 15 | 4 | 6 |
| 1A02 | 1 | 3 | 7 | 44 | 7 | blank | 7 | 13 | 2 | 6 |
| 1A03 | 3 | 24 | 35 | 60 | 3 | 4 | 4 | 8 | 4 | 8 |
| 1A04 | 2 | 3 | 7 | 51 | 7 | 14 | 11 | 15 | 6 | 7 |
| 1A05 | 3 | 26 | 60 | 62 | 3 | blank | 4 | 15 | 6 | 8 |
| 1A06 | 2 | 3 | 8 | 60 | 3 | 7 | 13 | 17 | 2 | 6 |
| 1A07 | 1 | 24 | 49 | 65 | 7 | 8 | 1 | 15 | 5 | 6 |
| 1A08 | 1 | 2 | 8 | 27 | 2 | 7 | 11 | 17 | 2 | 7 |
| 1A09 | 3 | blank | 35 | 44 | 4 | 5 | 1 | 4 | 5 | 7 |
| 1A10 | 2 | 3 | 7 | 64 | 7 | 8 | 7 | 15 | 2 | 6 |

**Incorrect assignments in 2013: HLA-A, B, C, DR & DQ by sample**

| Sample | Misassignment | Lab | Consensus |
|---|---|---|---|
| 1A04 | B51 called B52 | a | B7, B51 |
| 1A04 | Missed Cw14 | b, c | Cw7, Cw14 (Detected by DNA) |
| 1A07 | B65 called B14 | d, e | B49, B65 |
| 1A07 | B65 called B64 | f | B49, B65 |
| 1A10 | B64 called B14 | g, h | B7, B64 |

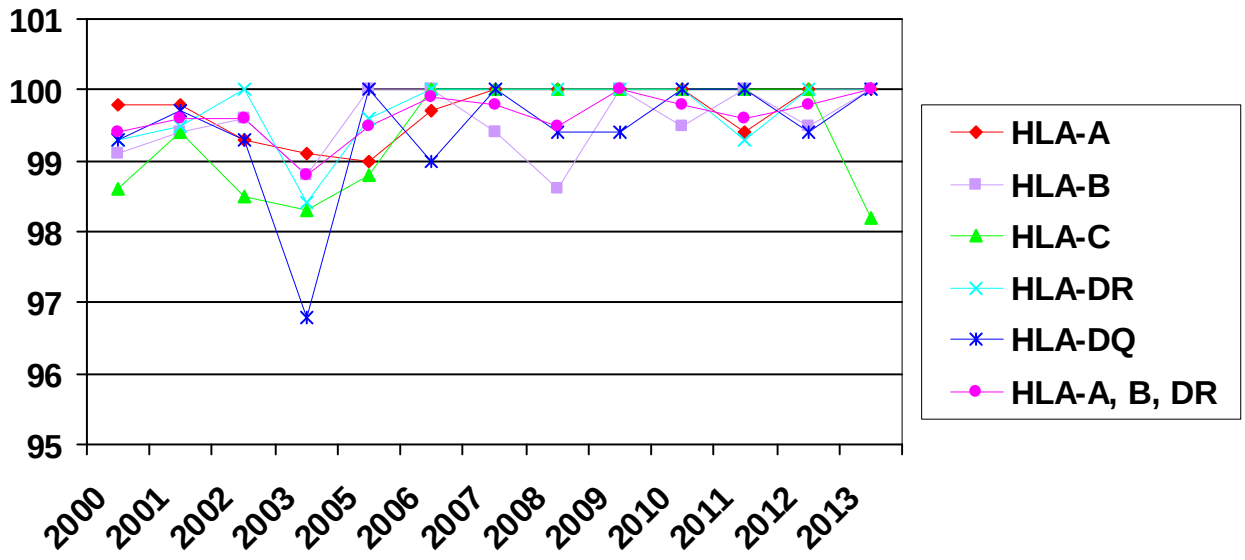
**Performance in 2013**

There were eight incorrect assignments made in 2013 involving 1 UK and 7 non-UK laboratories. There was no correlation with reagents or testing strategy used and the misassignments reported. This year was the first year where all participating laboratories achieved satisfactory performance.

**Overall 2013 Accuracy Rates**

| Antigen | Error | Assignments | Accuracy Index (%) | 2013 (%) |
|---|---|---|---|---|
| **Overall** | | | | |
| HLA-A | 0 | 512 | 100 | 99.5 |
| HLA-B | 6 | 512 | 98.8 | 99.1 |
| HLA-C | 2 | 148 | 98.6 | 99.3 |
| HLA-DR | 0 | 316 | 100 | 100 |
| HLA-DQ | 0 | 316 | 100 | 99.3 |
| HLA-A,B,DR | 6 | 1340 | 99.7 | 99.5 |
| | | | | |
| **UK** | | | | |
| HLA-A | 0 | 200 | 100 | 100 |
| HLA-B | 0 | 200 | 100 | 99.5 |
| HLA-C | 1 | 56 | 98.2 | 100 |
| HLA-DR | 0 | 180 | 100 | 100 |
| HLA-DQ | 0 | 180 | 100 | 99.4 |
| HLA-A,B,DR | 0 | 580 | 100 | 99.8 |

**Overall Accuracy rates in UK**




Overall accuracy is very good for this scheme. In the UK, only 1 error for HLA –C occurred in 2013.

*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.

