UK NEQAS

Bi-ennial Report
2005 - 2007

UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME

for Blood Transfusion Laboratory Practice

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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.

The Scheme is advised by the Steering Committee (see Appendix I for composition) for Blood Transfusion Laboratory Practice, which meets four 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 2005 to March 2007.

2. STAFF

Chair of the Steering Committee – Mrs Helen Barber
Scheme Director - Dr Megan Rowley
Scheme Manager - Mrs Clare Milkins
Deputy Scheme Manager - Ms Jenny White
BMS 2 – Mrs Dalila Benkhaled
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3. PARTICIPANTS

The number of participants registered at March 2007 is shown in table 1. Overseas participation by country is shown in table 2. The Scheme also distributes 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 2007

Type of Participant	Number Registered
UK clinical (including Eire and Channel Islands)	465
Overseas clinical (including 3 BFPO*)	215
Miscellaneous others	7

*British Forces Posted Overseas

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

Country	No. Participants	Country	No. Participants
Bahrain	1	Italy	12
Belgium	2	Macau	1
China	1	Malta	3
Croatia	2	Netherlands	3
Denmark	44	Norway	7
Faroe Islands	1	Oman	2
Finland	4	Portugal	38
France	2	Saudi Arabia	1
Germany	1	Slovenia	1
Gibraltar	1	South Africa	2
Greece	13	Spain	2
Greenland	1	Sweden	2
Hong Kong	1	Switzerland	3
Iceland	1	Turkey	41
Israel	23	United Arab Emirates	2
		USA	1

4. PERFORMANCE SUMMARIES

4.1 Exercises Distributed:

Table 3 – Summary of exercises distributed

Exercise Code	Date Distributed	Contents	Main aim to assess: (Q indicates that a questionnaire was included)
05E4	18 April 05	AS, ABID	ID of antibody mixtures.
05R5	23 May 05	ABO/D, AS, ABID, XM, PH	Assessment of crossmatching for detection of ABO and IgG antibodies. Also D grouping of r'r cell. Q
05E6	20 June 05	AS, ABID	Potential for 'carryover' of a strong antibody
05E7	18 July 05	AS, ABID	To assess the detection different dilutions of a monoclonal anti-D and a weak anti-K
05R8	26 Sept 05	ABO/D, AS, ABID, XM, PH	To assess proficiency in D typing of a DAT positive rr sample. Q
05E9	17 Oct 05	AS, ABID	Assessment of routine and additional methods. Q
05R10	21 Nov 05	ABO/D, AS, ABID, XM, PH	Assessment of crossmatching for detection of ABO and IgG antibodies. UI trial for ABID
06E1	16 Jan 06	AS, ABID	ID of antibody mixtures.
06R2	20 Feb 06	ABO/D, AS, ABID, XM, PH	Assessment of crossmatching for detection of ABO antibodies *vs* A_2 cells, and ID of an antibody mixture.
06E3	20 March 06	AS, ABID	To assess detection and ID of weak antibodies.
06E4	10 April 06	AS, ABID	ID of antibody mixtures.
06R5	22 May 06	ABO/D, AS, ABID	Assessment of provision of blood in an emergency situation. Q
06E6	12 June 06	AS, ABID	ID of an antibody mixture including anti-c.
06R7	3July 06	ABO/D, AS, ABID, XM, PH	Assessment of crossmatching for detection of ABO and IgG antibodies.
06E8	18 Sept 06	AS, ABID	ID of an antibody mixture and detection of UK NEQAS 'standard' anti-D
06R9	16 Oct 06	ABO/D, AS, ABID, XM, PH	Recognition of a mixed field reaction and detection of incompatibility due to anti-E
06E10	20 Nov 06	AS, ABID	To assess detection and ID of weak antibodies.
07E1	15 Jan 07	AS, ABID	To assess detection and ID of weak antibodies.
07R2	19 Feb 07	ABO/D, AS, ABID, XM, PH	D typing of an r"r sample. Assessment of crossmatching for detection of ABO and IgG antibodies.
07E3	19 March 07	AS, ABID	To assess detection of a weak antibody and ID 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 (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.

- The total number of errors may be greater than the number of participants with errors as some laboratories make more than one error.

