UK NEQAS

Bi-ennial Report
2007 - 2009

UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME
for Blood Transfusion Laboratory Practice

UK NEQAS (BTLP)
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1. INTRODUCTION

The Blood Group Serology Scheme was first recognised in 1979 when it was based at the BGRL in Oxford. The first exercises distributed covered crossmatching only. Over the next few years the Scheme expanded to include ABO and D grouping, antibody screening and antibody identification.

The Scheme completed a move from NIBSC at Potters Bar to Watford General Hospital in Watford in September 1995 to be sited alongside UK NEQAS for General Haematology. This coincided with a change in management and staff, although the Scheme Organiser remained the same. A change of name from Blood Group Serology to Blood Transfusion Laboratory Practice was made in April 1999 to encompass the non-serological aspects of transfusion practice that are assessed, particularly through non-scoring exercises and questionnaires. Red cell phenotyping was introduced in 2007.

The Scheme is advised by the Steering Committee (see Appendix I for composition) for Blood Transfusion Laboratory Practice, which meets three times a year. The meetings include discussion of past and future exercises; aims and objectives for the year; the organisation of the annual educational meeting; comments and complaints from participants; overall levels of performance.

In April 2001, the Scheme's UK activity was integrated fully into West Hertfordshire Hospitals NHS Trust reporting to the CEO through the Pathology Board. At the same time, the administrative aspects of the non-UK activity have been dealt with by a new company, Educational and Quality Laboratory Services (Blood Transfusion) Ltd. [EQUALS (BT) Ltd], through a contract for services with the Trust.

This report presents data for two financial years, April 2007 to March 2009.

2. STAFF

Chair of the Steering Committee – Dr Ann Benton
Scheme Director - Dr Megan Rowley
Scheme Manager - Mrs Clare Milkins
Deputy Scheme Manager - Ms Jenny White
Senior BMS – Mrs Dalila Benkhaled
Scheme Co-ordinator - Mrs Glynis Thorne (retired)

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3. PARTICIPANTS

The number of participants registered at March 2009 is shown in table 1. Overseas participation by country is shown in table 2. The Scheme also distributed the four ′R′ coded exercises to 31 WHO sponsored laboratories. The results from these laboratories are not included in this report.

Table 1 - Participation March 2008

Type of Participant	Number Registered
UK clinical (including Eire and Channel Islands)	452
Overseas clinical (including 3 BFPO*)	224
Diagnostic companies	7

*British Forces Posted Overseas

Table 2 - Overseas Participation by Country (excluding BFPO; including non-clinical)

Country	No. Participants	Country	No. Participants
Australia	1	Italy	25
Bahrain	1	Kuwait	13
Belgium	2	Malawi	1
Bolivia	1	Malta	3
Chile	1	Netherlands	4
China	2	New Zealand	1
Croatia	2	Norway	5
Cyprus	7	Oman	2
Denmark	38	Portugal	41
Estonia	2	Saudi Arabia	1
Faroe Islands	1	Serbia	2
Finland	4	Slovenia	1
France	2	South Africa	1
Germany	1	Spain	1
Gibraltar	1	Sweden	2
Greece	18	Switzerland	3
Greenland	1	Turkey	9
Hong Kong	1	United Arab Emirates	2
Iceland	2	USA	1
Israel	21		

4. PERFORMANCE SUMMARIES

4.1 Exercises Distributed:

Table 3 – Summary of exercises distributed

Exercise Code	Date Distributed	Contents	Main aim: (Q indicates that a questionnaire was included)
07E4	16 April 07	AS, ABID	Identification of antibody mixtures.
07R5	21 May 07	ABO/D, AS, ABID, XM, PH	Detection of weak Rh antibodies in screening and crossmatching. Rh phenotyping.
07E6	18 June 07	AS, ABID	Detection of 'standard' anti-D and potential for 'carryover' of a strong antibody; Identification of an antibody mixture.
07E7	9 July 07	AS, ABID	Identification of an antibody mixture, and titration of anti-K. Q
07R8	17 Sept 07	ABO/D, AS, ABID, XM, PH	D typing of a DAT positive rr sample. Detection of weak IgG antibody in the crossmatch; Ss phenotyping.
07E9	22 Oct 07	AS, ABID	Identification of an antibody mixture and exclusion in presence of anti-c.
07R10	19 Nov 07	ABO/D, AS, XM	Detection of weak anti-Jk^a in the crossmatch in an 'urgent' situation.
08E1	21 Jan 08	AS, ABID	Identification of antibody mixtures.
08R2	18 Feb 08	ABO/D, AS, ABID, XM, PH	Detection of ABO and IgG antibodies in the crossmatch. Jk^a/Jk^b phenotyping.
08E3	17 March 08	AS, ABID	Identification of an antibody mixture, and detection of 'standard' anti-D.
08R4	21 April 08	ABO/D, AS, ABID, XM, PH	ABO/D typing of a DAT positive rr sample. Detection of IgG antibodies in the crossmatch. Fy^a/Fy^b phenotyping.
08E5	19 May 08	AS, ABID	Identification of an antibody mixture.
08E6	16 June 08	AS, ABID	Reporting of anti-S in combination with an enzyme non-specific antibody. Detection and identification of a weak Rh antibody.
08E7	14 July 08	AS, ABID	Detection and identification of a weak antibody mixture. Identification of a single antibody.
08R8	22 Sept 08	ABO/D, AS, ABID, XM, PH	Recognition of mixed field reactions. IgG antibodies in the crossmatch; Jk^a/Jk^b phenotyping. Q re techniques.
08E9	20 Oct 08	AS, ABID	Identification of antibody mixtures.
08R10	17 Nov 08	ABO/D, AS, ABID	Testing undertaken where blood is required in an emergency. Q re emergency testing.
09E1	19 Jan 09	AS, ABID	Identification of an antibody mixture. Detection of a weak antibody.
09R2	16 Feb 09	ABO/D, AS, ABID, XM, PH	Detection of ABO and IgG antibodies in the crossmatch. Ss phenotyping.
09E3	16 March 09	AS, ABID	Detection of anti-D standard. Identification of an antibody mixture.

AS - Antibody Screen ABID - Antibody Identification
XM - Crossmatch PH – Red Cell Phenotyping Q - Questionnaire

4.2 General Information Relating to Exercise Summaries and material (4.3 - 4.12)

- Data is split between two categories, namely, *UK laboratories* and *non-UK laboratories*, with the following definitions:
    i. UK laboratories – clinical laboratories within the UK (NHS and private) and Eire.
    ii. Non-UK laboratories – overseas clinical laboratories (including BFPO) and commercial companies (UK and overseas).

- Antibody titres quoted are those obtained in the UK NEQAS laboratory on the closing date, by LISS spin tube, against red cells bearing heterozygous expression of the relevant antigen, unless otherwise stated.

- Numbers of errors reported includes late results, and any amendments to scores made following appeals.

- Numbers of participants include those who returned late results, which would not have been included in the exercise specific reports distributed at the time.

- Each 'Patient' whole blood sample comprises a pool of four or five donations, which may be diluted in ABO compatible FFP.

- Each 'Patient' plasma sample comprises a pool of ABO compatible plasma, some of which may contain red cell antibodies.

- Each 'donor' sample comprises a single red cell donation, diluted in modified Alsever's solution to make approximately 2 litres in total, giving a red cell concentration of 7-10%.

4.3 07E4

Material

'Patient' 1: Inert
'Patient' 2: Inert
'Patient' 3: Anti-D+C (titre 32 and 8 respectively)
'Patient' 4: Anti-K+Fy^a (titre 32 and 16 respectively)

Results

Table 4 – Summary of results for 07E4

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples**	**1/453**	**2** [1]	**1/92**	**1**
	- P1 - inert		1		
	- P3 - anti-D+C		1		
	- P2 - inert		0		1
Antibody ID	**All Samples**	**2/390**	**2**	**3/79**	**3**
	- P3 - anti-D+C		1		1
	- P4 - anti-K+Fy^a		1		2

[1] 07E3 tested in error

UK Errors:

Antibody identification:

- One participant reported anti-D only, for P3 – a positive reaction with an r'r cell was overlooked.
- One participant missed the anti-K, where it was masked by the anti-Fy^a.

Lessons:

- Antibody identification usually involves manual transcription and interpretation steps, making it particularly vulnerable to human error; SOPs should take this into account.

4.4 07R5

Material

'Patient' 1: O D negative, anti-D (titre 2)
'Patient' 2: B D positive, inert
'Patient' 3: A D positive, anti-E (titre 8)

'Donor' W: O D negative, rr
'Donor' Y: O D positive, R_2r
'Donor' Z: O D positive, R_1R_2

Results

Table 5 – Summary of results for 07R5

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
ABO Grouping	**All Samples**	**4[1]/458**	**7**	**1/201**	**3**
D Grouping	**All Samples**	**3/458**	**3**	**0/201**	**0**
	- False pos		1 [2]		
	- False neg		2		
Antibody Screening	**All Samples**	**0/453**	**0**	**4/188**	**4**
	- P1 - anti-D				2
	- P2 - inert				1
	- P3 - anti-E				1
Antibody Identification	**All Samples**	**0/396**	**0**	**2/137**	**2**
	- P1 - anti-D				1
	- P3 - anti-E				1
Incompatibilities	**All Samples**	**4/438**	**5**	**8/172**	**15**
	- P1DY (D)		1 [3]		1
	- P3DY (E)		1 [3]		5
	- P3DZ (E)		3		7
	- P1DZ (D)		0		2
Compatibilities	**All Samples**	**3/438**	**4 [4]**	**7/172**	**8**
Phenotyping (Rh)	**All Samples**	**6/237**	**8**	**0/10**	
	- False pos		3 [5]		
	- False neg		5 [5]		

[1] - Three made transposition errors and one a 'tick-box' error
[2] - Due to 'tick-box' error
[3] - Due to donor sample transposition error
[4] - Two due to donor sample transposition error
[5] - One due to donor sample or result transposition error

UK Errors:

ABO/D grouping

- Three laboratories made sample transposition errors:
  - Two did not book the EQA samples into the computer or assign accession numbers
  - One transposed the samples whilst labelling them and failed to include the usual check when loading them onto the automation.
- The two false negative D types were made in manual systems, with no clear cause.

Crossmatching

- One laboratory transposed two 'donor' samples, resulting in two missed incompatibilities and two missed compatibilities.
- Three laboratories missed the incompatibility between 'Patient' 3 (anti-E) and 'donor' Z (R_1R_2).

Red Cell Phenotyping (Rh CcEe)

- In addition to the errors shown in the table, three laboratories recorded the correct serological reactions but the incorrect 'shorthand' for the probable genotype.

4.5 07E6

Material

'Patient' 1: Anti-D (titres >32; 17iu)
'Patient' 2: Inert
'Patient' 3: Anti-s+Fy^a (titre 8 and >32, respectively)
'Patient' 4: Anti-D (UK NEQAS standard; titre 1)

Results

Table 6 – Summary of results for 07E6

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples**	**2/448**	**3**	**10/88**	**10**
	- P2 - inert		1 [1]		0
	- P3 - anti-s+Fya		1 [1]		0
	- P4 - anti-D		1		10
Antibody ID	**All Samples**	**25/393**	**25**	**7/76**	**7**
	- P1 - anti-D		1		0
	- P3 - anti-s+Fy^a		22 [2,3]		6
	- P4 - anti-D (P4)		2 [2]		1

[1] – Due to mislabelling of all four samples
[2] – One due to result transposition
[3] – One due to transcription error

UK Errors (excluding transcription/transposition error):

Antibody screening

- One laboratory missed the weak anti-D; however, this was detectable on repeat.

Antibody identification (standard anti-D):

- One laboratory reported anti-C+D for 'Patient' 1.
- One reported anti-E for 'Patient' 4; this was based on weak reactions with R_2R_2 cells only.

Antibody identification (anti-s+Fy^a)

- Twelve laboratories reported anti-s only:
  - in seven cases this was due to interpretation error where the anti-Fy^a was masked
  - the other five stated that they were unable to exclude anti-Fy^a but did not submit any supporting evidence.
- Four reported one of the two antibodies plus a second incorrect specificity, all due to interpretation error.
- Four made an interpretation of UI (submitting additional panel sheets for assessment), with which the Scheme disagreed.
- A further 11 made an interpretation of UI (± anti-s), with which the Scheme did agree.

Lessons:

- The antibody identification process must take into account the patient phenotype, the differential reactivity of antibodies, and the results of all cells tested (including screening cells).
- The presence of additional antibodies of clinical significance must be excluded before a final interpretation is made.

4.6 07E7

Material

'Patient' 1: Anti-K (titre 16)
'Patient' 2: Inert
'Patient' 3: Anti-E+K (titre 4 and 64, respectively)
'Patient' 4: Inert

Results

Table 7 – Summary of results for 07E7

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples**	**0/448**	**0**	**0/92**	**0**
Antibody ID	**All Samples** - P1 - anti-K - P3 - anti-E+K	**2/394**	**2** 2 [1]	**1/78**	**1** 0 1

[1] – One due to transcription error

Errors:

Antibody identification

- One laboratory reported anti-E+K for 'Patient' 1, correctly reflecting the reactions recorded on the worksheet; however, this was not repeatable, and the participant suspected that the results for 'Patient' 3 had been recorded twice.

This exercise included a non-scoring antibody titration exercise and practice questionnaire. See Appendix 2 for details of the questions and report.

4.7 07R8

Material

'Patient' 1: A D negative, DAT positive, anti-Fy^a (titre 8)
'Patient' 2: O D negative, inert
'Patient' 3: B D positive, inert

'Donor' W: O D negative, Fy(a+b-), Ss
'Donor' Y: A D negative, Fy(a-b+), Ss
'Donor' Z: O D negative, Fy(a+b+), SS

Results

Table 8 – Summary of results for 07R8

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
ABO Grouping	**All Samples**	**1/462**	**3**[1]	**1/211**	**3**
D Grouping	**All Samples**	**17/462**	**18**	**18/211**	**18**
	- P1 - false pos		16		14
	- P2 - false pos		1 [1]		3
	- P3 - false neg		1 [1]		1
Antibody Screening	**All Samples**	**1/456**	**1**	**4/193**	**5**
	- P1 - anti-Fy^a				4
	- P2 - inert		1		1
Antibody Identification	**All samples**	**1/397**	**1**	**2/145**	**2**
Incompatibilities	**All Samples**	**12/442**	**23**	**15/183**	**26**
	- P1DW (Fy^a)		4 [2]		10
	- P1DZ (Fy^a)		8 [3]		12
	- P2/P3 DY (ABO)		11[4]		4
Compatibilities	**All Samples**	**10/442**	**20** [5]	**9/183**	**12**
Phenotyping (Ss)	**All Samples**	**7/239**	**15**	**1/55**	**2**
	- False pos		2		1
	- False neg		13		1

[1] - Due to transposition of all three samples at the labelling stage
[2] - One due to 'donor' transposition error, and one due to testing the whole blood sample
[3] - Five due to transcription/transposition errors and one due to testing the whole blood sample
[4] - Eight due to transposition of the 'donor' units, two due transcription error and one to inappropriate use of EI
[5] - At least 14 due to transcription/transposition errors

UK Errors (excluding transcription/transposition errors):

D Typing 'Patient' 1 – D negative (rr), DAT positive

- Ten laboratories reported this sample as D positive and six as D variant (defined as weak or partial D on the EQA result sheets). All 16 were using BioVue for their initial testing: In many cases the reaction with the control reagent was weaker than that obtained with the anti-D reagent(s).
  - Testing was fully automated in 11 of these laboratories, and in each of these the cassette was brought forward for review by the automation and the result manually edited.
  - 7 assigned a D type without further testing.
  - 9 laboratories (all using automation) performed confirmatory tests:
    - 5 used ABD/ABD cassettes (no control reagent) – all obtained positive results.
    - 5 used saline IgM monoclonal anti-D reagents by tube (including one also using an ABD/ABD cassette for confirmation).
      - 4 obtained false positive results (one reading microscopically), that were all found negative on repeat;
      - One obtained negative results, but recorded these as positive due to a transcription error.

Following discussion with participants it appears that the following factors contributed solely or in combination to the wrong D type being reported:

- Potentiated anti-D reagents causing false positive reactions with the IgG coated cells.
- Reagent control giving negative reactions, or reactions weaker than anti-D reagent(s).
- Lack of understanding of the significance of a positive control.
- Reporting D positive or D variant based on a weak positive result with one anti-D reagent, contrary to BCSH guidelines and manufacturer's instructions.
- Reporting D positive or D variant based on mixed field reactions with anti-D reagent(s).
- Making manual edits to results from automation.
- Not following policy for repeat testing.
- Weak false positive results with IgM monoclonal anti-Ds (possibly 'over-reading' in anticipation of a positive result).

Incompatibility due to ABO

One of the laboratories transposing the donor units, plus one other, issued one or both of the ABO incompatible donors without undertaking any serology, indicating that electronic issue has been used; however, neither actually assigned barcodes to the 'donor' samples or booked them into the computer, and one did not book the 'patient' sample into the computer.

Incompatibility due to anti-Fy^a

- Two missed both incompatibilities, but detected them on repeat, with no obvious cause identified.

Phenotyping

Three laboratories reported the rare phenotype S-s-.

Discussion

See 08R4 discussion.

4.8 07E9

Material

'Patient' 1: Anti-K+Fy^a (titre 4 and 16 respectively)
'Patient' 2: Inert
'Patient' 3: Anti-c (titre 8)
'Patient' 4: Inert

Results

Table 9 – summary of results for 07E9

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples** - Inert	**0/447**	**0**	**2/91**	**2** 2
Antibody ID	**All Samples** - P1 - anti-K+Fy^a - P3 - anti-c	**5/391**	**5** 3 [1] 2	**4/79**	**4** 4

[1] - One due to probable transcription error

UK Errors:

Antibody identification

- Two laboratories reported anti-Fy^a only, in 'Patient' 1. They were presumably unaware that anti-K was masked by the anti-Fy^a.
- Two laboratories reported an additional antibody in 'Patient' 3.