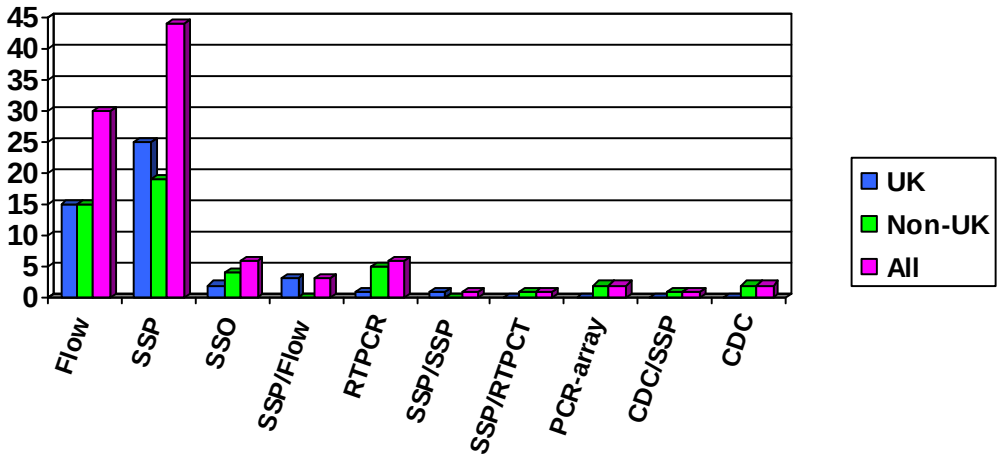
In 2013 there were 93-96 participants in the scheme (47-48 UK laboratories).

### 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: 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: Assignments outside of consensus

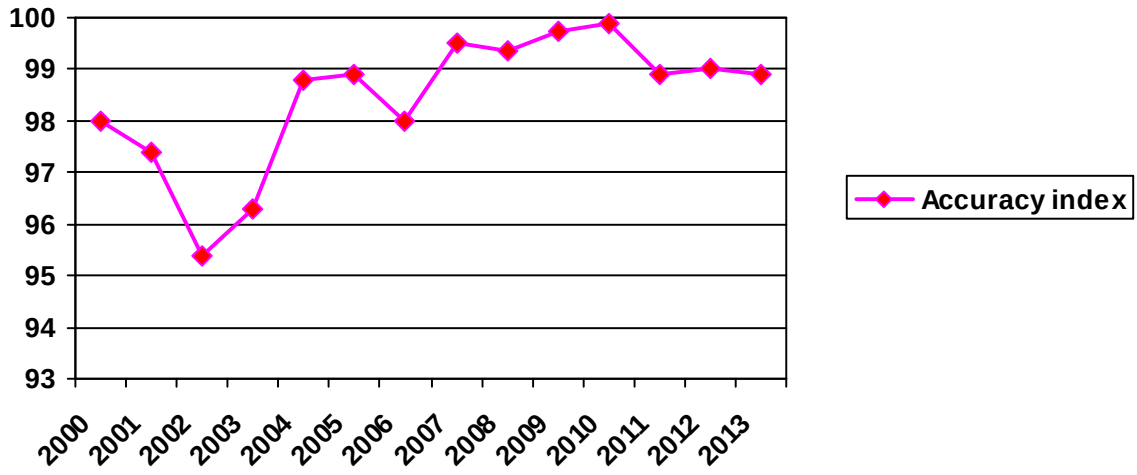
In 2013, four samples distributed were HLA-B27 positive. Four assignments were made outside of consensus involving 1 UK laboratory and 3 non-UK laboratories (see table below). Two misassignments were made by laboratories using flow cytometry, one by cytotoxicity and one by PCR-SSP.

| Sample | Result | No. of labs | Technique | HLA Type |
|---|---|---|---|---|
| 1B03 | false pos | 1 | CDC | B18, B47 |
| 1B07 | false pos | 1 | flow | B7 |
| 1B08 | false neg | 2 | flow, SSP | B8, B27 |

**Overall performance**

Eighty seven laboratories achieved acceptable performance and four were considered to have unacceptable performance.

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




*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 2013 there were 63 PBL/T cell and 49 B cell participants. The increased number of participants in the scheme means that it is no longer possible to supply all laboratories with the same cell/sera set and therefore form the 2nd cycle onwards UK and overseas laboratories received different crossmatch material.

**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 the 63 laboratories there were 15 different combinations – last year there were 16. Around 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%. Result were not returned on 49 occasions (8%): 20 due to poor viability, 2 insufficient cells, 17 technical problems, 8 samples lost, and 2 due to staff shortages.

**B cell methodology:** Forty nine laboratories submitted B cell data. Again incubation times varied between laboratories, there were 13 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 (59 occasions; 13%) as compared with T cells: poor viability accounting for almost 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 for UK laboratories and Table 2 for overseas laboratories. The B cell results are in Table 3 and 4. Overall performance for the calendar year is shown in Figures 1 and 2. Six 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. Three of these laboratories plus two others also failed to achieve greater than 85% for B cell results as shown in Figure 2.