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

4.3 05E4

Material

'Patient' 1: Inert
'Patient' 2: Anti-E+Fy^a (titre 8 and 2, respectively)
'Patient' 3: inert
'Patient' 4: Anti-D+K (titres both 8)

Results

Table 4 – Summary of results for 05E4

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 – inert - P2 – E+Fy^a - P3 – inert - P4 – D+K	**6/450**	**8** 2 [1] 1 [1] 4 [2,3] 1 [2]	**0/83**	**0**
Antibody ID	**All Samples** - Anti-E+Fy^a - Anti-D+K	**8/377**	**8** 4 [4,5] 4 [1,4]	**1/75**	**2** 1 1

[1] – One participant tested 05E3 in error
[2] – One participant transposed the results for P3 and P4
[3] – One participant made a tick-box error
[4] – One participant transposed the ID results for P2 and P4
[5] – Two incorrect results appear to be due to tick-box errors

Errors: (Excluding transcription, transposition and wrong samples tested)

There were three antibody identification errors:

- The significance of the screening cell panel results was overlooked and anti-K subsequently missed.
- False negative reactions by IAT, combined with the results of the enzyme panel not being taken into account, lead to an interpretation of anti-D+E instead of anti-E+Fy^a.
- Anti-K was not identified in the mixture, with no clear cause identified.

4.4 05R5

Material

'Patient' 1: B D positive, inert
'Patient' 2: A D negative, r'r, inert
'Patient' 3: O D positive, anti-Fy^{a} (titre 4)

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

Results

Table 5 – Summary of results for 05R5

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**	**1** [1]	**2/171**	**3**
D Grouping	**All Samples (all P2)**	**3/462**	**3** [1]	**2/171**	**3**
Antibody Screening	**All Samples** - P3 – anti-Fy^{a}	**2/455**	**2** 2 [2]	**2/169**	**2** 2
Antibody Identification	**All samples**	**1/388**	**1** [3]	**4/125**	**4**
Incompatibilities	**All Samples** - P1DY (ABO) - P3DY (ABO) - P3DW - P3DZ	**27/448**	**36** 4 [4] 3 [4] 18 [1] 11 [1]	**11/147**	**14** 1 0 9 4
Compatibilities	**All Samples**	**7/448**	**7**	**5/147**	7

[1] – One due to transcription error onto the EQA form
[2] – One due to a mix-up in recording results, where the usual procedure was not followed
[3] – This appears to be due to a tick-box error
[4] – Six of seven missed ABO incompatibilities due to transcription or donor sample transposition

Errors:

D grouping:

- One D grouping error appears to have occurred during setting-up, reading or recording the results
- One incorrect D grouping error, D^{Var} (representing weak or partial D) was due to a weak false positive reaction with one of two anti-D reagents.
- There were no errors due to misinterpretation of reactions with anti-CDE reagents; it was not known at this time whether such reagents are still in routine use in the UK. An ABO/D typing questionnaire was subsequently issued with 05R8, and the data can be found in Appendices IV and V.

Crossmatching

- In at least two cases where procedural errors resulted in missed ABO incompatibilities, the BMS involved was working alone, using manual systems.
- One participant ignored equivocal reactions, despite a positive antibody screen.

A questionnaire relating to transfusion of patients with sickle cell disease was distributed with this exercise. A summary of the questions can be found in Appendix II and the report in Appendix III.

4.5 05E6

Material

'Patient' 1: Anti-D (titre >512)
'Patient' 2: Inert
'Patient' 3: Inert
'Patient' 4: Anti-K+S (titre 32 and 16 respectively)

During the course of this exercise 'Patient' 4 plasma developed non-specific enzyme activity. No penalties were incurred as a result of detecting or reporting this.

Results

Table 6 – Summary of results for 05E6

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**	**7/451**	**10**	**3/79**	**3**
	- P1 – anti-D		1		
	- P2/3 – inert		7 [1]		3
	- P4 – anti-K+S		2 [2]		
Antibody ID	**All Samples**	**2/388**	**2**	**2/74**	**3**
	- Anti-D		2		1
	- Anti-K+S		0		2

[1] – Four due to transcription or result transposition error (with P4)
[2] – Two due to transposition of results (with P3)

Errors:

Antibody screening
Three laboratories recorded a false negative antibody screen:

- One laboratory missed the strong anti-D using a semi-automated system. A full Quality Incident investigation did not reveal the cause; however, it is assumed that the plasma was not aspirated due to an air bubble, and the visual check was omitted or failed to detect the reduced volume of liquid in the column.
- Two laboratories transposed the results for samples 3 and 4; in neither case were the samples given accession numbers or booked into the laboratory computer.