Acceptable UI:
One laboratory made a 'UI submission', with which we agreed – they were unable to confirm the presence of anti-K.

Lessons

- Exclusion of additional antibodies of likely clinical significance is a vital part of antibody identification.

4.9 07R10

Material

'Patient' 1: O D positive, inert
'Patient' 2: A D positive, inert
'Patient' 3: O D positive, anti-c+Jk^a (titre >32 and 8 respectively)

'Donor' W: O D positive, R_1R_1, Jk(a+b+)
'Donor' Y: A D positive, R_1R_1, Jk(a-b+)
'Donor' Z: O D positive, R_1R_1, Jk(a+b-)

This was to be undertaken as an 'urgent' exercise, with no time for antibody identification or phenotyping.

Results

Table 10 – Summary of results for 07R10

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
ABO Grouping	**All Samples** - P1/3 - Gp O	**1/460**	**1** 1[1]	**1/221**	**1** 1
D Grouping	**All Samples**	**0/460**	**0**	**0/221**	**0**
Antibody Screening	**All Samples** - P3 - anti-c+Jk^a - P1/P2 - inert	**3/454**	**3** 3 [2]	**5/202**	**6** 4 2
Incompatibilities	**All Samples** - P1DY (ABO) - P3DY (ABO) - P3DW (Jk^a) - P3DZ (Jk^a)	**10/454**	**13** 2 [3] 0 8 [4] 3	**32/202**	**52** 3 3 28 18
Compatibilities	**All Samples**	**4/454**	**5** [5]	**11/202**	**20**

[1] - Due to 'tick-box' error
[2] - Two due to transcription error
[3] - Both due to transposition error
[4] - Three due to transcription error
[5] - Two due to transcription/transposition error

UK Errors *(excluding transcription/transposition errors):*

Antibody screening

- One laboratory suspected that they had not added plasma to the column in a manual BioVue technique

Crossmatching (anti-Jk^a)

- Three laboratories missed both incompatibilities; none were repeatable
  - One suspected that they had tested 'Patient' 1 twice by mistake.
  - In one case the automation software alerted that there were too few red cells available for testing, but this was overridden by the operator.
  - One used the cells provided, directly in their system, without preparing in the appropriate diluent or to the correct cell suspension.
- Two laboratories missed the incompatibility against 'donor' W only; these were non-repeatable errors, with no clear cause identified.

Lessons:

- The importance of treating EQA samples in the same way as clinical samples wherever possible.
- Procedures should be in place to control manual editing of automated results, to include responses to 'warnings' issued by the software.

4.10 08E1

Material

'Patient' 1: Inert
'Patient' 2: Anti-D+Jk^a (titre >32 and 4, respectively)
'Patient' 3: Anti-E+K (titre 8 and 16, respectively)
'Patient' 4: Inert

Results

Table 11 – Summary of results for 08E1

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples** - P4 inert	**1/446**	**1** 1	**0/96**	**0**
Antibody ID	**All Samples** - P2 - anti-D+Jk^a - P3 - anti-E+K	**5/390**	**5** 2 3 [1]	**10/84**	**11** 10 1

[1] – Two due to apparent 'tickbox' error

UK Errors:

Antibody identification (excluding 'tickbox' errors)

- There were two UI submissions for antibody identification:
  - in one case it was agreed that anti-D could not be positively identified
  - the other was not agreed, since the presence of anti-Jk^a was not considered.
- There were two further identification errors:
  - one missed anti-K
  - one result of UI with no supporting submission.

4.11 08R2

Material

'Patient' 1: A D negative, inert
'Patient' 2: O D positive, inert
'Patient' 3: B D positive, anti-Fy^a (titre 8)

'Donor' W: O D negative, Fy(a+b+), Jk(a+b-)
'Donor' Y: B D negative, Fy(a-b+), Jk(a+b+)
'Donor' Z: O D negative, Fy(a+b-), Jk(a-b+)

Results

Table 12 – Summary of results for 08R2

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
ABO Grouping	**All Samples**	**2/460**	**2** [1]	**4/211**	**5**
D Grouping	**All Samples**	**3/460**	**3**	**2/211**	**2**
	- False pos		2 [2]		0
	- False neg		1		2
Antibody Screening	**All Samples**	**1/455**	**1**	**1/201**	**1**
	- P3 false neg		1		1
Antibody Identification	**All Samples**	**1/394**	**1**	**1/152**	**1**
Incompatibilities	**All Samples**	**7/439**	**10**	**11/185**	**18**
	- P3DW (Fy^a)		6 [3]		8
	- P3DZ (Fy^a)		4 [4]		7
	- P1DY(ABO)				1
	- P2DY(ABO)				2
Compatibilities	**All Samples**	**4/439**	**5** [4]	**11/185**	**17**
Phenotyping (Jk^a/Jk^b)	**All Samples**	**10/257**	**15**	**0/59**	**0**
	- False pos		3 [5]		
	- False neg		12 [5]		

[1] – both due to transcription error
[2] – two due to transcription error
[3] – one due to transcription error
[4] – one due to transposition of results
[5] – two due to apparent transposition of samples or results

UK Errors (excluding transcription/transposition errors):

D typing
One laboratory recorded a false negative result; no cause for the error was established.

Antibody screening
The false negative antibody screen was non-repeatable, and no cause was established.

Antibody identification
One laboratory reported an additional specificity.

Crossmatching
- Three laboratories missed the anti-Fy^{a} against both 'donors':
    - One used an automated method, but was unable to follow their usual method, as they were unable to make a suitable packed cell suspension from the 'donor' cells.
    - Two used manual methods.

Phenotyping
- Three laboratories reported results of Jk(a-b-):
    - Two may have been due to transcription errors.
    - One reported all three 'donors' as Jk(a-) suggesting that the reagent may not have been working or correctly controlled.

Lessons:
- Serological crossmatching is usually undertaken manually, and is less standardised than antibody screening, making it vulnerable to technical and procedural error
- Where unlikely results are obtained, e.g. rare phenotypes, the possibility of procedural errors should be considered, and test and control results checked.

4.12 08E3

Material

'Patient' 1: Anti-E+S (titre 8 and 2 respectively)
'Patient' 2: Inert
'Patient' 3: Inert
'Patient' 4: Anti-D (UK NEQAS standard, titre 1)

Results

Table 13 – Summary of results for 08E3

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples** - P4 – anti-D	**0/442**	**0**	**7/95**	**7** 7
Antibody ID	**All Samples** - P1 - anti-E+S - P4 -anti-D	**9/387**	**12** 8 [1,2] 4 [2]	**2/83**	**2** 2 0

[1] - one due to 'tick-box' error
[2] - one due to result transposition

Errors (excluding transcription/transposition errors):

Anti-E+S

- Five laboratories did not identify anti-S
  - Two made UI submissions with which the scheme disagreed: one recorded false positive and false negative reactions; the other stated that anti-S could not be excluded, where it was possible to positively identify anti-S.
  - One further laboratory stated that anti-S could not be excluded but did not submit any supporting evidence
  - Two did not mention the possibility of anti-S
  - One reported an additional specificity as present

'Standard' anti-D

- One laboratory misidentified the antibody as anti-E
- Two included additional Rh antibodies

4.13 08R4

Material

'Patient' 1: B D positive, inert
'Patient' 2: A D positive, inert
'Patient' 3: O D negative, DAT positive, anti-S (titre 2)

'Donor' W: O D negative, Ss, Fy(a+b+)
'Donor' Y: O D negative, SS, Fy(a-b+)
'Donor' Z: O D negative, ss, Fy(a+b-)

'Patient' 3 was withdrawn from scoring for screening, identification and crossmatching, due to a combination of poor sample quality and significant deterioration of the antibody during the course of the exercise.

Results

Table 14 – Summary of results for 08R4

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
ABO Grouping	**All Samples**	**3/455**	**5** [1,2]	**1/199**	**3**
D Grouping	**All Samples** - P1/P2 false neg - P3 false pos	**21/455**	**23** 4 [3,4] 19 [3,5]	**12/199**	**13** 4 9
Antibody Screening	**All Samples** - False pos	**0/451**	**0**	**1/198**	**2** 2
Incompatibilities	**All Samples** - ABO only	**0/438**	**0**	**0/177**	**0**
Compatibilities	**All Samples**	**5/438**	**6** [6]	**2/177**	**3**
Phenotyping (Fy^a/Fy^b)	**All Samples** - False pos - False neg	**5/270**	**11** 3 [7] 8 [7]	**3/66**	**9** 3 6

[1] - Four due to transposition of results
[2] - One due to transcription error
[3] - One due to transposition of results
[4] - Three due to transcription error
[5] - Two due to transcription error
[6] - Three due to transposition of results
[7] - One due to transposition of results

UK Errors (excluding transcription/transposition errors):

D typing P3 (rr DAT+)

- 16 laboratories reported a result of D positive (n=12), or D variant (n=4)
  - Fifteen of these used a BioVue technique, 14 in an automated system.
  - One used two IgM monoclonal reagents by tube, but obtained negative reactions with these reagents on repeat after the closing date.
- Of those using BioVue:
  - All recorded a weak or MF reaction with the anti-D reagent.
  - With two exceptions, this was combined with a negative control (the other two also recorded a weak(er) reaction with the control).
  - In four cases the test was repeated, using different reagents:
    - in three cases similar potentiated reagents were used, two without a control and one where the control was positive.
    - in the fourth case, a saline reagent was used in a tube technique and negative results obtained, but not taken into account.
  - In eight laboratories it would appear that policy was not followed with respect to repeating the test with saline reagents or treating these results as D negative pending confirmation.

Antibody Screening (withdrawn from scoring)

- Two laboratories reported a negative antibody screen for 'Patient' 3 using automated Immucor CRRS. In house testing showed variable reactions with S+ cells from 0 to 5 (on a scale of 0 to 10), by manual CRRS on the closing date.

Crossmatching (P3 withdrawn from scoring)

- One laboratory missed both incompatibilities by testing the 'donor' cells directly from the EQA sample without making a suspension in the appropriate medium for their IAT technology.
- One laboratory missed both incompatibilities based on a negative antibody screen for 'Patient' 3 and selection of the 'donor' units by electronic issue.
- A further 32 laboratories did not detect the incompatibility between 'Patient' 3 and 'donor' W (Ss).

Red Cell Phenotyping

- One laboratory reported all three donors as Fy(a-b-) due to inappropriate use of an IAT typing reagent by direct agglutination.

Discussion

The error rate for D typing the rr DAT positive sample was the same as for 07R8, although the cohort of laboratories making the errors was different. BCSH guidelines state that an interpretation of D positive should not be made on the basis of a weak positive reaction with a single anti-D reagent. Ortho Clinical Diagnostics advise that (in addition to a positive reaction with the control invalidating the test) weak positive reactions (<= 2+) with anti-D in BioVue cassettes may indicate 'spontaneous agglutination' and should be confirmed using a different technology. This clearly requires use of non-potentiated saline reacting anti-D reagent(s) in the first instance, and may require further testing at a reference centre. Until the D type is confirmed, no interpretation should be recorded (other than D negative, if essential to issue blood) and only D negative blood should be transfused, at least to women of child bearing potential.

4.14 08E5

Material

'Patient' 1: Inert
'Patient' 2: Inert
'Patient' 3: Anti-c+Jk^a (titre 2 for both)
'Patient' 4: Inert

Sample 3 was withdrawn from scoring for antibody identification – see discussion below

Results

Table 15 – Summary of results for 08E5

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples** - P2/P4 inert - P3 anti-c+Jk^a	**3/442**	**3** 2 1	**0/96**	**0**

Anti-c+Jk^a – withdrawal from scoring

Twenty-two participants reported anti-c+Fy^b, rather than anti-c+Jk^a, and all but one included an enzyme panel. At least two sets of panels in common use, each had two R_1R_1 Jk(a+), Fy(b+) cells (one on the screening panel and one on the identification panel), making it impossible to distinguish between anti-Jk^a and anti-Fy^b by IAT alone. Since Fy^b is papain sensitive and Jk^a papain resistant, and there were only two antibodies present, it was considered not unreasonable to assign the second specificity as anti-Fy^b rather than anti-Jk^a, where negative reactions were obtained in the enzyme panel.

The enzyme activity of the anti-Jk^a was diluted out, and in-house testing gave misleading negative reactions using a 2-stage enzyme technique. For this reason the sample was withdrawn from penalty scoring for antibody identification.

UK Errors (excluding those reporting anti-c+Fy^b):

Antibody screening

No clear cause was established for the screening errors.

Antibody identification

- Six laboratories reported anti-c+UI and made 'UI submissions':
  - 5 stated that anti-Jk^a (+/- others) could not be excluded, and were agreed by the Scheme.
  - 1 stated that anti-M could not be excluded, but did not mention anti-Jk^a.

- A further 19 reported anti-c only:
  - 12 stated that they were unable to exclude anti-Jk^a, but these participants did not make UI submissions.
  - 5 did not mention the possibility of a 2nd specificity.
  - 2 stated that they were unable to exclude a 2nd specificity, but not anti-Jk^a (or anti-Fy^b)
- 14 reported anti-c plus a second, incorrect specificity, and did not mention being unable to exclude any other specificity: anti-M, -C^w, -Fy^a, -Lu^a.
- 2 made probable transcription errors.

Of particular concern were the five participants who submitted a result of anti-c only, and did not consider the possibility of a second specificity; either the anti-Jk^a was completely masked by the anti-c, or there were positive reactions with c negative cells that were unaccounted for. Also of concern, were the 13 who reported an incorrect 2nd specificity (as either present or not excluded), without mentioning anti-Jk^a and without having performed an enzyme panel; this includes one of the 22 who reported anti-c+Fy^b.

Lessons:
Resources do not allow the distribution of undiluted material for EQA, and an unfortunate consequence of this is that the enzyme activity of Kidd antibodies is often diluted out. Although Kidd antibodies would normally be expected to react by enzyme, it is advisable to exclude their presence by IAT where possible, since the sensitivity of 2-stage enzyme techniques can be somewhat variable.

Kidd antibodies are often weak and frequently demonstrate a ‘dosage’ effect, making them difficult to detect and identify, particularly in a mixture. More sensitive techniques may be required, and an IAT using enzyme treated cells is an excellent tool to confirm their presence or absence.

When interpreting antibody identification results all available information should be taken into account, including patient phenotype, differential reaction by technique, and results of all cells tested (including the screening panel). It is vital that the presence of an additional clinically significant antibody(ies) is excluded before a final interpretation is made.

4.15 08E6

Material

'Patient' 1: Inert
'Patient' 2: Anti-S + enzyme non-specific (anti-S titre 16)
'Patient' 3: Anti-E (titre 4)
'Patient' 4: Inert

Results

Table 16 – Summary of results for 08E6

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples**	**0/445**		**0/95**	
Antibody ID	**All Samples** - P2 - anti-S+ENS - P3 - anti-E	**7/394**	**7** 2 5 [1]	**3/83**	**3** 3

[1] – two due to probable transcription errors

Results of enzyme testing

Both anti-S and anti-S+ ENS (enzyme non-specific) were considered acceptable antibody identification results for 'Patient' 2.

The same proportion of laboratories (78%) used an enzyme panel for 'Patient' 2 (anti-S) as for 'Patient' 3 (anti-E). 55% of those using an enzyme panel for 'Patient' 2 reported an ENS.

UK Errors (excluding transcription/transposition errors):

Antibody Identification – 'Patient' 2

- One laboratory reported 'UI' with anti-S as 'not excluded', where it could have been positively identified from the panel profiles submitted.
- One laboratory reported anti-S+k.

Antibody Identification – 'Patient' 3

- One laboratory reported anti-c+/-E: an enzyme panel was performed (subsequent to the original IAT panel) and anti-c clearly identified; it is possible that 'Patient' 3 sample from exercise 08E5 was tested in error.
- One laboratory identified and reported Anti-K; however, on re-examination of the antibody panel results after the closing date, the reactions clearly showed anti-E.

Discussion

It is not uncommon to encounter 'non-specific' enzyme antibodies in clinical practice. Where an enzyme pan-agglutinin is detected, there is no need to be concerned about additional

specificities being 'masked' in the enzyme panel, as long as all clinically significant antibodies can either be positively identified and / or excluded by IAT. BCSH compatibility testing guidelines[1] state that 'the majority of antibodies detectable only by an enzyme technique are unlikely to be of clinical significance'.
Those using an enzyme panel for 'Patient' 2 but not reporting ENS, may have chosen not to do so, possibly due to a difference in the way EQA samples are reported (especially as this is the first time we have distributed an EQA sample including an ENS). It is also possible that the ENS was not detected by all of those using an enzyme panel, since we have no data regarding the enzyme method used (two stage enzyme, enzyme IAT, papain / ficin etc.) or its influence on the results obtained.

[1]Guidelines for compatibility procedures in blood transfusion laboratories.
Transfusion Medicine, 2004, **14**, 59-73. www.bcshguidelines.com

4.16 08E7

Material

'Patient' 1: Anti-D+C (titre 2 and 1 respectively)
'Patient' 2: Inert
'Patient' 3: Anti-Fy^a (titre >32)
'Patient' 4: Inert

Results

Table 17 – Summary of results for 08E7

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples** - P1 - anti-D+C	**0/443**	**0**	**1/96**	**1** 1
Antibody ID	**All Samples** - P1 - anti-D+C - P3 - aAnti-Fy^a	**7/395**	**7** 7 0	**10/84**	**11** 10 1

UK Errors (excluding transcription/transposition errors):

Antibody identification – 'Patient' 1

- Six laboratories did not record the presence of anti-C
  - two stated that anti-C could not be excluded
    - one was able to positively identify anti-C on re-examination of the panel results
    - one obtained one positive and one negative reaction with r'r cells by both IAT and one-stage enzyme techniques.
  - four did not report the potential presence of anti-C or say that they would have referred this sample
    - 2 used an enzyme panel
      - one reported anti-D+Lu^a based on a false positive and a false negative reaction
      - one overlooked the enzyme reactions when interpreting the results.