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

| Cycle | % Range Correct | Mean % Correct | % of Labs 100% Correct | Number of Unacceptable Results | False Positives / False Negatives |
|---|---|---|---|---|---|
| 1st | 71.4 – 100 | 97.1 | 85 | 4 | 4 / 10 |
| 2nd | 62.5 – 100 | 92.6 | 68 | 13 | 10 / 3 |
| 3rd | 75 – 100 | 98 | 89 | 3 | 3 / 0 |
| 4th | 0 – 100 | 91 | 73 | 7 | 2 / 5 |
| 5th | 0 - 100 | 89.6 | 82 | 9 | 5 / 4 |

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

| Cycle | % Range Correct | Mean % Correct | % of Labs 100% Correct | Number of Unacceptable Results | False Positives / False Negatives |
|---|---|---|---|---|---|
| 1st | 42.9 – 100 | 90.4 | 64 | 22 | 12 / 10 |
| 2nd | 57.1 – 100 | 92.2 | 70 | 18 | 17 / 1 |
| 3rd | 75 – 100 | 92.5 | 54 | 22 | 12 / 10 |
| 4th | 50 – 100 | 92.2 | 61 | 20 | 10 / 10 |
| 5th | 16.7 - 100 | 97.8 | 97 | 5 | 1 / 4 |

**Table 3: B Cell Performance Results by Cycle: UK laboratories**

| Cycle | % Range Correct | Mean % Correct | % of Labs 100% Correct | Number of Unacceptable Results | False Positives / False Negatives |
|---|---|---|---|---|---|
| 1st | 87.5 – 100 | 95.5 | 70.5 | 6 | 6 / 0 |
| 2nd | 0 – 100 | 87.1 | 70 | 4 | 4 / 0 |
| 3rd | 57.1 – 100 | 95.1 | 82.3 | 5 | 5 / 0 |
| 4th | 50 – 100 | 92.6 | 66.6 | 8 | 0 / 8 |
| 5th | 85.7 - 100 | 95.7 | 70 | 7 | 2 / 5 |

**Table 4: B Cell Performance Results by Cycle: Overseas laboratories**

| Cycle | % Range Correct | Mean % Correct | % of Labs 100% Correct | Number of Unacceptable Results | False Positives / False Negatives |
|---|---|---|---|---|---|
| 1st | 62.5 – 100 | 88.6 | 38 | 18 | 7 / 11 |
| 2nd | 0 – 100 | 82.6 | 70.8 | 7 | 2 / 5 |
| 3rd | 71.4 – 100 | 92 | 65 | 13 | 1 / 12 |
| 4th | 50 – 100 | 91.4 | 64 | 16 | 6 / 10 |
| 5th | 14.3 - 100 | 93.1 | 76 | 11 | 2 / 9 |

**Figure 1: 2013 Participant PBL/T Performance**

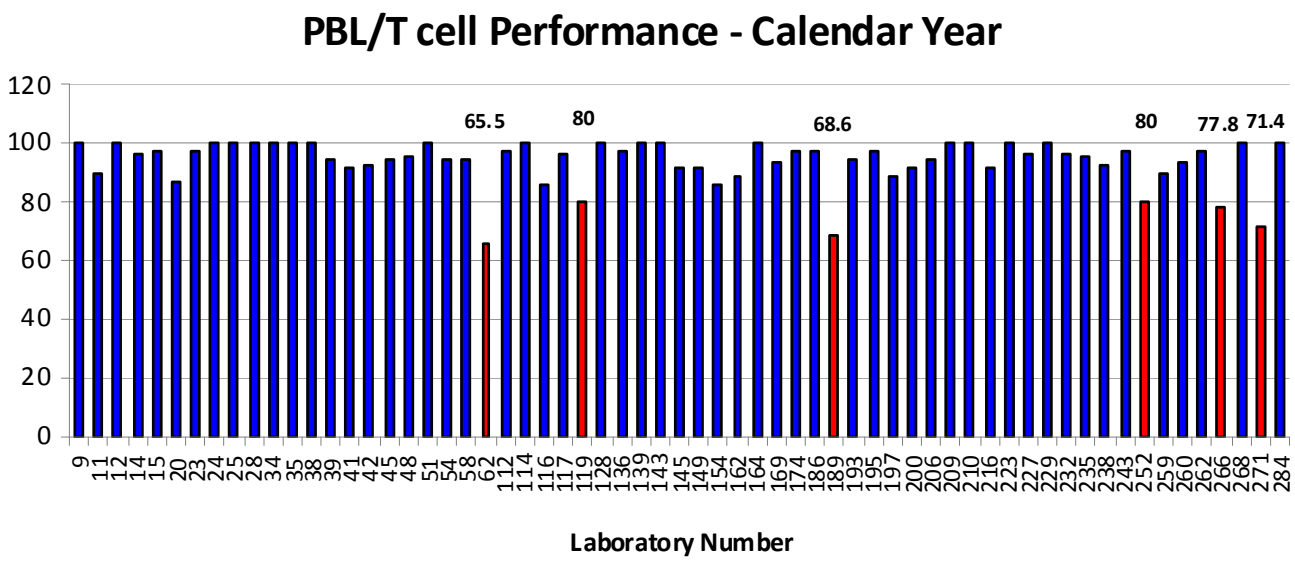
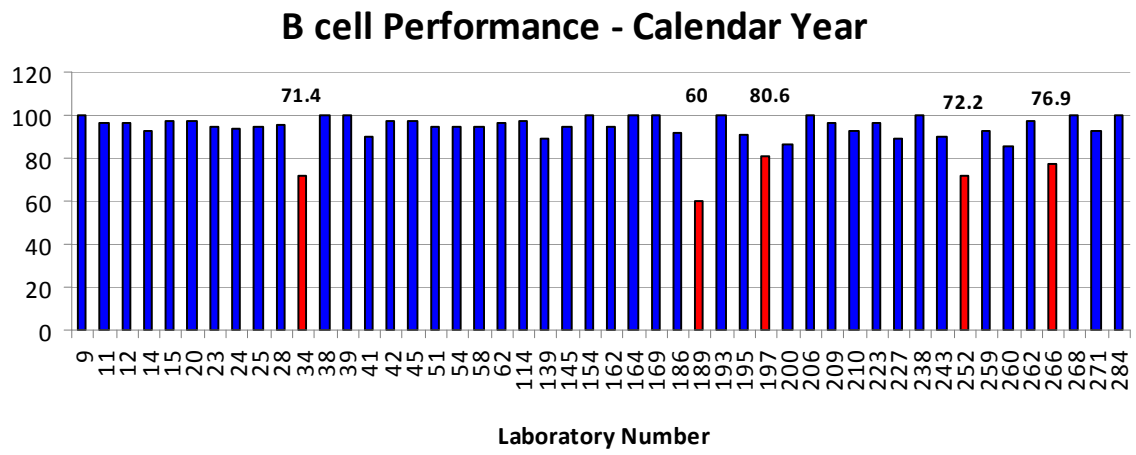