Only three laboratories reported any problems with 'carryover' of the strong anti-D

- One was using a manual system
- Two were using automated systems

Antibody identification
Both identification errors involved an additional specificity being recorded (anti-C and -E)

4.6 05E7

Material

'Patient' 1: Anti-D (titre 16)
'Patient' 2: Anti-D (titre 1)
'Patient' 3: Inert
'Patient' 4: Anti-K (titre 8)

The main aim of this exercise was to assess the suitability of a diluted monoclonal anti-D reagent for EQA use.

Results

Table 7 – Summary of results for 05E7

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**	**0/453**	**0**	**4/81**	**4**
Antibody ID	**All Samples** - Anti-K	**1/390**	**1** 1 [1]	**0/72**	**0**

[1] – This was due to a transcription error between the worksheet and the EQA form

Outcome:

Both dilutions of the antibody gave acceptable results throughout the course of the exercise. The weakest of the two dilutions gave a mixture of weak and strong positive results by all IAT technologies.

4.7 05R8

Material

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

'Donor' W: O D negative, SS
'Donor' Y: O D negative, ss
'Donor' Z: O D negative, Ss

The main aim of this exercise was to assess the level of false positive D grouping of a rr DAT positive cell. For this purpose 'Patient' 1 whole blood was coated with IgG anti-c to give a 3+ DAT.

A result of UI was considered acceptable for ABO and/or D typing of 'Patient' 1 due to the positive DAT. The anti-S was not reacting with all Ss panel cells by all technologies by the closing date and therefore UI was an acceptable result for antibody identification for 'Patient' 1.

Results

Table 8 – Summary of results for 05R8

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**	**0/459**	**0**	**1/168**	**2**
D Grouping	**All Samples**	**8/459**	**9**	**7/168**	**7**
	- P1 rr DAT +ve		8 [1]		6
	- P2 D pos		1 [1]		1
Antibody Screening	**All Samples**	**2/452**	**3**	**6/167**	**6**
	- P1 – anti-S		2 [1]		6
	- P2 - inert		1 [1]		
Antibody Identification	**All Samples**	**2/388**	**2**	**0/123**	**0**
Incompatibilities	**All Samples**	**35**	**38**	**2/142**	**4**
	- P1DW		4 [2]		2
	- P1DZ		34 [3]		2
Compatibilities	**All Samples**	**12/439**	**25** [4]	**4/142**	**10**

[1] - One due to sample transposition
[2] - Three due to transcription/transposition errors
[3] - Four due to transcription/transposition errors
[4] - Twelve due to transcription/transposition errors

Errors (excluding transcription/transposition errors):

D Typing

- 7 labs reported an incorrect D type for 'Patient' 1 (DAT positive), 3 D positive and 4 D^{Var}

A supplementary report was issued detailing these results, shown in Appendix IV

Antibody screening

- One laboratory missed the anti-S: the initial screen was positive by DiaMed, but the DiaMed panel was negative as was repeat testing by tube IAT.

Crossmatching

- 30 labs missed the incompatibility against 'donor' Z (Ss) and one also missed it against 'donor' W (SS). There was some correlation with IAT technology with the following detection rates:
  - Overall: 91.5%
  - DiaMed: 95.2%
  - BioVue: 84.5%
  - Tube LISS: 66.7%

Comparison with 04R2 (also rr DAT positive):

- There were seven D typing errors compared with 17 in 04R2
- Use of an IAT for D typing contributed to two errors on this occasion, compared with none in 04R2.

A questionnaire relating to ABO/D typing was distributed with this exercise. A summary of the questions can be found in Appendix V and the report in Appendix VI.

4.8 05E9

Material

'Patient' 1: Anti-E (titre 16)*
'Patient' 2: Inert
'Patient' 3: Anti-E (titre 16)*
'Patient' 4: Inert

* - Samples prepared from the same plasma pool. The purpose was to assess two different techniques or procedures on a trial basis. A short questionnaire was included.