- One laboratory reported an additional specificity not actually present.

4.17 08R8

Material

'Patient' 1: B D positive/negative (75:25), inert
'Patient' 2: A D positive/O D negative (50:50) inert
'Patient' 3: A D negative, anti-Fy^a (titre 2)

'Donor' W: O D negative, Fy(a-b+), Jk(a+b+)
'Donor' Y: A D negative, Fy(a+b+), Jk(a+b-)
'Donor' Z: O D negative, Fy(a+b+), Jk(a-b+)

Results

Table 18 – Summary of results for 08R8

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
ABO Grouping	**All Samples**	**1/450**	**1** [1]	**2/215**	**2**
D Grouping	**All Samples**	**2/450**	**2**	**2/215**	**2**
	- False pos		2 [1]		2
Antibody Screening	**All Samples**	**1/447**	**1**	**4/210**	**4**
	- P3 - anti-Fy^a		1 [2]		4
Antibody Identification	**All Samples**	**1/393**	**1**	**1/160**	**1**
Incompatibilities	**All Samples**	**10/433**	**14**	**14/193**	**23**
	- P1DY (ABO)		2 [3]		1
	- P3DY (Fy^a)		6 [3,4]		9
	- P3DZ (Fy^a)		6 [3,5]		13
Compatibilities	**All Samples**	**6/433**	**7** [6]	**9/193**	**13**
Phenotyping (Jk^a/Jk^b)	**All Samples**	**11/267**	**18**	**1/80**	**2**
	- False pos		7 [7]		0
	- False neg		11 [7]		2

[1] - All due to transcription/result transposition error
[2] - Due to testing whole blood sample instead of plasma sample
[3] - One due transposition of results
[4] - Two due to transcription error
[5] - Three due to transcription error
[6] - Three due to transposition of results
[7] - Four probably due to transposition of samples or results

Performance Monitoring
'Patients' 1 and 2 were intended to represent the not uncommon clinical situation, where red cells compatible but non-identical for ABO and/or D red cells are transfused. UI was the expected interpretation for D typing for 'Patient' 1, and for both ABO and D typing for 'Patient' 2, since it is not possible to establish the true ABO and/or D type without clinical information. However, these tests were not subject to performance monitoring. 'Patient' 2 was not scored for compatibility testing, since it is reasonable to 'deselect' anything other than group O where the ABO group of the patient cannot be confirmed.

UK Errors (excluding transcription/transposition errors):

Crossmatching

- One laboratory missed the ABO incompatibility whilst apparently using electronic issue.
- Two laboratories missed both incompatibilities between 'Patient' 3 and 'donors' Y and Z:
  - One identified a problem with storage of the batch of cards
  - The other manipulated the samples by adding the plasma to the whole blood, potentially causing dilution and/or adsorption of the antibody.
- One laboratory missed the anti-Fy^a against 'donor' Y only, but this was not reproducible.

Phenotyping

- Nine laboratories recorded 3 false positive and 7 false negative results
  - 1 reported all 3 as Jk(b-) suggesting that the reagent was not working.

A report supplement was distributed separately with a full discussion of the mixed field reactions. This is reproduced in Appendix 3. A standard practice questionnaire was also distributed with this exercise; the report is reproduced in appendix 4.

4.18 08E9

Material

'Patient' 1: Inert Anti-E+Fy^a (titre 4 and 8 respectively)
'Patient' 2: Inert
'Patient' 3: Inert
'Patient' 4: Anti-D+K (titre >32 and 8 respectively)

Results

Table 19 – Summary of results for 08E9

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples**	**0/440**	**0**	**0/95**	**0**
Antibody ID	**All Samples** - P1 - anti-E+Fy^a - P4 - anti-D+K	**5/392**	**6** 4 [1,2] 2 [1]	**2/85**	**3** 2 1

[1] – One due to transposition of results
[2] – Two due to transcription error

UK Errors (excluding transcription/transposition errors)

Antibody Identification 'Patient' 1

- One laboratory reported anti-E only; retrospective examination of results clearly showed anti-E+Fy^a.

Antibody Identification 'Patient' 4

- One laboratory reported anti-D only; only one of the positive reactions with D-Kk cells was transcribed correctly, resulting in anti-K being incorrectly excluded. The remaining positive reaction was attributed to an antibody to a low frequency antigen without further investigation.

4.19 08R10

Material

'Patient' 1: A D positive, inert
'Patient' 2: O D positive, inert
'Patient' 3: A D negative, anti-D (titre 32)

'Donor' W: Not provided
'Donor' Y: Not provided
'Donor' Z: Not provided

This was an 'emergency exercise', where 'patient' samples were provided with clinical details, and blood crossmatched from each laboratory's stock fridge; therefore crossmatching was not assessed.

Results

Table 20 – Summary of results for 08R10

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
ABO Grouping	**All Samples**	**4/439**	**4** [1]	**7/203**	**9**
D Grouping	**All Samples** - False pos - False neg	**0/439**		**4/203**	**5** 2 3 [2]
Antibody Screening	**All Samples** - False pos - False neg	**1/436**	**1** 1	**5/201**	**6** 1 5
Antibody Identification	**All Samples**	**1/391**	**1**	**0/154**	**0**

[1] - All due to data entry errors via the website
[2] – Includes one 'D variant'

UK Errors (excluding transcription/transposition errors):

Antibody screening

- One laboratory reported a false positive screen based on a weak positive reaction by IAT.

Antibody Identification

- One reported anti-D+C for 'Patient' 3.

Analysis of the emergency testing data was distributed separately and is reproduced in Appendix 5.

4.20 09E1

Material

'Patient' 1: Inert
'Patient' 2: Anti-D+E (titre 8 for both)
'Patient' 3: Anti-s (titre 8)
'Patient' 4: Inert

Results

Table 21 – Summary of results for 09E1

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	**Total No. of errors**	**Participants with errors**	**Total No. of errors**
Antibody Screening	**All Samples** - P1/4 – inert - P3 – anti-s	**2/433**	**3** 2 [1] 1 [1]	**1/98**	**1** 0 1
Antibody ID	**All Samples** - P2 - anti-D+E - P3 - anti-s	**9/389**	**9** 4 [2] 5	**1/87**	**1** 1 0

[1] – One due to transposition of results
[2] – Two due to transcription error

UK Errors (excluding transcription/transposition errors):

Antibody identification – anti-D+E

- Two laboratories reported anti-D only:
  - One stated that they were unable to exclude ant-E, but did not provide any supporting evidence
  - One overlooked a positive reaction with an r"r cell.

Antibody identification – anti-s

- Two reported anti-s+UI and made UI submissions, with which the scheme disagreed.
- Three reported an additional specificity not actually present.

4.21 09R2

Material

'Patient' 1: B D positive, inert
'Patient' 2: O D negative, inert
'Patient' 3: A D positive, anti-Jk^b (titre 16)

'Donor' W: O D negative, Jk(a+b+),ss
'Donor' Y: O D positive, Jk(a-b+), Ss
'Donor' Z: A D positive, Jk(a+b+), SS

Results

Table 22 – Summary of results for 09R2

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	Total No. of errors	Participants with errors	Total No. of errors
ABO Grouping	**All Samples**	**2/444**	**3** [1,2]	**2/214**	**4**
D Grouping	**All Samples** - False pos - False neg	**4/444**	**5** 3 [1,3] 2 [2,3]	**1/214**	**1** 0 1[9]
Antibody Screening	**All Samples**	**0/440**	**0**	**0/210**	**0**
Antibody Identification	**All Samples**	**4/392**	**4**	**3/164**	**3**
Incompatibilities	**All Samples** - P1DZ (ABO) - P2DZ (ABO) - P3DW (Jk^b) - P3DY (Jk^b) - P3DZ (Jk^b)	**7/430**	**15** 1 [2] 2 [4] 4 [4,5] 5 [5,6] 3 [2,5]	**13/196**	**24** 2 3 8 5 6
Compatibilities	**All Samples**	**5/430**	**10** [4]	**4/196**	**7**
Phenotyping (Ss)	**All Samples** - False pos - False neg	**6/267**	**10** 6 [7,8] 4 [7]	**3/83**	**6** 1 5

[1] – Two due to transposition of results
[2] – One due to transcription error
[3] – One due to transposition error
[4] – Two due to transcription error
[5] – Two due to testing of whole blood samples in error
[6] – Three due to transcription error
[7] – Three due to apparent transposition of samples or results
[8] – Two due to testing 'patient' instead of 'donor' samples
[9] – Includes one 'D variant'

UK Errors (excluding transcription/transposition errors):

Antibody identification

- One laboratory reported UI, but did not submit any supporting evidence.
- One made a UI submission, with which the Scheme disagreed.
- Two reported an enzyme non-specific antibody in addition to anti-Jk^b.

Phenotyping

- Two laboratories each made one phenotyping error - one false positive and one false negative.

Deselection of 'donor' Y for 'Patient' 2

50 laboratories deselected this D positive 'donor' for the D negative 'patient'. The penalty points were manually removed on this occasion; however this is not a satisfactory long-term solution, since genuine false positive results may not be assessed and the process is prone to error.

4.22 09E3

Material

'Patient' 1: Anti-D (titre >32)
'Patient' 2: Anti-D (UK NEQAS standard, titre1)
'Patient' 3: Inert
'Patient' 4: Anti-K+Fy^a (titre >32 and 4 respectively)

Results

Table 23 – Summary of results for 09E3

Test category		UK Laboratories		Non-UK Laboratories	
		Participants with errors	Total No. of errors	Participants with errors	Total No. of errors
Antibody Screening	**All Samples**	**0/430**	**0**	**9/99**	**11**
	- P1 - anti-D				1
	- P2 - anti-D				8
	- P4 - anti-Fy^a				1
	- Inert				1
Antibody ID	**All Samples**	**7/387**	**7**	**6/87**	**6**
	- P1 - anti-D		0		1
	- P2 - anti-D		2		1
	- P4 - anti-K+Fy^a		5 [1]		4

[1] - Four due to transcription error

UK Errors (excluding transcription/transposition errors):

Antibody identification – standard anti-D

- One laboratory reported anti-D+E due to misinterpretation of results – in this case the only positive reaction recorded by IAT was with an R_2R_2 cell, whilst the enzyme result showed a clear anti-D and exclusion of anti-E with a r"r cell.
- One reported anti-D+UI based on a stronger reaction with the R_2R_2 cell.

Antibody identification – anti-K+Fy^a

- One laboratory reported anti-Fy^a, due to misinterpretation of results, where a positive reaction with a Fy(a-) K+ cell was overlooked.

Lessons

On average R_2R_2 cells have a higher density of D antigen sites than other common D positive phenotypes, therefore preferential reactivity with R_2R_2 cells is not unexpected with a weak anti-D. A negative reaction vs. enzyme treated r"r cells and confirmation of anti-D in the enzyme panel is sufficient to exclude anti-E.

5. SUMMARY OF OVERALL RETURN AND ERROR RATES

The error rate is based on the number of opportunities for error by all participants returning results. Figures shown in brackets following the error rate for UK laboratories are the percentages known to be due to transcription or transposition errors (Tx). This information is not available for non-UK laboratories, as these participants are not contacted regarding errors made.

Return rates (2007/2009)

- for 'E' exercises = 98.1%
- for 'R' exercises = 97.9%

Tables 24 and 25 compare error rates over the last four financial years for UK and non-UK participants respectively, where n = the number of tests distributed in each category, that were suitable for scoring; e.g. there were 12 samples (suitable for scoring) distributed for ABO grouping during 2007/08, but only 11 during 2008/9.

***Table 24* – UK error rates** (Tx = transcription or sample transposition, or incorrect sample tested)

Analyte	08E4 – 09E3		07E4 – 08E3		06E4 – 07E3		05E4 – 06E3	
	n	error rate (%Tx)	n	error rate (%Tx)	n	error rate (%Tx)	n	error rate (%Tx)
ABO	11	0.26 (100%)	12	0.24 (77%)	12	0.15 (63%)	12	0.11 (50%)
D	10	0.67 (43%) [1]	12	0.45 (20%) [1]	12	0.13 (71%)	12	0.25 (36%) [1]
False Neg Ab Screen	16	0.05 (67%)	18	0.09 (71%)	17	0.16 (67%)	18	0.20 (75%)
False Pos Ab Screen	20	0.06 (20%)	18	0.05 (50%)	18	0.09 (71%)	18	0.28 (44%)
ABID (single)	9	0.6 (10%)	9	0.4 (23%)	9	0.7 (20%)	12	0.3 (31%)
ABID (dual)	5	1.1 (36%)	8	1.3 (17.5%)	8	1.8 (7%)	6	0.7 (50%)
Missed Incompatibility	8	0.8 (79%)	16	0.7 (47%)	12	0.8 (52%)	13	1.6 (30%)
Missed Compatibility	13	0.4 (61%)	20	0.4 (56%)	12	0.6 (41%)	20	0.6 (42%)
False Pos Phenotyping	6	1.0 (44%)	6	0.6 (50%)	Not applicable			
False Neg Phenotyping	12	0.7 (22%)	18	0.7 (13%)	Not applicable			

[1] - Includes one DAT positive cell. Adjusted figures for error rate, excluding the DAT positive cell are:
0.14% (71% tx) for 2005/06
0.18% (56% tx) for 2007/08
0.27% (100% tx) for 2008/09

***Table 25* - Non-UK error rates**

Analyte	08E4 – 09E3		07E4 – 08E3		06E4 – 07E3		05E4 – 06E3	
	n	error rate %	n	error rate %	n	error rate %	n	error rate %
ABO	11	0.79	12	0.47	12	0.61	12	0.58
D	10	1.02 [1]	12	0.79 [1]	12	0.19	12	0.67 [1]
False Neg Ab Screen	16	1.1	18	1.34	17	0.9	18	1.2
False Pos Ab Screen	20	0.1	18	0.3	18	0.1	18	0.3
ABID (single)	9	1.0	9	0.6	9	1.2	12	0.7
ABID (dual)	5	4.0	8	4.2	8	6.3	6	2.5
Missed Incompatibility	8	3.0	16	3.7	12	2.5	13	1.4
Missed Compatibility	13	1.0	20	1.5	12	1.0	20	0.9
False Pos Phenotyping	6	0.9	6	0.5	Not applicable			
False Neg Phenotyping	12	1.4	18	0.2	Not applicable			

[1] - Includes one DAT positive cell. Adjusted figures for error rate, excluding the DAT positive cell are 0.1% for 2003/04, and 0.42 % for 2005/06
0.42% for 2005/06
0.26% for 2007/08
0.64% for 2008/09

6. LEARNING POINTS FROM EXERCISE RESULTS

Antibody identification

- Antibody identification usually involves manual transcription and interpretation steps, making it particularly vulnerable to human error; SOPs should take this into account.
- The antibody identification process must take into account the patient phenotype, the differential reactivity of antibodies, and the results of all cells tested (including screening cells).
- The presence of additional antibodies of clinical significance must be excluded before a final interpretation is made; Exclusion of additional antibodies of likely clinical significance is a vital part of antibody identification, as highlighted in 07E6, 07E9 and 08E5.
- All positive reactions should be accounted for, without assumptions being made; e.g. in 08E9 the positive reaction with a K positive, D negative cell was incorrectly assumed to be due to an antibody to a low frequency antigen.

D typing

- 07R8 and 08R4 highlighted the problems associated with using D typing reagents that contain levels of potentiators sufficient to cause false positive reactions with IgG coated cells. 3% of participants recorded a false positive interpretation of the D type, despite in some cases, a positive control, and in other cases, a policy that should have prevented this from happening. The use of ABD/ABD cassettes for investigating the initial anomalous results, exacerbated the problem in some laboratories, as these cassettes contain similar potentiators but no control column.

Manual intervention in automated systems

- In 07R10, the automation alerted the operator that too few cells were available for testing, but this warning was overridden, demonstrating that procedures should be in place to control manual edits.
- 07R8 and 08R4 demonstrated the dangers of manual editing; the majority of laboratories reporting an interpretation of D positive (or D variant) for the rr DAT positive sample, made a manual edit of an automated result, where the software had brought the cassette forward for review.

Unexpected results

On two occasions, rare phenotypes have been incorrectly recorded for red cell phenotyping: S-s- in 07R8, and Jk(a-b-) in 08R2. This highlights the importance of including appropriate controls, using reagents according to the package inserts and validated methods, and considering the possibility of procedural error when faced with an unlikely result.

Manual systems

- Several exercises have highlighted the vulnerability of manual systems to transcription, transposition and other procedural errors in all aspects of serological testing; this should be taken into account when establishing SOPs for manual procedures; e.g. anti-c+Jk^{a} was missed in a manual BioVue technique in 07R10, probably due to plasma not being added to the microcolumn.

Testing of EQA samples

- Several exercises demonstrate the need to treat EQA samples as much like clinical samples as possible. Some errors would not have occurred had the EQA 'Patient' samples been given accession numbers, booked into the computer or been subject to the same testing procedures as clinical samples.

Antibody screening

- The error rate for antibody screening is extremely low, reflecting the quality of both reagent red cells and IAT technology. This, combined with secure automation for blood grouping and antibody screening, paves the way for the safe implementation of electronic issue.

7. SCHEME DEVELOPMENT AND QUALITY INDICATORS

7.1 Accreditation

Unconditional CPA accreditation of the Scheme has been maintained through a fourth cycle, including two inspections under the new standards, the most recent being in July 2008.

7.2 IT and communications

By March 2009, 84% of UK and 71% of non-UK laboratories had taken advantage of web-based entry of results and receipt of reports.

A new on-line tool (Survey Monkey) for collecting survey data was used in a small trial to approximately 80 UK participants in December 2008. The tool worked well, and the feedback was positive. The surveys can be sent by e-mail or linked to a website, and are now in routine use.