**Figure 2: 2013 Participant B cell performance**




**Changes to Scheme 2A for 2014:** For 2014 participants will be invited to submit results after DTT treatment. The DTT results will be considered independently from the non DTT treated results and in 2014 there will be no formal assessment of the DTT results

*Judith Worthington, Transplantation Laboratory, Manchester Royal Infirmary*

## SCHEME 3: HLA ANTIBODY SPECIFICITY ANALYSIS

### Purpose of scheme

Scheme 3 focuses on HLA antibody specificity analysis. In compliance with EFI standards, 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. Negative specificities are not assessed for EFI purposes. Individual laboratory performance is assessed via agreement with consensus, being either acceptable or unacceptable. Overall, laboratories must detect 75% of the positive specificities for acceptable performance.

### Participants

This year 66 laboratories registered for scheme 3, 26 UK and 40 overseas labs. This represents an increase of 16 since 2012, entirely due to increased participation from overseas. For class I antibody analyses, all 66 laboratories participated. Sixty three laboratories registered for class II antibody specificity analysis, 24/26 UK labs and 39/40 from overseas. This is the same as 2012 for UK labs, but representative of the overall increase of scheme 3 participation in overseas laboratories. 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 2012. It is the sole technique in 21 laboratories, and increase of 3 since 2012. CDC remains in use in 4 labs (-1 cf 2012), all in combination with Luminex. One laboratories used Luminex plus ELISA (-1 cf 2012), but for cycle 1 only. ELISA was eliminated as a technique of choice in the UK for cycle 2. A single laboratory continues to use flow as a stand alone technique.

The number of overseas laboratories using Luminex stood at 31 (+8 cf 2012), and this increased to all 40 in cycle 2 (+17 cf 2012). Twenty one (21) labs used Luminex as a stand alone technique (+5 cf 2012) for cycle 1 and this increased to 29 in cycle 2. Nine (9) laboratories continue with CDC (+2 cf 2012), 7 in conjunction with Luminex (+1 cf 2012), 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 2013.

The switching of methods of sample analysis was virtually static in UK laboratories, with just the one laboratory dropping ELISA for cycle 2. The situation was more fluid in overseas laboratories. One (1) laboratory dropped CDC for cycle 2, whilst 2 others added it. A further laboratory added flow analysis for cycle 2, but by far the biggest increase was the addition of Luminex testing in 9 laboratories in cycle 2 that had not used it in cycle 1. In total, 17 overseas laboratories added Luminex to their testing repertoire across the two cycles.

### Performance

Overall performance for UK laboratories was excellent for both class I and class II analyses across both cycles. For class I analysis, only 1 laboratory received an unsatisfactory

performance (75% present; +1 cf 2012). All UK laboratories achieved a satisfactory performance for 95% absence (identical to 2012). This was repeated for class II antibody analysis where all UK laboratories achieved satisfactory performance for 75% consensus positive and 95% consensus negative.

The situation in the overseas laboratories shows further deterioration since 2012 and remains of concern. For class I antibody analysis, 10 laboratories had unsatisfactory performance for 75% present (+4 cf 2012). Unsatisfactory performance persisted in 4 of these laboratories from 2012. For 95% consensus negative, performance was much better with just 2 laboratories unsatisfactory (no change cf 2012). However, these same two laboratories were also unsatisfactory performers in 2012. The deterioration trend continued for Class II antibody analysis with 5 laboratories achieving unsatisfactory performance for 75% presence (+3 cf 2012). This was largely due to new laboratories joining scheme 3, with only 1 previous participant carrying over poor performance from 2012. The situation was much improved for 95% absence, where all laboratories achieved satisfactory performance.

**Key Findings**

- A number of overseas laboratories are still finding detection difficult both for class I and class II, but as for last year, this is particularly prevalent for class I.
- This is not restricted to laboratories joining scheme 3 for the first time
- Much better performance for antibody absence
- For Luminex testing, One Lambda and Gen-Probe (Immucor) kits can give different results. The majority of laboratories use One Lambda kits, mostly solo, with a small number using both. Of the 11 laboratories achieving unacceptable performance in 2013, nine (9) were using Genprobe (Immucor) kits only.

*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.

There are 10 samples 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 and for 1st field or presence of DRB3, DRB4 and DRB5. Participating laboratories will only be assessed on the loci for which they have registered.

Alleles that fail to reach 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 listed in the latest full HLA nomenclature report will be assessed.

**Class I 1st Field Misassignments**

| Misassignment | Consensus |
|---|---|
| A*01, A*25 | A*01, A*26 |
| A*01, A*02 | A*02, A*03 |
| A*02, A*26 | A*01, A*02 |
| A*02, A*23 | A*01, A*25 |
| A*01, A*26 | A*02, A*26 |
| A*01, A*24 | A*02, A*24 |
| A*01, blank | A*01, A*30 |
| A*24, A*69 | A*24, A*68 |
| | |
| B*08, B*55/56 | B*08, B*55 |
| B*08, B*51 | B*08, B*55 |
| B*08, blank | B*08, B*55 |
| B*07, B*08 | B*08, B*15 |
| B*08, B*40 | B*07, B*08 |
| B*07/42, B*08 | B*07, B*08 |
| B*14, B*37 | B*07, B*08 |
| B*08, B*15 | B*08, blank |
| B*08, B*55 | B*14, B*37 |

**Class II 1st Field Misassignments**

| Misassignment | Consensus |
|---|---|
| DRB1*03, blank | DRB1*03, DRB1*08 |
| DRB1*03, DRB1*15 | DRB1*03, DRB1*04 |
| DRB1*07, DRB1*13 | DRB1*03, DRB1*15 |
| DRB1*03, DRB1*04 | DRB1*03, blank |
| DRB1*03, DRB1*08 | DRB1*07, DRB1*13 |
| | |
| DQA1*05, DQA1*06 | DQA1*04, DQA1*05 |
| DQA1*05, blank | DQA1*01, DQA1*05 |
| | |
| DQB1*03, DQB1*05 | DQB1*02, DQB1*03 |
| DQB1*03, blank | DQB1*02, DQB1*03 |
| DQB1*06, blank | DQB1*02, DQB1*06 |
| DQB1*02, DQB1*04 | DQB1*02, blank |

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 10/10, there were three laboratories classified as unacceptable performers. Laboratories submitting incorrect results used a mixture of SSP and SSOP approaches.

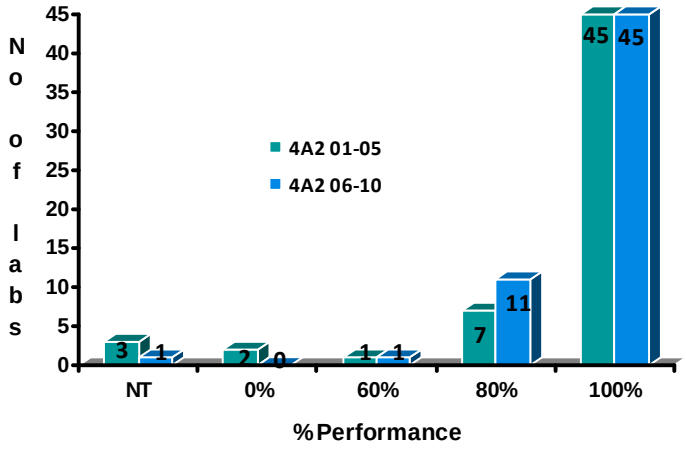
*Leigh Keen, H&I Laboratory, Filton*

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

The aim of the scheme is to assess the participant's ability to correctly determine HLA alleles to the 2nd field level. A total of ten blood samples are sent as two distributions of five blood samples.

***Performance***

58 laboratories (international and UK) participated in 2013, and overall laboratory performance is shown below. 3 labs provided no returns (1 lab did not test samples 01-05 & 06-10, and 2 labs did not test samples 01-05). 45/58 laboratories achieved 100% performance for both scheme cycles.




Overall there were more Class I misassignments in 2013 than 2012 (7 v 4) and less Class II misassignments in 2013 than 2012 (14 v 16).

Class I misassignments 2nd Field

| ***Misassignments*** | ***Consensus*** |
|---|---|
| A*29:01 | A*29:02 |
| A*29:02/10 | A*29:02 |
| B*13:01 | B*13:02 |
| B*18:02 | B*18:01 |
| B*08:01/47 | B*08:01 |
| C*06:02 | C*07:02 |
| C*07:01 only | C*07:18 or string that includes *07:01 |

Class II misassignments 2nd Field

| ***Misassignments*** | ***Consensus*** |
|---|---|
| DQB1*02:01/05 | DQB1*02:01 |
| DQB1*03:03/20 | DQB1*03:03 |
| DQB1*02:01/07 | DQB1*02:01 |
| DQA1*01:01 | DQA1*01:02 |
| DQA1*05:05/08/09/10 | DQA1*05:09 or DQA1*05:05/08/09 |
| DRB1*07:01/03 DRB1*07:01, *07:14 | DRB1*07:01 |
| DRB1*04:01 | DRB1*04:04 |
| DRB4*02:02 | DRB3*02:02 |
| DRB3*02:02/23 | DRB3*02:02 |
| DRB3*01:01/12/15 DRB3*01:01/02/15 | DRB3*01:01 |
| DPB1*04:01/120:01N | DPB1*04:01 |
| DPB1*04:02/82:01 | DPB1*04:02 |

***Developments***

In 2014, laboratories can register for DPA1 assessment.