7.3 Delegate fee in annual subscription

At re-registration in March 2008, UK participants were asked to indicate whether they would like to include one delegate fee (at a reduced rate) for the Annual Participants' meeting within the annual subscription for 2008/09. This followed a straw poll at the 2006 annual meeting and a written questionnaire after that meeting. The option was taken up by 169 (42%) of UK participants. Unfortunately, approximately 40 of these laboratories were unable to send a delegate, mainly due to acute staffing problems. This registration option will continue to be offered by the Scheme.

7.4 UI Submissions

A total 39 UI submissions were received during this two year review period. On review of the panel sheets and explanations, the Scheme agreed with 26 submissions (66.7%) and disagreed with 13 (33.3%). There were two appeals, both of which were upheld by the Scheme. Appendix 6 lists all the UI submissions, and provides further details on the 13 where there was no agreement; the current version of the 'Rules' are in appendix 7. This data will be continue to be reviewed by the Steering Committee.

7.5 ABO titration Pilot

Following discussions with the Steering Committee, and collaboration with UK NEQAS for Histocompatibility and Immunogenetics, a small number of participants from the UK and overseas were recruited into an exploratory pilot exercise for titration of anti-A and anti-B

antibodies, between December 2008 and March 2009. The primary purpose of this pilot is to assess the variability in techniques and titration levels, where these are used to determine suitability for ABO incompatible renal transplantation. The Pilot was planned for May 2009 and will be reported on in the next annual report.

7.6 Performance Targets

All internal performance targets were met with the exception of report distributions. See footnote 3 to table 26 for details.

Table 26 – Performance targets from April 2007 to March 2009

Category	No. of Events	Target	Target Achievement Rate	Actual Achievement Rate
Exercise Distributions	20	On schedule	100%	**100%**
Report Distributions	20	Within 6 days of C/D [1]	90%	**70%** [3]
Complaints	16	Dealt with in 4 weeks	70%	**100%**
New Unsatisfactory Performers	100	Make telephone contact	90%	**94%**
		Within 5 days of C/D [1,2]	80%	**100%**
Borderline Performers	76	Make telephone contact	50%	**57%**
		Within 10 days of C/D [1,2]	80%	**100%**
Reported Sample Quality – Plasma	66	≤2% unsatisfactory	90% of samples	**94%**
Reported Sample Quality – Whole Blood Samples	21	≤2% unsatisfactory	90% of samples	**95%**
Reported Sample Quality – Red cells in Alsever's	21	≤2% unsatisfactory	90% of samples	**100%**
Integrity of Samples	54170	<0.5% unsuitable for testing per exercise	90% (i.e. 9/10 exercises)	**100%**

[1] - C/D = Closing Date
[2] - Of those contacted
[3] - Target was changed in February 2009 to 8 days for 'R' exercises, to reflect the additional work involved in reporting these complex exercises using the new computer system. The target remains at 6 days for 'E' exercises.

8. QUESTIONNAIRES AND NON-SCORING ELEMENTS

8.1 Titration exercise and questionnaire

07E7 included a non-scoring antibody titration exercise and questionnaire. The aims of the exercise with respect to titration, and the questionnaire, were to:

- Monitor compliance with BCSH guidelines in the follow-up of an antenatal patient with anti-K.
- Monitor compliance with BCSH technical guidance in performing titrations.
- Make a direct comparison between methodology and results.
- Investigate the circumstances in which anti-A and anti-B titrations are performed .

There was a wide range of titration results reported, and huge variation in practice. See Appendix 2 for full details of the questions and report.

8.2 Standard Practice questionnaire

A standard practice questionnaire was distributed with 08R8. The purpose of this questionnaire was to gather basic information on routine pre-transfusion grouping and antibody screening techniques (not necessarily the testing performed on exercise 08R8). Respondents were requested not to include information regarding testing performed on antenatal, cord or reference samples. This information will be updated on an annual basis. See Appendix 4 for full details.

8.3 Emergency Exercise

08R10 was an 'emergency' exercise and included additional result sheets and questionnaires. Participants were requested to crossmatch blood from their stock fridge, so no 'donor' cells were provided. The aim was to establish what pre-transfusion testing is performed when blood is requested in an emergency situation. See Appendix 5 for full details.

9. TRENDS IN USE OF TECHNIQUES IN UK NEQAS EXERCISES

The data for 1996, 2001 and 2005 are taken from one exercise in each calendar year and therefore only include laboratories returning results. 2008 data are derived from the questionnaire distributed with 08R8. Historically, questionnaire data have shown that some participants use different or additional techniques for UK NEQAS samples than for clinical samples.

9.1 IAT techniques used for antibody screening in the UK (data prior to 1999 does not include Eire)

Table 27 – IAT techniques used for antibody screening over time

Technique	1996		2001		2005		2008	
	No.	% of total	No	% of total	No	% of total	No	% of total
LISS spin-tube	127	29%	29	6%	8	1%	1	<1%
LISS addition	19	4%	7	2%	0	0%	0	0%
NISS tube	15	3%	1	<1%	0	0%	0	0%
DiaMed	131	30%	265	58%	313	66%	247	63%
BioVue	78	18%	105	23%	115	24%	105	27%
Microplate-LP	21	5%	7	2%	0	0%	0	0%
Microplate-SPAT	2	<1%	4	1%	1	<1%	0	0%
Capture RRS	6	1%	15	3%	25	4%	31	8%
Biotest SS	1	<1%	3	1%	5	1%	3	1%
More than one	33	8%	21	5	N/A	3%	1	<1%
Other/not stated	3	1%	3	1	2	0%	1	<1%
Total	436	100%	460	100	454	N/A	389	100%

LP - liquid phase
SPAT - solid phase antiglobulin test
Capture RRS – Capture R Ready Screen
Biotest SS – Biotest Solid Screen
N/A - Not applicable

9.2 IAT techniques used for crossmatching in the UK

Table 28 – IAT techniques used for crossmatching over time

Technique	1996		2001		2005		2008	
	No.	% of total	No.	% of total	No.	% of total	No.	% of total
LISS spin-tube	201	47%	68	15%	25	6%	10	3%
LISS addition	14	3%	7	2%	4	1%	0	0%
NISS tube	15	4%	2	<1%	2	<1%	0	0%
DiaMed	105	25%	255	56%	300	67%	266	70%
BioVue	72	17%	96	21%	109	24%	98	26%
More than one	17	4%	21	5%	7	2%	2	<1%
Other	2	<1%	6	1%	4	1%	5 [1]	1%
Total	426	100%	455	100%	451	100%	381	100%

1 – includes 3 using CRRS

9.3 Use of enzyme techniques in the UK

Table 29 – Use of enzyme techniques over time

Procedure	% of participants using enzyme techniques			
	1996	2001	2005	2008
Screening	56%	30%	12%	6%
Crossmatching	20%	No data	No data	No data

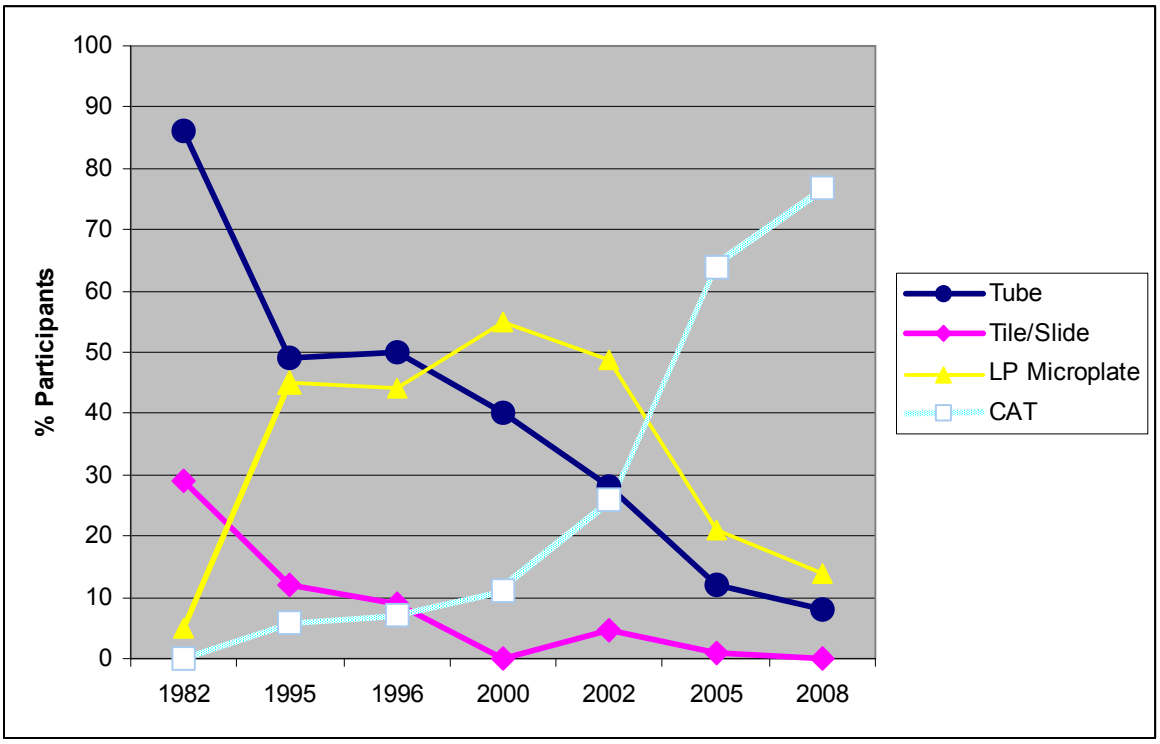
9.4 ABO and D grouping technologies in the UK

From 2002 onwards, data relates to routine testing only. Prior to this, where two or more techniques have been cited, the figures are included in each technique, and some may relate to rapid testing.

Table 30 – ABO grouping technology used over time

Technique	1982	1996	2002	2005	2008
Tube	86%	50%	24%	12%	8%
Slide	29%	8%	<1%	1%	0%
LPM	5%	44%	41%	21%	14%
CAT	0%	7%	33%	64%	77%
Other	<1%	<1%	1%	1%	1%

Figure 2 – ABO/D grouping techniques over time



10. INFORMATION, EDUCATION AND PUBLICATIONS/PRESENTATIONS

Education

- Annual meeting November 2007: See appendix 8 for programme details.
- Annual meeting November 2008: See appendix 9 for programme details.
- MRCPath teaching
- A history of the Scheme was displayed at the 25th anniversary BBTS meeting in Glasgow in September.

Publications

Abstract (poster presentation)
*ABO Grouping - Serological Detection of a Mixed Field Reaction within the UK, Portugal and Denmark. C.E. Milkins, J. White, M.R. Rowley. 2007, Vox Sang **89**, Supplement*

Abstract (poster presentation)
Trends in Practice and Performance in UK NEQAS (BTLP).
*J. White, C.E. Milkins, M.R. Rowley. 2008, Vox Sang **95**, Supplement 1*

Abstract (poster presentation)
Misinterpretation of RhD Negative DAT Positive Samples in UK NEQAS Exercises – New techniques, Old Problems
*C.E. Milkins, J. White, M.R. Rowley. 2008, Vox Sang **95**, Supplement 1*

Presentations/teaching

In addition to those already included in the publications section, Scheme staff made several contributions through oral presentations and teaching to many different organizations.

UK NEQAS (BTLP) was also represented on the following groups/bodies:

- SHOT Standing Working Group
- National Transfusion Laboratory Collaborative
- BCSH Blood Transfusion Task Force
- BBTS SIG for Blood Bank Technology
- Writing group for the BCSH FMH guidelines
- National Occupational Standards
- Healthcare Science Career Pathways workshops
- Modernising Scientific Careers
- Specialist Advisory Committee for Immunohaematology (SACIH)
- CMOs National Transfusion Committee IT Working Group (Joint with the NPSA)

11. FINANCIAL STATEMENT

Income and Expenditure Summaries for the two-year period (to the nearest £500)

Income:

Participant Type	2007/09
UK Clinical Laboratories:	613,000
Non-UK Clinical Laboratories:	288500
Non-Clinical Laboratories:	8000
Grand Total	**£909,500**

Expenditure:

Category	2007/09
Capital Expenditure	0
Salaries:	470500
Revenue:	273000
Overheads:	157000
Education/R&D (inc. books meetings etc.)	9000
Grand Total	**£909,500**

Appendix I

Composition of Steering Committee at March 2009

Dr Ann Benton (Chair), Morriston Hospital, Swansea/Welsh Blood Service
Mrs Clare Milkins (Secretary), Scheme Manager, UK NEQAS
Dr Megan Rowley, Scheme Director, UK NEQAS
Ms Jenny White, Deputy Scheme Manager, UK NEQAS
Dr Peter Baker, Royal Liverpool University Hospital
Mrs Heather Cawley, BUPA Murrayfield, Wirral
Mrs Samantha Harle-Stephens, Derriford Hospital, Plymouth
Dr Edwin Massey, NBS Bristol/United Bristol Healthcare Trust
Dr Kieran Morris, Northern Ireland BTS
Mr Mark Williams, RCI, NHSBT, Leeds
Mr Stephan Bates (NQAAP representative), Cheltenham General Hospital
Mr Malcolm James (co-opted), NHSBT Reagents, Birmingham

Appendix 2

Report of antibody titration exercise and questionnaire distributed with Exercise 07E7 – July 2007

Introduction

Antibody titration was included as an optional, non-scoring addition to Exercise 07E7 for all laboratories registered for antibody identification. Two of the exercise samples, detailed below, were to be titrated against the cells provided, assuming that 'Patient' 1 plasma was an antenatal 'booking' sample taken at 12 weeks of pregnancy, and 'Patient' 3 plasma, a further sample from the same woman at 28 weeks of pregnancy. A questionnaire was also included for those undertaking the antibody titration part of the exercise, with some questions relating to titration of antenatal samples in general, some directly to the methods used for the titrations performed in this exercise, and others to the follow-up of the 'Patient' (summary attached as appendix 1). The titrations were performed by 155 laboratories, however as not all respondents completed all questions, the numbers in the result tables do not always match the number of questionnaires analysed; and due to rounding, totals may not be exactly 100%.

Aims

The aims of the exercise with respect to titration, and the questionnaire, were to:

- Monitor compliance with BCSH guidelines[1] in the follow-up of an antenatal patient with anti-K
- Monitor compliance with BCSH technical guidance[2] in performing titrations
- Make a direct comparison between methodology and results
- Investigate the circumstances in which anti-A and anti-B titrations are performed

Material

Distribution 07E7 was also a routine antibody identification exercise for the majority not undertaking titration, and therefore an additional antibody was included in 'Patient' 3 (the 28 week sample) to increase the complexity of this aspect of the exercise. Table 1 shows the material provided.

Table 1

Sample labelled:	Representing	Specificity/ Phenotype
07E7 'Patient' 1	Booking sample	Anti-K
07E7 'Patient' 3	28 week sample	Anti-K+E
Titration 1	Cells for titration	rr (cde/cde), Kk

All laboratories were requested to use the same red cell sample for titration to reduce variables when comparing results; however, it was not possible to give the Rh and K phenotype of this cell without prejudicing performance monitoring for antibody identification. A second cell was not provided for the titration of anti-E, although this clearly would have been required in clinical practice. However, given the potential clinical significance of anti-K in pregnancy, this anti-E detected at 28 weeks should not have affected the follow-up actions taken.

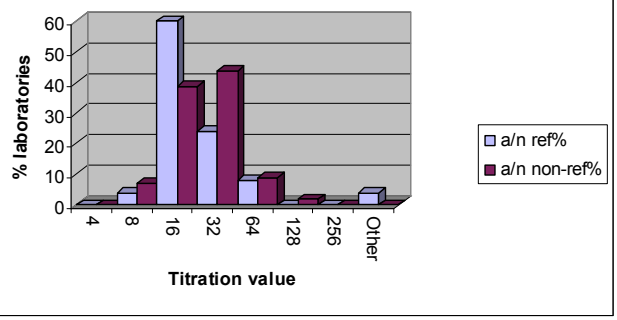
Appendix 2

The anti-K in 'Patient' samples 1 and 3 was from the same pool, with the volume of anti-K containing plasma in the final pool for 'Patient' 3 being approximately twice the volume added to that for 'Patient' 1. Since the cells provided for titration were E negative (rr), the titration values obtained were representative of the level of anti-K alone.

Titration Results

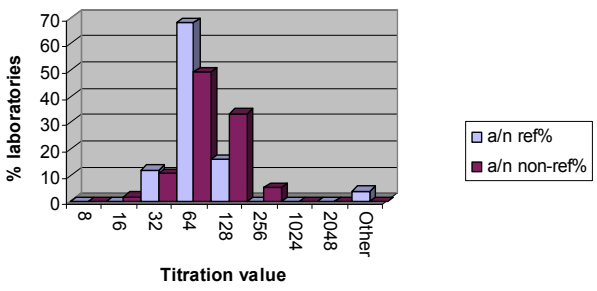
Figures 1 and 2 show the titration values obtained for 'Patient' 1 (12 week sample) and 'Patient' 3 (28 week sample) by reference laboratories (n=25) and non-reference laboratories (n=57) that routinely titrate antenatal samples. The median and range for laboratories not testing antenatal samples (n=71) is also given.

Fig 1 - 'Patient' 1 titration values for antenatal reference and non-reference laboratories



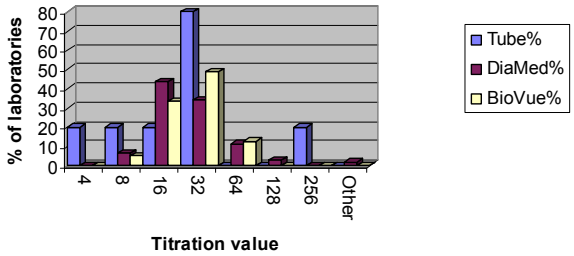
	Median	Range
A/n ref	16	8 - 64
A/n non-ref	32	8 - 128
Not testing A/n samples	32	4 - 256

Fig 2 - 'Patient' 3 titration values for antenatal reference and non-refernece laboratories



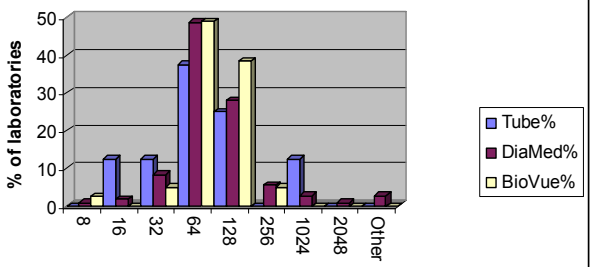
	Median	Range
A/n ref	64	32 - 128
A/n non-ref	64	16 - 256
Not testing A/n samples	64	8 - 2048

Fig. 3- 'Patient' 1 titration value by IAT technology



	Median	Range
Tube	32	4 - 256
DiaMed	32	8 - 128
BioVue	32	8 - 64

Fig. 4- 'Patient' 3 titration value by IAT technology



	Median	Range
Tube	64	16 - 1024
DiaMed	64	8 - 2048
BioVue	64	8 - 256

Questionnaire Results

Tables 2 – 5 give technical details of how titrations for 'Patients' 1 and 3 were performed, and Table 6 a comparison of median titration values for those using DiaMed systems with different plasma diluents.

Table 2 - IAT technology used

Technology	Number (%)
DiaMed	107 (69%)
Tube	8 (5%)
BioVue	39 (25%)
DiaMed and BioVue	1 (1%)
Total	**155 (100%)**

Table 3 – Red cell diluent vs. technology

Technology	Red cell diluent (% using technology)						
	LISS	PBS	DiaMed Diluent 2	DiaMed CellStab	Ortho 0.8%	Ortho OAES	Other
Tube	6 (75%)	2 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
DiaMed	1 (1%)	4 (4%)	60 (56%)	40 (37%)	0 (0%)	0 (0%)	2 (2%)
BioVue	5 (13%)	13 (33%)	0 (0%)	0 (0%)	14 (36%)	2 (5%)	5* (13%)
Total	**12 (8%)**	**19 (12%)**	**60 (39%)**	**40 (26%)**	**14 (9%)**	**2 (1%)**	**7 (5%)**

* Ortho BLISS

Table 4 - Plasma diluent

Technology	Number (%)
PBS	118 (77%)
LISS	1 (<1%)
AB serum / plasma	3 (2%)
DiaMed CellStab	4 (3%)
DiaMed Dil 2	23 (15%)
Ortho 0.8%	4 (3%)
Other	1* (<1%)
Total	**154 (100%)**

*1% albumin in DiaMed CellStab

Table 5 – Reaction grade at titration end-point vs IAT technology

IAT Technology	Reaction grade at end point of titration - number (% of those using IAT technology)		
	2+	1+	Weak
Tube	1 (13%)	5 (62%)	2 (25%)
BioVue	2 (6%)	20 (55%)	14 (39%)
DiaMed	9 (8%)	49 (46%)	49 (46%)
Overall	**12 (8%)**	**74 (49%)**	**65 (43%)**

Table 6 – Titration value vs. plasma diluent used in DiaMed systems

Diluent	Median titration value Patient 1	Median titration value Patient 3
PBS (n=74)	16	64
Other diluents (n=32)	32	128

- 148 determined the end point of the titration macroscopically and 4 microscopically.
- 141 prepared dilutions using an automatic pipette and 9 used drops from a pasteur pipette.
- 6 laboratories stated that the NIBSC anti-D standard was titrated in parallel with the exercise samples, with results ranging from 1 in 2 to 1 in 800.
- Overall 58/82 (71%) laboratories that routinely titrate antenatal samples stated that the previous sample is titrated in parallel with the current sample, where possible. This includes 20/25 (80%) reference laboratories, and 38/57 (67%) non-reference laboratories.
- 5/82 (6%) of those that routinely titrate antenatal samples select 'homozygous' cells, with one of these five using either heterozygous or homozygous cells depending on the antibody specificity.

Follow-up

The 'follow-up' sections were completed by 151 and 150 laboratories at 12 and 28 weeks respectively. Tables 7 – 9 show the numbers of laboratories taking actions at 12 weeks, 28 weeks and in total respectively.

Table 7 Follow-up at 12 weeks (completed by 151 laboratories)

Action	Number
Refer for confirmation of specificity	56
Request transfusion history	104
Advise re-test monthly until 28 weeks	134
Advise next re-test at 28 weeks	7
Phenotype partner (if available)	142
Refer for fetal genotyping (assuming paternal sample unavailable)	28
Refer to a fetal medicine unit	61
No further action until delivery	0
Other, please specify	31

Table 8 - Follow-up at 28 weeks (completed by 150 laboratories)

Action	Number
Refer for confirmation of specificity	51
Advise next re-test at 34 weeks	6
Advise two weekly re-test	134
Phenotype partner (if not already done at 12 weeks)	103
Refer for fetal genotyping (Assuming paternal sample not available, and not already done at 12 weeks)	34
Refer to a fetal medicine unit (if not already done at 12 weeks)	71
Request transfusion history (if not already done at 12 weeks)	63
No further action until delivery	1
Other	31

Table 9 - Total number (%) of labs taking actions at either 12 or 28 weeks

Action	Number (%)
Refer for confirmation of specificity	59 (47%)*
Phenotype partner	142 (95%)
Refer for fetal genotyping	40 (27%)
Refer to a fetal medicine unit	88 (59%)
Request transfusion history	104 (69%)

* % excludes labs that accept antenatal reference samples

- A total of 10 laboratories stated follow-up intervals outwith guidelines, with three of these following up only at 28 and 34 weeks.

Titration of anti-A and anti-B

Overall 40/155 (26%) laboratories perform titrations of anti-A and or anti-B:

- 16 for solid organ transplant
- 21 for BMT
- 14 for investigating ABO HDN
- 3 for immune deficiencies and one for platelet donors
- 13/40 (33%) are reference laboratories

Discussion

The 1996 BCSH guidelines for blood grouping and antibody testing in pregnancy were updated in 2006[1] and recognise the impact on testing strategies of changes in practice such as the universal use of RAADP, and new developments in testing, such as non-invasive fetal genotyping. The specificity most commonly implicated in HDN is still anti-D followed by anti-c and then anti-K. The new guidelines advise specifically on follow-up for women with anti-K since in these cases antibody titre is not always predictive of the outcome of the pregnancy. The mechanism for fetal anaemia due to anti-K (and other antibodies within the Kell system) may be due to inhibition of K positive early progenitor cells and/or to promotion of their immune destruction. Guidance on the methodology for performing titration remains essentially unchanged from the 1999 addendum[2] to the 1996 guidelines, except for the suggestion that D negative cells may be required for titration when women have received RAADP. The data from this questionnaire also shows that there has been little change in

practice since the last 'titration' exercise in 2004, and a wide variation in methodology for titration continues.

Titration methodology
The BCSH technical recommendations were primarily written with tube testing in mind, however, they include aspects that apply to all technologies:

'The end point should be (macroscopic) and well defined'
Not surprisingly, given that 95% are using column agglutination technology for titration, 97% of respondents read the end point of the titration macroscopically. However, the reaction grade used to define this end-point varies, with 8% using a 2+ reaction, 49% a 1+ reaction and 43% a 'weak' reaction; as in 2004, this varies both within and between IAT technologies.

'Dilutions should be made in phosphate buffered saline (PBS)'
All eight tube users prepare plasma dilutions in PBS; however, 10% using BioVue dilute in Ortho 0.8% solution, and 25% using DiaMed dilute in either DiaMed CellStab or Diluent 2. Whilst the numbers using BioVue diluents were too small for analysis, the median results for 'Patients' 1 and 3 were 16 and 64 respectively by DiaMed in combination with PBS, compared to 32 and 128 where another diluent was used. Whilst it cannot be confirmed without undertaking trials in parallel, this would appear to support the data from the 2004 questionnaire that use of low ionic strength diluents increases the titration result.

'Wherever possible each sample should be tested in parallel with the previous sample'
The previous sample (where available) is titrated in parallel with the current sample by 71% laboratories routinely undertaking antenatal titrations (*cf.* 68% in 2004). Since a rise in titre rather than the absolute value obtained is a significant indicator that the fetus may be affected during the pregnancy, it is essential to have reproducible and comparable results.

'The NIBSC standard anti-D should be used to validate sensitivity of the IAT method employed and can serve as an internal control'
Only six laboratories stated that the NIBSC anti-D standard was titrated in parallel with the exercise samples, with results ranging from 1 in 2 to 1 in 800.

'Wherever possible, the same cell sample (heterozygous for the antigen) should be used'
Five (6%) of those routinely testing antenatal samples stated that they deliberately selected cells with apparent homozygous expression of the relevant antigens (*cf.* 10% in 2004 and 22% in 1999*)*. One of the five stated that they selected heterozygous cells only for titration of Rh antibodies, and homozygous cells for all other specificities. None of these are reference laboratories. The guidelines suggest titrating with heterozygous cells to promote inter-laboratory standardisation.

Also, homozygosity is difficult to substantiate, and more importantly, heterozygous cells are easier to obtain, especially where antibodies of more than one specificity are present and each specificity has to be titrated with cells that do not carry the antigen(s) corresponding to the other antibody(ies). With the widespread implementation of antenatal anti-D prophylaxis, passive anti-D may be present in addition to alloantibodies, and this should also be considered when selecting cells for titration. Although zygosity of the cells will affect

the titration value obtained with most antibodies, it is not a factor affecting the results of this exercise, since all participants used the red cells provided.

Follow-up of anti-K detected at booking

The following algorithm for follow up of a woman with anti-K detected at antenatal booking is based the BCSH guidelines 2006[1]. However, fetal genotyping is not a firm recommendation in the guidelines due to the service being under development at the time of writing.

- Perform an initial titration
- Obtain transfusion history (most examples of anti-K are stimulated by transfusion)
- Phenotype putative father if available and paternity can be established
    - If the father is K-, then repeat titration at 28 weeks, and test to exclude further antibody specificities
    - If the father is K+, refer to a specialist Fetal Medicine Unit (FMU), and repeat the titration monthly to 28 weeks and then two weekly until delivery
- Now fetal genotyping is available as a routine service, this should be requested at 20 weeks where the K status of the fetus cannot be determined, i.e. where the partner is Kk or is unavailable for testing, and in cases where paternity cannot reliably be established. IBGRL advise testing for the K antigen from 20 weeks onwards, rather than from 16 weeks as for Rh antigens, to ensure that there is sufficient fetal DNA to obtain a reliable K typing result.
    - If the fetus is K-, repeat the titration and check for additional specificities at 28 weeks (repeat fetal genotyping may be advised at this stage)
    - If the fetus is K+, refer to a FMU and repeat the titration monthly up to 28 weeks, and then two weekly until delivery
    - If the titre of anti-K is rising significantly (under standard conditions) then it should be suspected that the fetus is K+, regardless of any 'evidence' to the contrary.

Compliance with BCSH guidelines in terms of obtaining a transfusion history for the mother was 69%, and for phenotyping the partner 95%, with all laboratories that took these actions doing so at the 12 week 'booking' stage. Recommendations for timing of follow-up samples were complied with by 93% participants, with some stating that they would follow blood service recommendations when requesting further samples (these were taken as compliant even if 'monthly to 28 weeks and then two weekly to term' boxes were not ticked). Those that gave more than once response dependent on the results of phenotyping and/or genotyping have also been included as compliant.

It is recognized that responses to questions regarding genotyping of the fetus would depend on the availability and results of the father's phenotype, and that referral to a FMU would depend on the father's phenotype and / or the genotype of the fetus. Referral to a FMU may be entirely a clinical decision and some participants commented that this was the case. It is also recognized that some centers participating would have FMUs on-site. However, only 59% of laboratories stated that they would refer to an FMU, despite the median anti-K

titre increasing at 28 weeks in 149/155 (96%) of laboratories, and reaching a level where the fetus may be at increased risk of being affected, i.e. >32 also in 149/155 (96%) of laboratories.

Conclusions

Where sequential samples are tested throughout a pregnancy, these may be subject to variables other than those inherent with choice of technology and reagents, such as being tested by different members of staff with subtle differences in methodology, or selection of cells with a different phenotype. It is therefore important that wherever possible the previous sample is re-tested in parallel and that the NIBSC standard anti-D is also titrated (vs. cells of the same Rh phenotype each time) in parallel, as an internal control.

Whilst the use of the NIBSC standard anti-D should enable some inter and intra laboratory comparison, it is not commonly used. It would appear that the wide variation in methodology used for titration, would make it difficult for reliable comparisons to be made. This is of concern since clinical decisions are being made, at least for antibodies other than Rh and K, on titration values as advised in BCSH guidelines. It is clear from this and previous UK NEQAS exercises that a titre of >32 (as an indication that the fetus is likely to be affected) has no consistent meaning within UK laboratories.

It may be that there is a need to review technical guidelines for titration as part of antenatal testing in view of the use of different IAT technologies, and also taking into account the use of titration in other settings such as BMT and solid organ transplant.

Antibodies within the Kell system should be considered to have the potential to cause HDN regardless of titre. Therefore, follow-up to establish the antigen status of the fetus (by paternal phenotype and / or fetal genotype) should always be undertaken. If the fetus is found to be antigen positive, then referral to a specialist FMU is essential, to allow potential fetal anemia to be monitored by middle cerebral artery Doppler ultrasonography, and treated if necessary. The possibility of mistaken paternity cannot be overlooked, and if results of sequential titrations are rising significantly in a case where the fetus is thought to be antigen negative, a referral to a FMU should be made.

References

[1]Guidelines for blood grouping and red cell antibody testing during pregnancy, 2006 www.bcshguidelines.com
[2]Addendum for guidelines for blood grouping and red cell antibody testing during pregnancy. *Transfusion Medicine,* 1999, **9**, 99 and www.bcshguidelines.com (archived guidelines)

Appendix 1 Summary of 07E7 titration questionnaire

Section 1 - Results:
Record titration values* and scores** for 'Patients' 1 and 3, and for NIBSC standard anti-D if used.

*reciprocal of the highest dilution giving a positive reaction e.g. 1 in 16 is the last dilution to give a positive result then the titration value = 16

** sum of scores allocated to reaction grades obtained in the titration

Section 2 – Follow-up:
Taking into account the antibody identification and titration results obtained on the booking ('Patient' 1) sample and 28 week ('Patient' 3) sample, please indicate in the text box below, what further tests and/or intervention you would suggest for this antenatal patient at booking (12 weeks) and at 28 weeks?

At 12 weeks

Refer for confirmation of specificity
Advise re-test at 28 weeks
Advise re-test monthly until 28 weeks
Phenotype partner (if available)
Refer for fetal genotyping (if paternal sample not available)
Refer to a fetal medicine unit
Request transfusion history
No further action until delivery
Other, please specify

At 28 weeks

Refer for confirmation of specificity
Advise re-test at 34 weeks
Advise two weekly re-test
Phenotype partner *(if not already done at 12 weeks)*
Refer for fetal genotyping *(if partner sample not available, and not already done at 12 weeks*)
Refer to a fetal medicine unit *(if not already done at 12 weeks)*
Request transfusion history *(if not already done at 12 weeks)*
No further action until delivery
Other

Section 3- Techniques used to obtain results for exercise 07E7

1. Indicate the IAT technology and red cell diluent used
2. Indicate the diluent used (i.e for diluting the plasma)
3. Were dilutions measured using Pasteur pipettes (drops) or an automatic pipette?
4. Was the end point of your titration macroscopic or microscopic?
5. Was the end point of your titration a 2+, 1+ or weak reaction?

General Questions (not relating specifically to Exercise 07E7)

1. Do you routinely titrate samples from antenatal patients?
2. If Yes do you:
   - Select (wherever possible) cells with apparent homozygous expression of relevant antigen(s)?
   - Select (wherever possible) cells with heterozygous expression of relevant antigen(s)?
   - Not specify the zygosity of the cells used for titration?
   - Titrate the previous sample (where available) in parallel with the current sample?

Appendix 2

3. Does your laboratory titrate antenatal samples referred from elsewhere?
4. Are you ever required to titrate anti-A and anti-B in patient samples?
5. If Yes are these for:
    - Solid organ transplant patients?
    - Bone marrow / stem cell transplant patients?
    - Other? (Specify)

Supplementary Report for Exercise 08R8 - Distributed 22 September 2008 - UK

Introduction

The samples provided for ABO/D grouping for 'Patients' 1 and 2 were designed to simulate a dual population of red cells, arising from the clinical situation where ABO and/or D non-identical red cells are transfused, either intentionally or unintentionally. The aim was to assess recognition of mixed field (MF) reactions, and both samples were non-scoring. For the purposes of the EQA exercise, UI was the expected interpretation for D typing for 'Patient' 1, and for both ABO and D typing for 'Patient' 2, since it was not possible to establish the true ABO and/or D type without clinical information to elucidate the cause of the mixed field (MF) reactions.

This analysis is based on data from 449 laboratories in UK and Eire, including two returning results after the closing date. It excludes our in-house registration and one laboratory testing for ABO only, using a molecular technique. Of these 449, one did not ABO/D type 'Patient' 2 due to poor sample quality. Reaction grades were not recorded by one laboratory for 'Patient' 1 and by two for 'Patient' 2.

Material

'Patient' 1: B D positive / negative (75:25)
'Patient' 2: A D positive / O D negative (50:50)*
*Prepared with group A D positive red cells, group O D negative red cells, and group A plasma

Results

1. Overall detection of MF reactions

The overall detection rates for the three mixed field reactions were:

- 25/448 (6%) for 'Patient' 1 cells and anti-D
- 176/446 (39%) for 'Patient' 2 cells vs. anti-A
- 116/446 (26%) for 'Patient' 2 cells vs. anti-D

Of those detecting the MF reaction vs. anti-D in 'Patient' 2, 111/116 (96%) also reported MF vs. anti-A.

Data for 'Patient' 2 is presented first since it is more complex, and many of the same points apply to 'Patient' 1.