*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 2013 there were 7 participants. All participants used PCR-SSP technology with 4 using commercial kits and the remainder using 'in house' developed primer sets.

One participant had unacceptable performance in 2013 due to an issue with 2 of the samples and submitted the following comment with their report

- Comment – With samples 01/2013 and 03/2013 we found a very doubtful result with our specific primers for the presence of blood group genotype O2. In this case we stated the doubtful result as negative, matching the blood group A1/Ax instead. As a phenotype we found blood group O in these samples. Routinely we would perform an absorption and dilution test to exclude the blood group Ax and ask for a new fresh sample to confirm our DNA results.

This participant was 1 of the 4 labs using commercial kit – however it was a different kit from the other 3 participants

*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

In 2013 there were 57 participants; 47% (n=27) reported codon 65 results. The most common technique used was RT-PCR and there we no errors

The numbers participating in this scheme slightly increased from last year with 1 lab reporting results for codon 168.

*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 2013 was achieved by obtaining four 'acceptable' classifications in the year.

***Participation and results***
Returns were received from 19 participants for the first distribution (scenarios 1 and 2) and 18 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 | 0 | penalty points |
| 4 | labs got | 1 | penalty point (0 points on 3 scenarios) |
| 4 | labs got | 2 | penalty points (0 points on 2 scenarios) |
| 5 | labs got | 3 | penalty points |
| 3 | labs got | 5 | penalty points |
| 2 | labs got | 6 | penalty points |

Average Annual total points was 2.8 (for comparison, average was 2.5 in 2012, and 3.4 in 2011).

Satisfactory performance was achieved by all but three participants. Two of these participants scored an "unacceptable" classification for scenario 1, and the third scored an "unacceptable" classification for scenario 2.

**Table 1. Performance Summary**

| | Scenario 1 | Scenario 2 | Scenario 3 | Scenario 4 |
|---|---|---|---|---|
| No. of labs with 0 penalty points | 6 | 7 | 6 | 11 |
| No. of labs with 1 penalty point | 10 | 10 | 10 | 7 |
| Labs with 0 or 1 penalty points | 84% | 89% | 89% | 100% |
| Average penalty | 0.95 | 0.79 | 0.78 | 0.39 |
| No. of labs with classification "unacceptable" (3+ pts) | 2 | 1 | 0 | 0 |

General deficiencies identified in reports included the following:

- Not relating genotype to phenotype
- Not referring to the variable penetrance of the HFE genotype – signs and symptoms may be due to HFE or have another cause
- Not recognising 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

There are no planned changes to Scheme 5B for 2014.

*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 2013 there were 66 participants in the first cycle and 68 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 80% 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, 8% flow and the vast majority (91%) are using luminex (Table 1). In terms of specific kit usage; for luminex the One Lambda (LSM12) kits are favoured although there is increased use of the GenProbe (LMX) kits particularly in combination with the LabScreen. There has also been an increase in the use of ID kits as oppose to the "yes/no" detection kits

**Table 1: Methodology**

| Technique | 1st Cycle (2012 FIGURES) | 2nd Cycle (2012 FIGURES) |
|---|---|---|
| **CDC** | 10 (8) | 10 (7) |
| **ELISA** | 6 (5) | 6 (7) |
| **FLOW** | 5 (3) | 4 (5) |
| **LUMINEX** | 62 (49) | 65 (51) |

| | | | | |
|---|---|---|---|---|
| ELISA | Quikscreen / B-Screen | = 2 | LUMINEX | LMS12 = 36 |
| | LATM | = 3 | | LMX = 21 |
| | Biotest | = 1 | | LSM+LMX = 3 |

76% of the laboratories are using a single technique for this scheme; the most popular single technique is luminex (Table 2). The other 24% 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 (2012 FIGURES) | 2nd Cycle (2012 FIGURES) |
|---|---|---|
| **CDC ONLY** | 0 (0) | 0 (0) |
| **ELISA ONLY** | 2 (3) | 2 (3) |
| **FLOW ONLY** | 3 (2) | 1 (3) |
| **LUMINEX ONLY** | 45 (38) | 49 (38) |

**Table 3: Combinations of Techniques Used**

| Technique | 1st Cycle | 2nd Cycle |
|---|---|---|
| **CDC + LS** | 9 | 9 |
| **ELISA + LS** | 4 | 3 |
| **FLOW + LS** | 2 | 3 |
| **CDC + ELISA + LS** | 0 | 1 |