2. 'Patient' 2 – Technology and automation

A total of 438/446 laboratories recorded reaction grades and technique(s) used for 'Patient' 2. Table 1 shows the numbers using each single technology and those using multiple techniques, and the number (%) of each of these detecting the MF reaction vs. anti-A and anti-D. Table 2 shows the use of automation by those using a single technology, and Tables 3 and 4 the percentage of those using a single technology detecting the MF reaction vs. anti-A and anti-D respectively, by manual and automated techniques.

Table 1 – Number detecting MF reactions by technology (% of those using each technology)

Technology	Number using technology	Number (%) recording MF vs. anti-A	Number (%) recording MF vs. anti-D
BioVue	85	50 (59%)	9 (11%)
DiaMed	181	58 (32%)	61 (34%)
Tube	41	7 (17%)	6 (15%)
Liquid Phase Microplate (LPMP)	34	4 (12%)	3 (9%)
Other	14	5 (36%)	4 (29%)
Multiple techniques	83	50 (60%)	31 (37%)
No technology stated	8	2 (25%)	2 (25%)
All	**446**	**176 (39%)**	**116 (26%)**

Table 2– Number (%) manual and automated testing by technology

Technology	Number using technology *	Number (%) Manual	Number (%) Automated	Number (%) Both
DiaMed	176	61 (35%)	99 (56%)	16 (9%)
BioVue	85	21 (25%)	53 (62%)	11 (13%)
LPMP	34	10 (29%)	22 (65%)	2 (6%)

*excluding 5 DiaMed users not stating whether testing was manual or automated.

Table 3 – Number (%) detecting MF vs. anti-A by technology and manual/automated testing

Technology	Number (%) detecting MF Manual	Number (%) detecting MF Automated	Number (%) detecting MF Both
DiaMed	19 (31%)	28 (28%)	10 (68%)
BioVue	6 (29%)	34 (64%)	10 (91%)
LPMP	2 (20%)	0 (0%)	2 (100%)

Table 4 – Number (%) detecting MF vs. anti-D by technology and manual/automated testing

Technology	Number (%) detecting MF Manual	Number (%) detecting MF Automated	Number (%) detecting MF Both
DiaMed	19 (31%)	32 (32%)	10 (63%)
BioVue	0 (0%)	4 (8%)	5 (45%)
LPMP	1 (10%)	0 (0%)	2 (100%)

3. 'Patient' 1 - Technology and automation

Table 5 shows the detection of the MF reaction vs. anti-D for 'Patient' 1 by technology.

Table 5 – Number detecting MF reaction by technology (% of those using each technology)

Technology	Number using technology	Number (%) recording MF
BioVue	96	0 (0%)
DiaMed	191	14 (7%)
Tube	44	1 (2%)
LPMP	40	1 (3%)
Other	14	2 (14%)
Multiple methods	56	7 (13%)
Technology not stated	7	0 (0%)
Total	**448**	**25 (6%)**

4. Interpretation of ABO/D typing results – 'Patients' 1 and 2

Table 6 shows the reaction grades recorded and D typing interpretations reported for 'Patient' 1, and Table 7 the interpretation reported for 'Patient' 2 vs. recording of MF reactions.

Table 6 - Reaction grades and interpretations for 'Patient' 1

Reaction grade 'Patient' 1	Interpretation			
	UI	**D positive**	**D Negative**	**Total**
MF	23	2	0	25
Strong positive	0	419	1	420
Weak positive	0	2	0	2
Negative	0	1	0	1
Total (%)	**23 (5%)**	**424 (95%)**	**1 (0<1%)**	**448 (100%)**

Table 7 – 'Patient' 2 detection of MF reactions vs. grouping interpretations

MF detected vs.	A D pos	A D UI	UI D pos	UI UI	Other	Total
Anti-A only	32 (49%)	0 (0%)	30 (46%)	3 (5%)	0 (0%)	65
Anti-D only	4 (80%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	5
Anti-A and anti-D	39 (35%)	5 (5%)	0 (0%)	67 (60%)	0 (0%)	111
Neither	262 (99%)	0 (0%)	0 (0%)	0 (0%)	3* (1%)	265
Total	337	6	30	70	3	446 (100%)

*one D variant, two D negative

- 71/176 (40%) detecting the MF reaction vs. anti-A reported 'Patient' 2 as group A.

5. Issue of group A blood for 'Patient' 2

Overall the crossmatch between 'Patient' 2 and 'Donor' Y (group A) was reported as compatible / suitable by 400/431 (93%) laboratories returning crossmatching results. Table 8 shows the proportion reporting compatible /suitable and issuing 'Donor' Y for 'Patient' 2, according to whether or not they reported the MF reaction vs. anti-A, and whether the group was reported as A or UI.

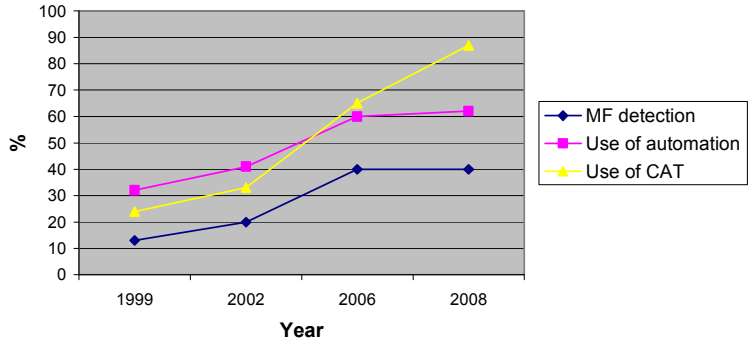
Table 8: Crossmatching results and issue of 'Donor' Y, vs. detection of MF reaction vs. anti-A and blood group assigned to 'Patient' 2

Grouping results and interpretations	Number	Number (%) reporting compatible / suitable	Number (%) that would issue 'Donor' Y*
'Patient' 2 reported as UI	99	68 (68%)	39 (39%)
Recording MF reaction vs. anti-A	171	140 (82%)	101 (59%)

*This is a minimum value as those not answering 'yes' are defaulted to 'no' at data entry.

6. Trends in EQA exercise including MF reactions

Figure 1 – Detection of MF, use of automation and of CAT over time



Discussion

Detection of MF reactions

For this analysis, the assumption has been made that if a MF reaction was not reported, then it was not detected. The detection rate of the MF reactions vs. anti-D in 'Patient' 1 (D pos / D neg 75:25) and 'Patient' 2 (pos/neg 50:50) was 6% and 26% respectively. This difference is not entirely unexpected, due to the proportions of D positive and D negative cells present, with the 6% detection rate in 'Patient' 1 being comparable to the 5% detecting a 75:25 MF vs. anti-D distributed in 2004 (04R8). However, the difference in detection rates for the MF reaction vs. anti-A (39%) and anti-D (26%) for 'Patient' 2 was not expected. For 'Patient' 2, the majority (96%) of those detecting the MF reaction vs. anti-D also reported a MF reaction vs. anti-A. Whilst the detection rate for the two MF reactions in 'Patient' 2 was very similar for DiaMed users (32% and 34%) this was not been the case for BioVue users. Although having the highest detection rate by technology for the MF reaction vs. anti-A (59%), BioVue users had a detection rate of only 11% for the MF reaction vs. anti-D. Those using multiple techniques including BioVue also showed the same picture.

A lower proportion of DiaMed users used automation (65%) *cf.* BioVue users (75%), and this might have influenced the overall MF detection rate, since data from exercise 06R9 and trend data from 1998 - 2006 indicated an increase in detection of MF reactions with the use of automation. However, data from this exercise does not follow this trend. Taking automation to include those using both manual and automated techniques, as well as those using automation alone, the detection rate of each of the MF reactions in 'Patient' 2 by DiaMed users was similar for manual and automated testing at 31% and 34% respectively. The detection rate for the MF reaction vs. anti-A for BioVue users was much higher using automation (as seen previously) at 69% *cf.* 29% for manual testing, and no manual BioVue users detected the MF vs. anti-D in 'Patient' 1.

Questions raised

There are unanswered questions surrounding this exercise, such as why did DiaMed automation not show the expected advantage over a manual technique, and why did BioVue users who detected the MF vs. anti-A not detect the MF vs. anti-D in the same sample? Some participants have reported that the MF reactions that they obtained with 'Patient' 2 did not appear to be 50:50, but seemed to have a higher proportion of cells 'positive' with anti-A and / or with anti-D. Others reported no detection of MF reactions with initial testing on automation (confirmed by re-examining images), but detection the MF reactions on repeat testing using the same sample and instrument, after receipt of the report.

There has been an ongoing debate regarding the effect of centrifugation on the distribution of patient and transfused cells in clinical samples. The EQA samples are prepared from pooled units from one donor session, to make samples with populations of cells of the same 'age', and with parameters such as haemoglobin and red cell volume within normal ranges. Therefore, the suspected reasons for a sometimes uneven distribution of the dual population in clinical samples, which contain disparate populations of patient and donor cells are unlikely to apply in this situation.

An alternative theory is that some or all of the 'negative' cells became trapped within the agglutinates formed by the reagent and 'positive' cells, affecting the proportion that passed through the gel or beads in CAT systems. It is possible that this could have been affected by the length of time that reactants were incubated before reading. The incubation time can vary using automated systems, depending on where in a 'run' the samples are placed. Where the EQA samples were tested routinely they might have been added to a batch of testing, whereas when tested as a 'one-off' repeat, they might have been put onto the automation as a single test. If this were the case, it could be an explanation for reports of variable reactions using the same sample and same instrument, and possibly also for the lower than expected detection rate of the MF reactions using DiaMed automation overall.

It is possible to speculate that the avidity of the reagent and the presence of any potentiators might also affect this process. This could account for the unexpectedly low detection rate of the MF reaction vs. the potentiated anti-D reagent in BioVue cassettes cf. the anti-A, where the level of PEG added to anti-D reagent(s) is higher than that added to anti-A. Further work is required to investigate the validity of these speculations, as it is important to ascertain whether anomalous results are artefacts of EQA, or whether they reflect clinical practice.

Selection of blood for transfusion

In clinical practice there are a number of causes of MF reactions in ABO/D grouping, the most likely being the transfusion of ABO/D compatible, non-identical blood. A rarer cause, but of utmost importance, is where the sample is from a post stem cell transplant recipient, either during the engraftment period or when the transplant is failing. Failure to recognise a MF reaction in this situation could lead to blood components of the incorrect ABO/D group being transfused, and other special requirements being overlooked, particularly where there is shared care or where the patient has been referred from a different centre and the laboratory is unaware of the diagnosis. Rarely, a MF reaction may be attributed to factors such as permanent chimerisms or ABO subgroups; however, it is still important to identify the cause before making a decision on the most appropriate blood group to transfuse.

A MF reaction may be the first sign that a clinically unrecognised ABO incompatible transfusion has taken place. Its recognition is therefore critical, to avert the potential for additional ABO incompatible units being transfused. This is especially relevant where no reverse group is performed in the presence of a historical group, as is routine practice for 25% of laboratories (08R8 Pre-transfusion testing questionnaire).

It is interesting to note that 43% of those detecting the MF reaction reported 'Patient' 2 as blood group A (*cf.* 60% in a similar exercise - 06R9). This may have been due to the lack of any anti-A in the reverse group, indicative of a 'group A' patient transfused with group O donor cells rather than another cause. However, without a history, it would have been prudent to defer drawing a conclusion or issuing blood other than group O. Despite the lack of clinical information on this sample, at least 59% of those detecting the MF reaction stated that they would have issued 'Donor' Y (group A) for 'Patient' 2, *cf.* 70% in the same situation in 2006 – exercise 06R9).

Appendices

Appendix 1 suggests a protocol for investigation and issue of blood where an ABO MF reaction is detected.

Appendix 3

Appendix 1 to supplementary report

Suggested protocol for investigation and issue of blood where an ABO MF reaction is detected.

1. A blood group should not be assigned nor red cells other than group O transfused until the cause of the MF is elucidated.
2. Clinical and transfusion history should be obtained, and consideration given to the possibility of ABO haemolytic transfusion reaction.
3. Testing should be repeated on the original sample, (and where possible on a second sample to rule out contamination of the original sample) including full forward and reverse group and DAT.
4. If the MF reaction is confirmed and clinical / transfusion history provides an explanation for the MF, red cells may be transfused as advised in the BCSH pre-transfusion compatibility testing guidelines[1], and summarised in Table 1.

Table 1 – Red cell transfusion in the presence of an ABO MF

Established Reason for MF	Group of red cells (and products contaminated with red cells) to be transfused
Transfusion of compatible, non-ABO identical red cells	Recipient group if possible
Transfusion of ABO ***incompatible*** red cells	Recipient group
BMT (Major mismatch e.g. group A donor and group O recipient)	Recipient group until no ABO antibodies to the donor group are detectable by IAT, and DAT is neg. Then donor group
BMT (Minor mismatch e.g. group O donor and group A recipient)	Donor group (plasma depleted until recipient red cells are no longer detectable).
BMT (Combined mismatch e.g. group A donor and group B recipient)	Group O until ABO antibodies to the donor ABO antigen(s) are not detectable by IAT, and DAT is negative. Then donor group.

5. If the patient has not been transfused, has not received a BMT and there is no other clinical reason for the MF reaction, then the MF reaction should be investigated serologically and may require a referral to a specialist centre for the following:
    a. Testing performed to investigate for rare subtypes, e.g. A_3 or A_{end}.
    b. Molecular typing and family studies to investigate the possibility of a chimera.

There are no BCSH guidelines for selection of blood for chimeric patients or those with rare ABO subtypes; however, red cells must be compatible by IAT with ABO antibodies in the plasma (including anti-A_1 if present). It would seem reasonable to give group O red cells in these rare situations.

Reference

[1] BCSH (2004) Guidelines for compatibility procedures in blood transfusion laboratories. *Transfusion Medicine,* 2004, **14,** 59-73 and www.bcshguidelines.co.uk

Appendix 4

Pre-Transfusion Testing Questionnaire
Distributed with exercise 08R8 – September 2008

Introduction

The purpose of this questionnaire was to gather basic information on routine pre-transfusion grouping and antibody screening, (not necessarily the testing performed on exercise 08R8). Respondents were requested not to include information regarding testing performed on antenatal, cord or reference samples. We intend to update this information on an annual basis.

Return Rate

Initially, 345/452 (76%) laboratories returned questionnaires. However, since the return rate was higher for laboratories not registered for web-entry (87%) than for web users (74%), the remaining web users were contacted by e-mail and requested to complete the survey on line as part of a trial of new software. A further 47 laboratories responded giving a final return rate of 392/452 (87%). Not all respondents answered all the questions, and therefore the numbers in the tables do not always total 392.

1. Summary and trend data

Table 1 shows a summary of current data compared to historical data where available

Table 1 – Trends in routine pre-transfusion testing

	2008 (n=392)	2005 (n=426)	2002 (n=446)
Automation for 'group and screen'			
Used during core hours[1]	68%	60%	41%
Proportion of full automation used 24/7	82%	N/a	N/a
Proportion of full automation interfaced to LIMS	89%	N/a	N/a
Routine ABO/D Grouping			
Liquid phase microplates	14%	21%	41%
Column Agglutination Technology (CAT)	77%	65%	33%
Omit reverse group on patients with historical groups	25%	20%	13%
Omit reverse group on patients without historical group	<1%	1%	1%
D typing reagents			
Single anti-D used once for patients with a historical group	45%	41%	15%
Single anti-D reagent used once for patients with no historical group	25%	23%	5%
Routinely include IAT for D typing on apparent D negatives	6%	5%	3%
Include and anti-CDE reagent	1%	6%	≥ 10%
Routine method of establishing compatibility			
Electronic issue	37%	26%	10%
'Immediate' spin	8%	11%	15%
IAT (± other technique(s))	55%	63%	75%
IAT technology antibody screening			
CAT	90%	92%	85%
SPMP	9%	8%	4%
IAT technology crossmatching			
CAT	96%	81%	77%[2]
Tube	3%	7%	17%[2]

[1] Full automation in 2008 *cf.* full or 'semi' automation in 2005 and 2002
[2] 2001 exercise data.

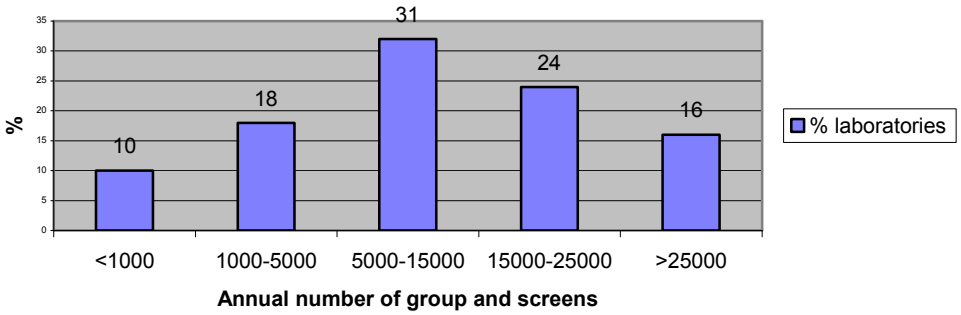
Appendix 4

2. Analysis of 2008 data

Q1 Number of 'group and screens' performed per annum.

Figure 1 shows the percentage of laboratories falling into workload categories based on the approximate number of group and screens performed per year.

Fig 1: Percentage of laboratories in each workload category



Q2. Use of automation

- 267/390 (68%) use full automation (i.e. for sample handling and reading) to perform 'group and screens' within core hours.
- Approximately 88% group and screens are tested with full automation during core hours (taking the number of group and screens performed by each laboratory to be the midpoint of the range reported).
- Of the 188/229 (82%) answering the second part of Q2, use the automation 24 hours a day seven days a week (24/7), including one laboratory stating that they are not open 24/7 but that the automation is used for all testing. The number of responses is reduced because this question was omitted from the on-line survey.

Table 2 shows the number (%) laboratories with an interface between the automation and laboratory information management system (LIMS).