**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.

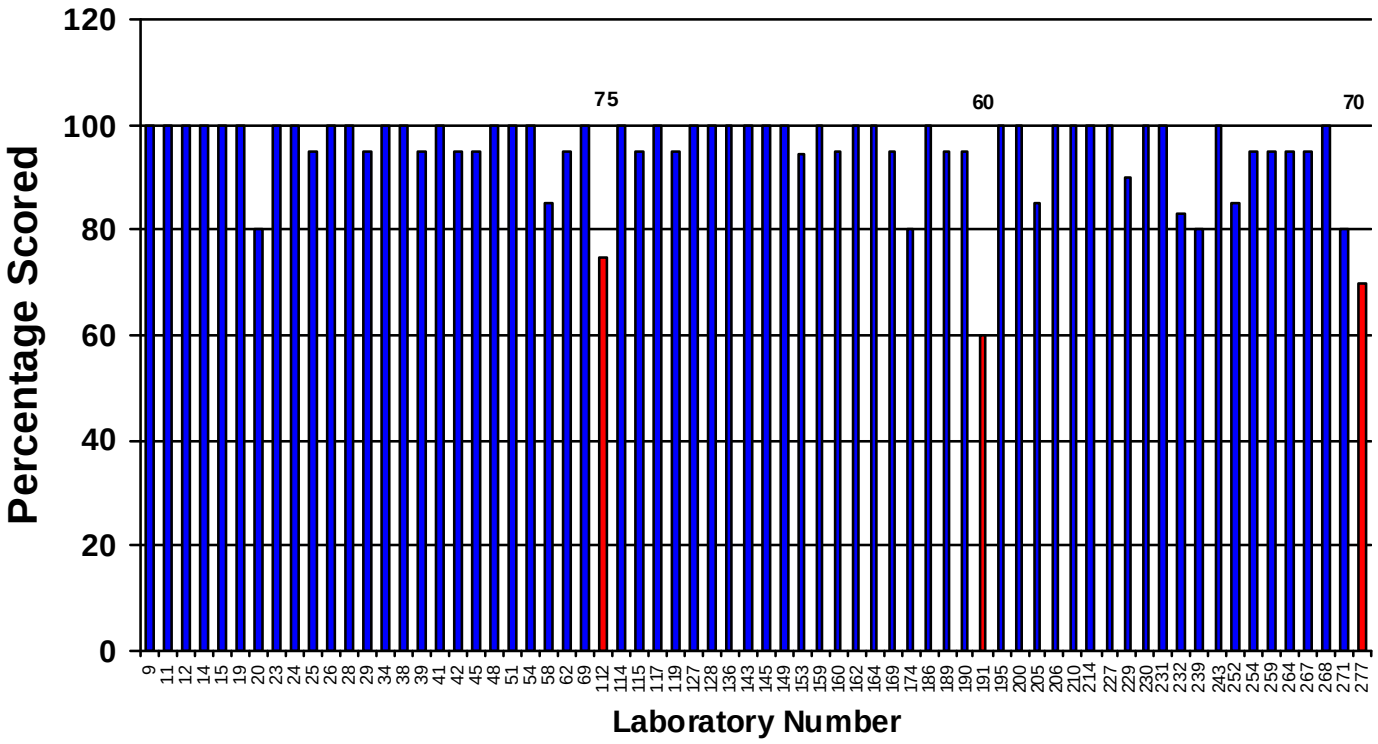
**Table 4: Provisional Specificities and Concordance**

| Sera ID | Provisional Specificity | % of labs detecting Ab | % class I positive | % class II positive |
|---|---|---|---|---|
| **601/13** | A66,34;B7;DQ3;DR4 | 100 | 71 | 100 |
| **603/13** | A9;Bw4;DR2,7 | 100 | 92 | 98 |
| **604/13** | DQ3 | 95 | 4 | 98 |
| **607/13** | A2,28;DR4 | 92 | 73 | 98 |
| **609/13** | | 6 | 1 | 5 |
| **611/13** | A1,9;B8;Cw17;DQ2 | 100 | 100 | 100 |
| **612/13** | B5;DQ1 | 100 | 100 | 100 |
| **614/13** | B62 | 96 | 96 | 8 |
| **615/13** | Cw3;DR2 | 90 | 74 | 91 |
| **616/13** | Cw17;DR1 | 76 | 9 | 80 |
| **617/13** | A2;B17;DR11;DQ3 | 90 | 88 | 80 |
| **620/13** | Bw6 | 100 | 100 | 2 |

The remaining 8 sera were AB serum or “hidden negative” – in 2012 the percentage of false positives was an all time low of 2.5 this year it was 5.0% and these were reported by 14 laboratories.

**Performance:** Satisfactory performance is making 80% of reports on all sera in agreement with consensus in a calendar year. In 2013 only 3 laboratories failed to reach 80% these are summarised in Figure 1.

**Figure 1: Performance Chart for Scheme 6**




**Changes to the Scheme for 2014:** None

*Judith Worthington, Transplant 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.

2013 was the Scheme's 6th year of operation – participation has increased year on year since 2008. Thus, in 2008 there were 28 participants (UK n=19, non-UK n=9). This climbed to 47 (UK n=23, non-UK n=24) in 2013.

The methods used in 2013 were: PCR-SSP - 57.5% (n=27), SSP & SSO -19.2% (n=9), SSO - 6.4, SSP & SBT - 6.4%, SSO & SBT - 6.4%, RT PCR - 4.3%.

In 2013 two distributions were made of 5 blood samples each.

Of the 10 samples supplied 2 were from B*57:01 positive donors and 8 were from B*57:01 negative donors. Two of the B*57:01 negative samples were B*58:01 – both were typed as B*57:01 negative by all participants.

There were no unsatisfactory performers in 2013.

Additionally, over the last three years, which involved 1,283 reports, there was 1 false B*57:01 negative assignment, apparently caused by a sample mix-up, and no false B*57:01 positive assignments.

There are no changes planned for Scheme 7 for 2014.

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

## 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.

For the first time in 2013 the scheme was operated as a fully assessed scheme – rather than a pilot scheme.

In 2013 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 2$^{nd}$ 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 2$^{nd}$ 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 19 participants in 2013 - 8 UK labs and 11 non-UK labs.

Of the 19 labs:

- 16 reports were in accord with the consensus findings (4 labs only tested the last 5 samples)
- 2 reports involved 1 error each
- 1 report involved 2 errors

Thus, there was 1 unsatisfactory performer for 2013 (this was a non-UK participant).
There are no changes planned for Scheme 8 for 2014.

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

## EDUCATIONAL SCHEME

In 2013 all 4 Educational Scheme samples were sent as DNA extracts. The alleles of interest were: ED01/13 – A*01:01:38L, ED02/13 – A*33:44 and DRB1*08:09, ED03/13 – B*49:03, ED04/13 – C*02:22. Between 25 and 30 laboratories reported on the samples – the findings were:

**ED01/13 - A*01:01:38L**

A*01:01:38L (differs from A*01:01:01:01 by a single silent change (705G>A) in exon 4 which causes a splice site and intron 3 and part of exon 4 are spliced out resulting in low expression). 12 out of 30 labs (40.0%) assigned A*01:01:38L or A*01:01/01:01:38L. There were 12 reports of A*01 and 6 reports of A*01:01 or A*01:01:01:01.

**ED02/13 – A*33:44 and DRB1*08:09**

A*33:44 (differs from A*33:01:01:01 by 3 bases in exons 3 and 4 causing 3 amino acid substitutions).

Just 2 out of 26 labs (7.7%) assigned A*33:44, 22 labs reported A*33 while 2 reported A*33 allele groups, of up to 25 alleles, although both groups lacked A*33:44.

DRB1*08:09 (differs from DRB1*08:01:01 by 7 bases in exons 1 and 2 causing 3 amino acid substitutions).

20 out of 29 labs (69.0%) assigned DRB1*08:09, 8 labs reported DRB1*08 and 1 lab reported DRB1*08:09/42.

**ED03/13 - B*49:03**

B*49:03 (differs from B*49:01 by 8 bases in exon 2 causing 4 amino acid substitutions).
23 out of 29 labs (79.3%) assigned B*49:03 while 6 reported B*49 only.

**ED03/13 - C*02:22**

C*02:22 (differs from C*02:02:02 by 3 bases in exon 3 causing 2 amino acid substitutions).
22 out of 25 labs (88.0%) assigned C*02:22 while 3 reported C*02 only.

It can be seen that while an average of 80% of participants identified the HLA-B, -C and -DRB1 alleles, assignment of the two ‘rare’ HLA-A alleles was only 7.7% of labs for A*33:44 and 40.0% of labs for A*01:01:38L.

This scheme has been operating for 12 years. A satisfaction questionnaire, in 2013, showed that 70% of participants considered that this scheme was worthwhile.

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

## 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 consider given the information presented in the scenario. Two scenarios were distributed in 2013 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 live donor kidney transplant case and was distributed to all participants of Scheme 2A – cytotoxic crossmatching and Scheme 2B – crossmatching by flow cytometry.

The HLA typing information of the recipient and potential live unrelated donor were presented along with HLA antibody testing and crossmatch results. Participants were asked to complete 5 questions based on the results provided. This included if they thought the results were a contraindication to transplantation, and the clinical advice they would give on expected outcome if the transplant proceeded.

Results were received from 46 participants.

### Clinical Scenario 2: Haematopoietic Stem Cell Transplantation

The second scenario was based on a based on a patient who was in need of a haematopoietic stem cell transplant (HSCT) and was distributed to participants of Scheme 4A2 – DNA HLA typing to the 2$^{nd}$ Field.

Information was provided on the 2$^{nd}$ field HLA type, and other patient specific factors, along with unrelated donor search results. Participants were asked to complete 6 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 27 participants.

### For 2014

A further two clinical scenarios are to be distributed in 2014. Existing participants of other UK NEQAS fro H&I schemes are able to register to receive a Solid organ transplant scenario and/or a HSCT scenario. These will continue to be not assessed in 2014.

*Deborah Singleton, UK NEQAS for H&I Manager*

## 3. NUMBER OF PARTICIPANTS DURING 2013

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 2013.

| Scheme | UK and ROI | International |
|---|---|---|
| Scheme 1A | 10 | 18 |
| Scheme 1B | 49 | 46 |
| Scheme 2A | 23 | 42 |
| Scheme 2B | 23 | 47 |
| Scheme 3 | 26 | 40 |
| Scheme 4A1 | 30 | 65 |
| Scheme 4B | 4 | 3 |
| Scheme 4A2 | 21 | 37 |
| Scheme 5A | 51 | 7 |
| Scheme 5B | 19 | 0 |
| Scheme 6 | 25 | 43 |
| Scheme 7 | 24 | 24 |
| Scheme 8 | 7 | 11 |
| Educational Scheme Samples | 23 | 5 |
| Educational Scheme Clinical Scenarios | 16 | 30 |

ROI – Republic of Ireland

## 4. 2013 ANNUAL PARTICIPANT MEETING – BRISTOL

67 participants representing 26 laboratories attended the UK NEQAS for H&I annual participant meeting in Bristol on the 3rd December 2013.

There was a special presentation and discussion concerning the new Interpretative Educational Scheme

There was also a scientific presentation from Dr Murali Somasundaram from the Nuffield Department of Surgical Sciences regarding Intestinal Tissue Engineering.

Slides from all of the annual meeting presentations may be downloaded from:

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

or requested from Deborah Singleton, Schemes' Manager.

The 2013 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 2014 PLEASE NOTE

Laboratories will retain their code numbers for 2014. 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 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 2014 Prospectus. Please see the 2014 Prospectus for full details of the assessment system which is available to download from the website

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