Table 2 – Number (%) laboratories with automation – LIMS interface

Interface between automation and LIMS	Number (%) of laboratories using automation
Bi-directional	137 (53%)
Uni-directional	110 (43%)
Not interfaced	11 (4%)
Total	**258 (100%)**

Q3. ABO/D typing techniques

Table 3 shows the number (%) of laboratories using each technology as their primary ABO/D typing technique for patients with a previous group, and the percentage of each using full automation.

Appendix 4

Table 3 – ABO/D typing techniques used by number (%) laboratories

Technology	Number (%) laboratories	Number (%) using automation in core hours	Number (%) of those with automation, using it 24/7
DiaMed	197 (51%)	147 (75%)	107/128 (84%)
BioVue	101 (26%)	85 (84%)	58/71 (82%)
Liquid Phase Microplate (LPMP)	53 (14%)	32 (60%)	20/27 (74%)
Tube	31 (8%)	0 (0%)	N/a
Solid phase microplate	4 (1%)	3 (75%)	3/3 (100%)
Other	2 (<1%)	0 (0%)	N/a
Total	**388 (100%)**	**267 (69%)**	**188/229* (82%)**

*Reduced numbers due to exclusion from on-line survey.

Q4. Inclusion of a reverse group

- 98/389 (25%) omit the reverse group for patients with a previous group
  - 91/266 (34%) using full automation omit the reverse group *cf.* 7/123 (6%) not using full automation.
  - 86/297 (29%) using column agglutination technology (CAT) omit the reverse group *cf.* 11/90 (12%) using other technologies.
- 371/372 (>99%) include a reverse group for new patients (22 did not answer this question).
  - The one laboratory not including a reverse group does not use full automation, and uses electronic issue (EI).

Q5 and Q6. D typing

Table 4 shows the number (%) using one anti-D reagent once, or testing for D in duplicate, either different reagents or one reagent twice, for patients with and without a previous group.

Table 4 – D typing protocol for patients with and without a previous group

D typing reagents	Patients with a previous group	Patients with no previous group
Use a single anti-D reagent once	175* (45%)	96* (25%)
Use two anti-D reagents or one twice	206 (53%)	283 (74%)
Ticked both categories	6 (2%)	3 (1%)
Total	**387 (100%)**	**382 (100%)**

*Includes one using two D's only when a crossmatch is requested

- 168/175 (96%) of those using a single anti-D once for patients with a previous group use CAT, as do 95/95 (100%) of those using a single anti-D for patients with no previous group.

- 4 laboratories are using anti-CDE reagents (three for all patients, and one only for new female patients of child bearing potential).

- 22/387 (6%) routinely use an anti-D reagent by the indirect antiglobulin technique (IAT) to confirm the D status of apparent D negative patients.
  - 13% of those grouping by tube include an IAT anti-D reagent compared to 5% of those grouping by other technologies.

Appendix 4

Q7. Method of establishing compatibility

Table 5 shows the number (%) of laboratories using electronic issue (EI), 'immediate spin' (IS) or IAT +/- IS as their primary method for establishing compatibility.

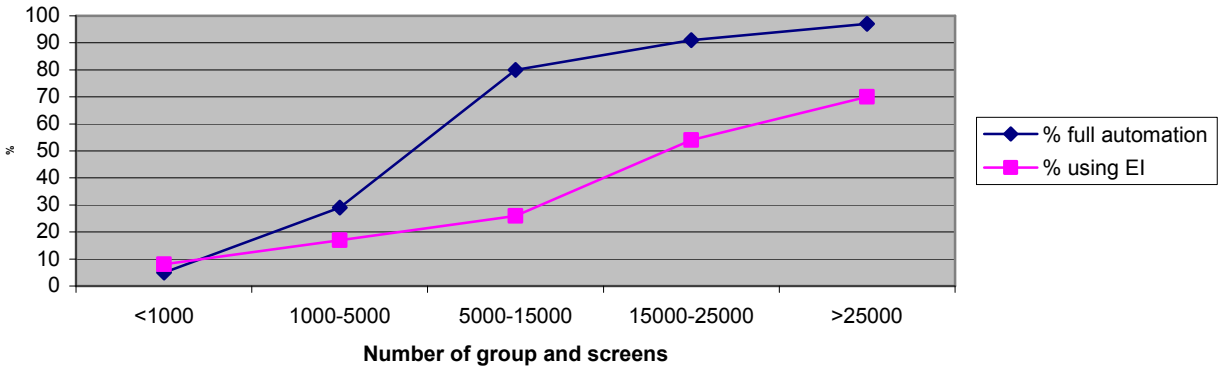
Table 5 – Method of establishing compatibility – number (%) laboratories

Method of establishing compatibility	Number (%)
Electronic issue	140* (37%)
'Immediate spin' (IS)	32 (8%)
IAT (+/- IS)	211 (55%)
Total	**383 (100%)**

* Includes 8 labs also stating 'IAT +/- IS', one of which uses EI for known patients only.

Figure 2 shows the relationship between laboratory workload and use of EI.

Fig 2: Use of EI and use of full automation by workload



Of those using EI as their primary method for establishing compatibility:

- 7/140 (5%) are not using full automation
- 7/110 (6%) that do use full automation, do not use it 24/7
- One laboratory does not have an electronic interface between automation and the LIMS
- 54/140 (39%) do not perform a reverse group on patients with one or more previous groups, and one of these also omits the reverse group on patients who do not have a historical group.

Q8. Technology used for antibody screening and crossmatching

Table 6 shows the number (%) using each IAT technology for antibody screening and crossmatching.

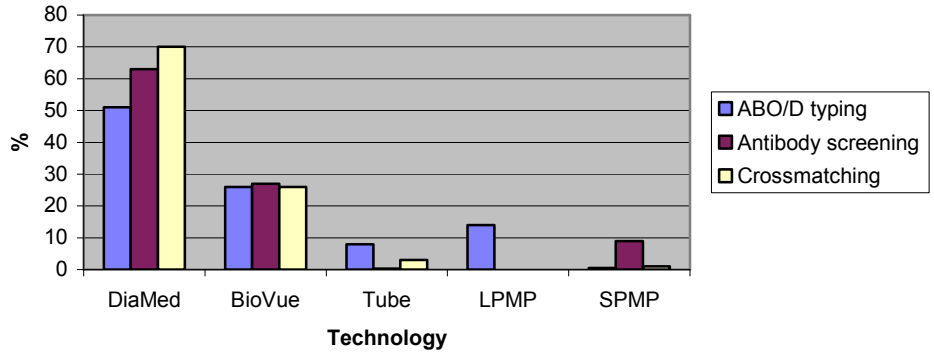
Appendix 4

Table 6 – IAT technology used for antibody screening and crossmatching

Technology	Number (%) laboratories IAT Antibody screen	Number (%) laboratories IAT XM
DiaMed	247 (63%)	266 (70%)
BioVue	105 (27%)	98 (26%)
Tube	1 (<1%)	10 (3%)
Solid phase microplate	34 (9%)	3 (1%)
Other	1 (<1%)	2 (<1%)
Multiple	1 (<1%)	2 (<1%)
Total	**389 (100%)**	**381 (100%)**

Fig 3 shows the percentage of laboratories using each technology for ABO/D typing, antibody screening (by IAT) and crossmatching (by IAT).

Fig 3: Use of technology for ABO/D, antibody screen and crossmatch



Q9. Use of an enzyme screen

- 22/387 (6%) routinely perform an antibody screen with enzyme treated cells.

3. Discussion

Full automation is used by 69% of laboratories for routine group and screens within core hours *cf.* questionnaire data from 2005 (05R8) where 60% were using automation (including semi-automated systems). The bias towards automation in larger laboratories means that an even higher percentage of samples (estimated at 88%) are tested using full automation. The majority (96%) have an electronic interface between the automation and laboratory information system (LIMS), affording security against transcription / transposition error. Of the 67% using full automation during core hours only 82% continue to use full automation during 'out of hours' sessions.

77% use CAT for routine ABO/D typing *cf.* 65% in 2005. Of the major users of automation, BioVue users are most likely to have automation (84%), followed by DiaMed users (75%), and LPMP users (60%). A reverse group was included by 75% where the patient has a previous group (*cf.* 80% in 2005), although this varied according to the use of full automation (66% full automation *cf.* 94% without full automation) and use of CAT (71% CAT

cf. 88% other technologies). Where the patient has no previous group on record all but one laboratory performs a reverse group (*cf.* 4 laboratories in 2005).

Patients with a previous group are tested with a single anti-D reagent (used once) by 45% (*cf.* 41% 2005), and for patients with no group on record by 25% of laboratories. The protocol for D typing varies according to the technology used, with 96% of those using a single anti-D and all of those using a single anti-D for patients with no previous group, typing by CAT. The use of anti-CDE reagents has declined following the publication of the 2004 BCSH guidelines for pre-transfusion compatibility testing[1] that recommend that anti-CDE is not used for patient testing, and is now <1% *cf.* 6% in 2005. The use of an anti-D reagent by IAT to confirm the D status of apparently D negative patients seems to be increasing over time, rising from 2% in 2002, to 5% in 2005 and now at 6%, although the BCSH guidelines contraindicate the use of an IAT anti-D for patient testing[1]. A higher proportion of those using an anti-D reagent by IAT use tubes for ABO/D grouping (13% *cf.* 5% overall).

The proportion of those establishing compatibility by electronic issue has increased since 2005 from 26% to 36%. Of these, 5% do not have full automation, and of those with fully automated systems, 6% do not use this automation 24/7, although it is possible that these laboratories do not undertake transfusion testing outside core hours. No reverse group is performed on patients with a historical group by 39% of those using electronic issue. Two laboratories using EI are of concern; one without full automation and omitting the reverse group even for new patients, and the other with no interface between automation and LIMS and no reverse group for patients with a historical group.

The technologies used for crossmatching are broadly similar to those for ABO/D typing, except that the majority (79%) of those using tube or LPMP for ABO/D typing, change to DiaMed for crossmatching by IAT. The technology used for antibody screening again reflects that for ABO/D typing, but with those ABO/D typing by LPMP using either SPMP (58%) or DiaMed (34%) for antibody screening, and the majority (90%) of those ABO/D typing in tubes, antibody screening by DiaMed. For the first time in many years there has been a slight decline in the percentage of laboratories using CAT for antibody screening with a corresponding slight increase in the use of SPMP, with the introduction of SPMP 'walk away' automation.

The information in this questionnaire will be updated annually.

4. References

[1] BCSH (2004) Guidelines for compatibility procedures in blood transfusion laboratories. *Transfusion Medicine,* **14**, 59-73.

Emergency Issue Questionnaire
Distributed with exercise 08R10 – November 2008

1. INTRODUCTION

The aim of this non-scoring emergency exercise and associated questionnaire was to establish what pre-transfusion testing is performed when blood is requested in an emergency situation.

2. RETURN RATE

418 questionnaires were returned. Of these, 22 were excluded from analysis: nine from reference laboratories, five from laboratories with more than one registration, five where emergency crossmatching is not undertaken, and three where not all pages were returned. The following analysis is based on the remaining 396, although since not all respondents answered all the questions, the total numbers in the tables do not always equal 396. A summary of the questions is attached as Appendix 1.

3. RESULTS

'Patient' 1: Steve UKNEQAS, age 40 (group A D positive, inert) Blood required within 10 – 15 minutes

Blood grouping summary

31/396 (8%) laboratories did not complete a group within 10 – 15 minutes

365/396 (92%) laboratories stated that they performed a rapid group
- 146/365 (40%) completed only one cell group (+/-reverse group) within 10-15 minutes
- 219/365 (60%) completed two cell groups (+/-reverse group) within 10-15 minutes
  - 14/221 (6%) used same aliquot for first and second group

All 363 recording a grouping result within 10-15 minutes correctly reported A D positive

Overall 98/365 (27%) did not perform a reverse group before issuing blood

At least 36/98 (37%) did not include a control with their forward group(s)

72/365 (20%) laboratories stated that they did not perform a second group or an 'immediate spin' crossmatch within 10-15 minutes, and 44 of these issued group A blood

Initial (rapid) group

Table 1 shows the use of controls and Table 2 the technology used for 'rapid' grouping, compared to the routine ABO/D grouping technology (data from 'Patient' 3 exercise 08R8).

Table 1: Use of controls for 'rapid' grouping

Criteria	Negative control included[1]
Cell group only (n=197)	79/174 (45%)
Cell and reverse group (n=168)	100/152 (65%)
Overall[1] (n=365)	**179/326 (55%)**

[1] Excludes 39 laboratories not stating whether a control was used, 23 cell group only, and 16 cell and reverse group

Table 2: Technology used for 'rapid' grouping *cf.* that used for routine testing (exercise 08R8)

Technology	Number (%) rapid group	Number (%) routine group (08R8)
Card/cassette	58 (16%)	286 (65%)
Microplate	13 (4%)	40 (9%)
Tube	248 (68%)	45 (10%)
Tile/slide	28 (8%)	0 (0%)
Multiple	15 (4%)	58 (13%)
Other	1 (<1%)	11 (2%)
Total	363 (100%)	442 (100%)

Confirmatory group

Of the 365 performing a rapid group, 219 (60%) completed a second cell group (+/- a reverse group) prior to issue. A further two laboratories performed a reverse group only, as a confirmatory group within 10-15 minutes. Table 3 shows combinations of rapid and confirmatory groups recorded at 10 - 15 minutes.

Table 3: Details of combination of rapid and confirmatory groups (n=364[1])

Rapid	'Confirmatory' group within 10-15 minutes			
	Cell only	**Full**	**Reverse only**	**None**
Cell only	37	97	2	61
Full group	38	47	0	82

[1] one did not record details of confirmatory group.

- 14/221 (6%) stated that they used the same aliquot of cells for both the rapid and confirmatory group.

Further tests completed prior to labelling blood for collection in 10-15 minutes

Of the 31 laboratories that did not perform a group prior to the issue of blood:

- 2/31 checked the group of the donor units
- 2/31 took samples from the donations for retrospective crossmatching.

Of the 365 laboratories that completed at least one group prior to the issue of blood:

- 137/365 (38%) performed an immediate spin crossmatch

- 45/365 (12%) group checked the donations
- 16/365 (4%) completed other tests; including: electronic group check, reduced incubation antibody screen and IAT crossmatch, K typing donations, sampling units for retrospective crossmatch, confirmed reading of rapid group, and DAT.

Units selected for transfusion

Table 4 compares the level of testing undertaken within 10-15 minutes, with the ABO/D group of the units selected.

Table 4 – Summary of donor units selected vs. level of grouping undertaken on Steve

Tests undertaken	Donor units selected				
	O D negative	O D positive	A D positive	A D Negative	Not stated
No group (31)	25	5	0	0	0
Rapid group only (144)	27	4	108	3	2
Rapid + confirmatory group (221)	16	5	198	1	1
Total (n= 396)	**68 (17%)**	**14 (4%)**	**306 (77%)**	**4 (1%)**	**4 (1%)**

- Of the 68/396 (17%) selecting group O D negative units:
  - 55 (81%) used blood designated as 'flying squad' or equivalent
  - 58 (85%) stated that it was K negative
  - 46 (68%) stated that it was rr (CDE negative).

- Of the 310 selecting group A units:
  - At least 44 (14%) did not perform a confirmatory group or an immediate spin crossmatch
  - At least 13 (4%) performed no reverse group, control or 'immediate spin' crossmatch before issue.

Retrospective testing - completed after the issue of blood, but before the next session of core hours

Table 5 shows testing completed after the issue of blood (10-15 minutes) but before the next session of 'core' hours.

Table 5 – Details of testing completed after issue and before the next session of core hours

Further testing	Manual testing	Automated testing	Manual and automated testing
Blood group (n=274)	105 (38%)	159 (58%)	10 (4%)
Antibody screen (n=384)	191 (50%)	169 (44%)	24 (6%)
IAT crossmatch (n=285)	250 (88%)	35 (12%)	0 (0%)
Antibody ID panel (n=5)	4 (80%)	1 (20%)	0 (0%)

'Patient' 2: Clare UKNEQAS, age 42 (group O D positive, inert) Blood required within 1 hour

ABO/D group

- All completed a group (rapid and/or routine) within 1 hour
- All recorded group O D positive
- 185/396 (47%) utilised automation for testing within 1 hour
- 371/396 (94%) performed at least one full (forward and reverse) group within 1 hour
- 275/396 (69%) performed two groups within 1 hour; however, 17/275 (6%) used the same aliquot for both groups
- 13/396 (3%) did not complete a reverse group within the hour, and two of these also omitted a serological crossmatch by immediate spin or IAT.

Further testing completed within the hour

- All completed an antibody screen, with 187 (47%) using automation.
- 289/396 (73%) completed an IAT (+/- an 'immediate spin') crossmatch', with 37 (13%) using automation
- 39/396 (10%) undertook an 'immediate spin' crossmatch only
- 5/396 (1%) stated that they completed an antibody identification panel
- 15 laboratories stated that they undertook other testing.

Electronic Issue (EI)

- 56/396 (14%) established compatibility using electronic issue
  - One performed only a single forward group (and no reverse group) within the hour
  - One did not use automation, but performed three manual groups within the hour.

Units selected for transfusion

- 7/395 (2%) selected O D negative blood; all had completed blood grouping and antibody screening
- The remaining 388 (98%) laboratories selected group specific (O D positive) blood.

Retrospective testing – completed after blood issued but before the next session of 'core' hours

- 66 laboratories repeated the group (two had completed only a rapid group within 1 hour)
  - 45 also completed a screen
  - 13 also completed an IAT crossmatch
  - One completed and antibody ID panel
- 3 laboratories stated that they undertook other testing: requested second sample; had results checked by a second member of staff ; and performed an 'auto' on the patient sample.

'Patient' 3: Megan UKNEQAS, Age 30 (Group A D negative, anti-D) 'Group and Save'

Work undertaken before the next session of core hours

- All those performing a group recorded A D negative
- 320/396 (81%) performed a group and screen

- 202/320 (63%) used automation for group and screen
- 251 undertook antibody identification
  - 74/251 (29%) used automation
- Of the 72 that performed no serology before the next session of core hours:
  - 43/396 (11%) did nothing with this request until the next session of core hours
  - 13 checked the sample identification and booked in the request
  - 4 booked in the request but did not check the sample identification
  - 11 checked the sample identification but did not book in the request

Routine testing on 'Patients' 1, 2, and 3 (carried out during the next session of core hours)

Table 6 shows the number (%) carrying out testing on the three 'Patients' during the next session of core hours.

Table 6 – number (%) testing for group, crossmatch antibody screen and ID in the next session of 'core' hours

	Group	Screen	IATXM	ID panel
Patient 1 - Steve	126 (32%)	100 (25%)	46 (12%)	5 (1%)
Patient 2 - Clare	113 (29%)	84 (21%)	29 (7%)	3 (1%)
Patient 3 - Megan	157 (40%)	132 (33%)	57 (14%)	161 (41%)

General Questions

Selection of blood for 'Unknown' patients

41 laboratories stated that units of a different blood group would have been selected for transfusion within 10 – 15 minutes if the patient had been labelled as 'Unknown male' with only an accident and emergency number for identification.

Use of automation

44/199 (22%) laboratories performing manual (non-rapid) grouping on 'Patient' 2 to issue blood within 1 hour, would have used automation if the request had been within core hours.

Specialist transfusion advice 'out of hours'

243/393 (60%) laboratories would always be able to call someone with a specialist transfusion qualification e.g. MSc, IBMS fellowship or IBMS higher specialist diploma, whilst 125 stated that they would usually be able to do so, 17 that this was unlikely, and nine that specialist advice was never available out of hours.

4. DISCUSSION

ABO/D grouping

A rapid group was performed for 'Patient' 1 by 92% of laboratories, and as expected, this involved a change from routine grouping techniques for the majority. A second cell group (+/- a reverse group) was completed within 10 –15 minutes by 60%; however, at least 6% of these performed the second test on the same aliquot of cells as the first group - a practice that would have perpetuated any error in selecting the correct specimen for the first group. This compares with 12% using the same aliquot in the last emergency exercise (06R9) and 18% in the one before (03R9). Overall, at least 27% did not perform a reverse group before issuing blood.

Issue of group specific blood within 10 – 15 minutes

Group A blood was issued for Patient 1 by 78% laboratories overall. However, at least 11% issued group A units based on a single group and no immediate spin crossmatch, and a further 1% based on a confirmatory group using the same aliquot of cells as that used for the rapid group, and no immediate spin crossmatch. These 12% laboratories are not compliant with BCSH guidelines[1] that state that group specific blood may be issued following a rapid group, plus either a confirmatory group (using a new aliquot from the original sample), or an immediate spin crossmatch. It is interesting to note that four laboratories selected group A D negative blood for Patient 1 (a 40 year old A D positive male), although it is possible this selection was due to the stock situation on the day, as the instructions required participants to crossmatch units from their own stock.

Issue of group O D negative blood

The NBTC recommends use of O D negative in emergency situations, only until the patient's blood group has been determined, with a limit of two units, if possible [2]. In this exercise, 17% selected O D negative units. Of the 68 selecting O D negative units, 24% had performed a rapid and confirmatory group and a further 4% performed a rapid group plus an immediate spin crossmatch, a level of testing that satisfies the BCSH guidelines for issue of group specific blood in an emergency [1]. Even with an hour available for testing, 2% laboratories stated that they selected group O D negative rather than O D positive red cells for Patient 2, despite all of these completing grouping and screening, and in one case using automated (presumably routine) techniques. SHOT data has demonstrated that more laboratory errors occur out of hours and with manual testing [3], possibly accounting for some of the caution noted in this exercise.

Laboratory policy for issue of O D negative or group specific blood in emergency situations, should be based on a risk assessment, with factors including the frequency with which emergency testing is undertaken, differences in methodology between routine and emergency testing, level of blood stocks, skill mix and case mix.

Level of testing undertaken for a group and save sample

The majority of laboratories undertook some work on the group and save sample (Patient 3 – Megan) before 'the next working day', with 81% performing a full group and screen (*cf.* 86% in 2006); approximately 63% utilised automation (*cf.* 50% in 2006). The 19% laboratories that did not perform an antibody screen on this sample until the next day, took the risk of delaying transfusion had the patient's status changed, requiring urgent transfusion, especially considering that the 'Patient' had an atypical red cell antibody.

Use of automation

Although not helpful for issuing blood in an extreme emergency (e.g. within 10 – 15 minutes), fully automated systems change the balance of risk during 'out of hours' situations. They allow for simultaneous testing of several urgent samples, using routine (and therefore safer, and more secure) techniques. Even where time-constraints require initial manual testing, results of repeat and additional tests can be available much more quickly, and require little 'hands-on' time. Approximately 47% (*cf.* 30% in 2006) of laboratories utilised automation to provide blood within the hour, and 63% to undertake an out of hours request for a 'group and screen' (*cf.* 50% in 2006). It is interesting to note that 43/199 (22%) undertaking a manual 'full' group within the hour would have used an automation had the request been received out of hours. The UK Transfusion Laboratory Collaborative[4] recommends that all laboratories have full walk away automation with bidirectional interfaces to the laboratory information system, in use 24 hours, 7 days a week, with the exception of "where the workload does not warrant such technology e.g. hospitals with a remote and rural location performing in the order of 10 group and screens per week then the collaborative expects all reasonable measures to be taken in order to mitigate laboratory errors".

5. REFERENCES

1. BCSH (2004) Guidelines for compatibility procedures in blood transfusion laboratories. *Transfusion Medicine,* 2004, **14,** 59-73 and www.bcshguidelines.co.uk
2. NBTC: The appropriate use of group RhD negative red cells http://www.transfusionguidelines.org.uk/docs/pdfs/nbtc_bbt_o_neg_red_cells_recs_09_04.pdf:
   (accessed 8/5/09)
3. SHOT reports, www.shotuk.org (accessed 8/5/09)
4. UK Transfusion Laboratory Collaborative: Recommended minimum standards for hospital transfusion laboratories – in press (Transfusion Medicine)

6. APPENDICES

Appendix 1 - Summary of instructions and questions

Appendix 5

Appendix 1 to Emergency Issue questionnaire

SCENARIO

You are on your own outside of core hours. You receive two requests for crossmatching and one for group and save (type and screen); these do not arrive simultaneously and may be tested separately. The request forms give patient and request details.

- Steve Ukneqas (Patient 1) has acute bleeding and requires 2 units of blood for theatre in 10 - 15 minutes of sample receipt, and may need more later.
- Clare Ukneqas (Patient 2) is also acutely bleeding and requires 2 units for theatre, but can wait for up to an hour.
- Megan Ukneqas (Patient 3) requires a group and save (type and screen) but may need blood for theatre at the start of the next session of core hours (e.g. first thing in the morning).

INSTRUCTIONS

- Book specimens into computer (or treat as usual). Assume they arrive at different times, and do not overlap.
- Prepare, label and issue 2 units for Steve within 10 - 15 minutes, and 2 units for Clare within 1 hour, ***using your own blood supplies***.
- Undertake whatever testing you would normally perform on Megan Ukneqas before the next session of core hours.
- Document results in the same way as you would normally document emergency testing.
- Complete the emergency results sheets/questionnaire and routine UK NEQAS result sheets or webpages later.

QUESTIONS ON ADDITIONAL RESULT SHEETS

Was the emergency exercise undertaken?

Patient 1 – Steve

1. Initial group:

a. Was an initial group performed?
b. If yes, did this include:
    i. forward group only or forward and reverse group
    ii. one, or more than one anti-D reagent
    iii. a control
c. Enter blood group interpretation
d. Which technology was used?

2. What further tests did you complete prior to labelling blood for collection in 10-15 minutes?

i. A repeat group
ii. Immediate spin crossmatch
iii. Group check donations
iv. Other

3 . Confirmatory group

a. If a confirmatory group was performed, did this include:
   i. forward group only or forward and reverse group
   ii. one, or more than one anti-D reagent
   iii. a control
b. Enter blood group interpretation
c. Did you resample the primary sample or use an aliquot already made?
d. Was this testing manual or automated

4. Units selected for transfusion

a. What ABO/D group units did you select for transfusion within 10-15 minutes?
b. Were these selected for any further phenotypes?
c. If O D negative was selected, was this blood specially reserved for emergency use?

5. Retrospective testing (completed after units issued but before next session of core hours)

a. Further ABO/D group – specify manual or automated
b. Antibody screen - specify manual or automated
c. IAT crossmatch - specify manual or automated
d. IAT crossmatch on units already issued
e. Antibody panel - specify manual or automated
f. Other

6. Would you have selected a different blood group for transfusion if the patient sample had been labelled as 'unknown male' and only had a A+E number for identification?

Patient 2 – Clare

1. Initial group:

a. Was an initial group performed?
b. If yes, did this include:
   i. forward group only or forward and reverse group
   ii. one, or more than one anti-D reagent
   iii. a control
c. Enter blood group interpretation
d. Which technology was used?

2. What further tests were completed within 1 hour?

a. ABO/D group (confirm or first group) – specify manual or automated
b. Antibody screen - specify manual or automated
c. IAT crossmatch - specify manual or automated
d. 'Immediate spin' crossmatch
e. Antibody panel - specify manual or automated
f. Electronic issue of units
g. Other

3. Grouping details

a. If a confirmatory group was performed, did this include:
   i. forward group only or forward and reverse group

ii. one, or more than one anti-D reagent
iii. a control

b. Enter blood group interpretation
c. Did you resample the primary sample or use an aliquot already made?

4. Units selected for transfusion

a. What ABO/D group units did you select for transfusion within 10-15 minutes?
b. Were these selected for any further phenotypes?
c. If O D negative was selected, was this blood specially reserved for emergency use?

5. Retrospective testing (completed after units issued but before next session of core hours)

a. Further ABO/D group – specify manual or automated
b. Antibody screen - specify manual or automated
c. IAT crossmatch on units already issued - specify manual or automated
d. Antibody panel - specify manual or automated
e. Other

6. If groups recorded in 2 a.) or 5 a.) were performed manually, would this testing be automated in core hours.

7. Is somebody with a specialist transfusion qualification in blood transfusion (e.g. MSc, IBMS fellowship or HSD available for advice out of hours?

Patient 3 – Megan

1. Group and Save

a. Was any work performed before the next session of core hours?
b. If yes what was done:
i. ABO/D group – specify manual or automated
ii. Antibody screen – specify manual or automated
iii. Antibody identification – specify manual or automated
iv. Just booked in (computer or manually)
v. Just sample ID checked vs. form

2. Grouping details

a. If a group was performed, did this include:
i. forward group only or forward and reverse group
ii. one, or more than one anti-D reagent
iii. a control
b. Enter blood group interpretationRoutine testing during next session of core hours

1. Were any of the following tests performed during the next session of core hours (repeat of out of hours or for first time)

a. Blood group
b. Antibody screen
c. IAT crossmatch
d. Antibody panel

Appendix 6

Summary of Data for UI submissions April 2007 to March 2009

Table 1 summarises the UI submissions and outcomes by exercise, and Table 2 details the reasons for not agreeing with the submission. All panels submitted were assessed based on BCSH guideline and the 'rules' outlined in Appendix 7

Table 1 – Details by exercise

Exercise Code	Antibodies	No. UI returns	No. agreed	No. disagreed	No. appeals
07E6	S+Fy[a]	15	11	4	0
07R8	Fy[a] (DAT+)	1	0	1	1 [1]
07E9	K+Fy[a]	1	1	0	0
07E9	c	1	0	1	0
08E1	D+Jk[a]	2	1	1	0
08E3	E+S	2	0	2	0
08E6	S+ENS	10	9	1	1 [1]
09E1	s	4	2	2	0
09R2	Jk[b]	3	2	1	0
Total		**39**	**26**	**13**	**2**

[1] – Appeals upheld

Table 2 – Reasons for disagreeing with the UI submissions

Category	No. submissions
Could have identified the antibody with the IAT panel results submitted	6
False positive or false negative reactions recorded	3
Could have excluded additional antibody (ies) based on enzyme results submitted	2
Could have excluded additional antibody (ies) based on IAT results submitted	1
Did not consider the presence of an antibody (actually present)	1
Total	**13**

Appendix 7

Acceptance of a result of UI for antibody identification

This process should only be used where antibodies of likely clinical significance cannot be fully elucidated or excluded.

The following rules will apply:

a. the following will incur penalties

- Misinterpretations contributed to by false negative or false positive reactions.
- If a specificity (actually present) is not entered as positively identified and we feel that it can be identified based on two positive and two negative reactions (as stated in BCSH guidelines) by whatever method is appropriate (e.g. IAT, OR enzymes in the case of Rh). This will be based on a maximum of 2 antibodies being present. (N.B: Serological reactions obtained with the antibody screening cells should be included in the interpretation).
- If a specificity not actually present is entered as positively identified.
- If a specificity is entered as 'cannot be excluded', but we feel that it can be excluded, either because of one or more negative reactions with an appropriate antigen positive cell, or because of one or more negative reactions by a particular method. For example, stating that an Rh antibody cannot be excluded from an antibody mixture in the presence of a negative result with an enzyme treated cell carrying the corresponding antigen would incur a penalty.
- If a specificity is entered as 'cannot be excluded', but the patient phenotype provided shows that the patient is positive for the corresponding antigen.
- Not positively identifying a clinically significant antibody in the presence of an enzyme non-specific antibody.

b. the following will not incur penalties

- Being unable to exclude a specificity in line with BCSH guidelines. E.g. having no homozygous cell available to exclude anti-Jk^a.
- Including a specificity (if actually present) even if the inclusion does not comply with BCSH guidelines (e.g. only one r'r cell).
- If an antibody (actually present) is not reacting with heterozygous cells, but with homozygous cells only, and is recorded as 'cannot be excluded', rather than as 'positively identified'. However, this would only apply if our in-house testing also found non-reactivity with heterozygous cells by the same technique; otherwise, this would be classed as a false negative result.

c. the following documentation is required for a UI submission to be considered

- The UI box should be marked in addition to any boxes for antibodies that you can confidently identify.
- Antibodies that cannot be positively identified, but cannot be excluded should be marked on the result sheet, and the result sheet must be completed with your explanation of why identification cannot be confirmed.
- Copies of all panel sheets showing the reactions recorded, (including those used for antibody screening) must be returned with your exercise result sheet and marked with your PRN.
- If supporting paperwork is not submitted, antibodies recorded as positively identified will be considered as your result for performance monitoring purposes.

Appendix 8

Joint meeting of SHOT, UK NEQAS (BTLP) and the NBTC

Tuesday 20th November 2007, National Motorcycle Museum, Birmingham

09.00	Coffee, Registration
09.00 to10.00	Shuttle bus available from Birmingham International railway station
10.00	Opening Remarks by Dr Adrian Copplestone, Chair of the NBTC
Session 1 – 10.05 – 11.45	**Chair: Adrian Copplestone**
10.05 – 10.25	Highlights from the SHOT report 2006 – Hannah Cohen
10.25 – 10.55	Zooming in on laboratory errors – SHOT and UK NEQAS. - Debbie Asher/Clare Milkins
	Keynote Speaker
10.55 – 11.35	Understanding errors and improving patient safety– Professor Charles Vincent, Director of the Clinical Safety Research Unit, Imperial College
11.35 – 11.45	Discussion
	11.45 – 12.05 Coffee
Session 2 – 12.05 - 13.10 A Time for Change	***Chair: Stephan Bates***
12.05 – 12.20	West Midlands audit of out-of-hours pre-transfusion testing – Craig Taylor
12.20 – 12.50	National Transfusion Laboratory Collaborative – How do we achieve 24/7 quality? – Bill Chaffe
12.50 – 13.10	Forum – your opportunity to contribute to the Collaborative
	13.10 – 14.10 Lunch
Session 3 – 14.10 – 16.00 Laboratory/Clinical Interface	***Chair: Ann Benton***
14.10 – 14.30	MHRA update – Clare Taylor
14.30 – 14.50	Investigation of ATRs – Derek Norfolk
14.50 – 15.10	Right patient, right component – Andrea Blest
15.10 – 15.40	Short presentations/case studies – solutions and corrective/preventive actions • Little samples, big problems – Edwin Massey • When the unthinkable happens… - Adrian Copplestone • Non haemolytic reactions – what is 'severe' and why test? – Phil Robson
15.40 – 16.00	Discussion and close

Appendix 9

Joint meeting of UK NEQAS (BTLP) and the BBTS Blood Bank Technology SIG

Your Place or Mine?

Tuesday 18th November 2008, Heriot-Watt University, Edinburgh

09.30 Coffee, Registration

10.20 Opening Remarks by Ann Benton (Chair UK NEQAS Steering Committee)

Session 1 – How will we provide Hospital Transfusion Services in the future? **Chair: Ann Benton**

10.30 Impact of the Carter Review on Blood Transfusion Laboratories – Dan Smith, John Radcliffe, Oxford

10.50 How well does remote issue support centralised transfusion services? - Tina Cave, Bangor

11.10 Managing transfusion services in the private sector – the 'hub and spoke' model – Judy Langham, Nuffield Diagnostics

11.30 – 11.50 Coffee

11.50 Debate chaired by Richard Gray

Motion: Networking is the answer for NHS transfusion laboratories

For: Raphael Ezekwesili, Consultant Haematologist, Darent Valley

Against: Jane Leftley, Haematology & Blood Transfusion Services Manager; Maidstone

12.50 – 14.00 Lunch

Session 2 – To refer of not to refer, that is the question ***Chair: Fiona Stribling***

14.00 Evidence from UK NEQAS - understanding the limitations of in-house resources – Jenny White, UK NEQAS

14.30 Preventing HDFN - Guideline update – Megan Rowley, UK NEQAS

14.50 Economics of mass fetal genotyping for RhD – Prof Ceri Phillips, University of Swansea

15.15 Could haematology analysers replace acid elution in screening for FMH? – Sue Redfearn/Jason Withers, Poole

15.35 How, when and where to investigate a positive DAT – Mark Williams, NBS Leeds

15.55 Discussion and close 16.15