Results

Table 9 – summary of results for 05E9

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**	**5/457**	**8**[1]	**1/79**	**2**
	- P1 – anti-E		2		1
	- P3 – anti-E		3		1
	- P2/4 inert		3		
Antibody ID	**All Samples**	**1/392**	**2**[2]	**0/72**	**0**
	- Anti-E		1		
	- Anti-E		1		

[1] - Six due to transposition of either samples or results and 2 due to transcription error
[2] - Both due to 'tickbox' error

There was no apparent difference in the proportion of errors made using 'routine' or 'additional' methods or procedures. See appendix VII for analysis of additional techniques used.

4.9 05R10

Material

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

'Donor' W: O D positive, R_1r
'Donor' Y: O D positive, R_1R_1
'Donor' Z: A D negative, rr

Due to an oversight on the part of the Scheme, a replacement 'Patient' 3 plasma was distributed one day after the main distribution. Since some participants had already submitted results on receipt of the replacement, the crossmatch for 'Patient' 3 *vs* 'Donor' Z was withdrawn from scoring.

Results

Table 10 – Summary of results for 05R10

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**	**3/464**	**3**	**6/174**	**6**
D Grouping	**All Samples**	**2/463**	**2** [1]	**2/174**	**2**
Antibody Screening	**All Samples** - Anti-c	**0/457**	**0**	**1/173**	**1** 1
Antibody Identification	**All Samples**	**3/392**	**3**	**1/131**	**1**
Incompatibilities	**All Samples** - P1DZ (ABO) - P2DW(c) - P2DZ (c)	**4/444**	**5** 1 [2] 2 [2] 2 [1]	**6/150**	**6** 1 3 2
Compatibilities	**All Samples**	**6/444**	**7** [3]	**4/150**	**6**

[1] - Both due to transcription/transposition error
[2] - One due to transcription/transposition error
[3] - Four due to transcription/transposition error

Errors:

ABO/D grouping

- Two ABO grouping errors were due to results being recorded incorrectly; both occurred in manual systems and where there was a lone worker.
- A third laboratory recorded a false positive weak reaction with A cells for 'Patient' 2, and was consequently unable to make an interpretation (result of UI).
- Two laboratories recorded false positive D types, due to transcription errors made in manual systems.

Antibody Identification

- Two laboratories recorded the presence of anti-Lu^a in addition to anti-c.
- One further laboratory recorded a false positive reaction in the panel, and consequently recorded a result of anti-c+UI

UI trial for antibody identification

This was the third trial, and the first where 'rules' were applied. See Appendix VIII for the rules and Appendix IX for the results.

4.10 06E1

Material

'Patient' 1: Inert
'Patient' 2: Anti-D+K (titre 4 and 8, respectively)
'Patient' 3: Inert
'Patient' 4: Anti-E+S (titre 16 and 2, respectively)

Results

Table 11 – Summary of results for 05E1

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** -	**0/455**	**0**	**1/80**	**1**
Antibody ID	**All Samples** - Anti-D+K - Anti-E+S	**4/391**	**4** 2 [1] 2	**3/75**	**3** 1 2

[1] - Two due to 'tickbox' errors

Errors:

Antibody identification (excluding 'tickbox' errors)

- One laboratory missed the anti-S in the mixture
- One laboratory recorded the presence of anti-Jk[a] in addition to anti-E+S

4.11 06R2

Material

'Patient' 1: O D positive, anti-K+Fy[a] (titres both >32)
'Patient' 2: A D negative, inert
'Patient' 3: B D positive, inert

'Donor' W: A_2 D negative K-, Fy(a-)
'Donor' Y: O D negative, K+, Fy(a-)
'Donor' Z: O D negative, K-, Fy(a+)

Results

Table 12 – Summary of results for 06R2

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**	**2/467**	**2** [1]	**1/179**	**1**
D Grouping	**All Samples**	**0/467**	**0**	**1/179**	**2**
Antibody Screening	**All Samples** - False positive - False negative	**6/460**	**6** [2]	**5/177**	**5** 4 1
Antibody Identification	**All Samples**	**4/394**	**4** [3]	**6/137**	**6**
Incompatibilities	**All Samples** - P1DW (ABO) - P1DY - P1DZ - P3DW (ABO)	**10/450**	**14** 1 [5] 3 [3] 4 [2] 6 [4]	**5/155**	**6** 1 3 2
Compatibilities	**All Samples**	**7/450**	**11** [4]	**4/155**	**4**

[1] - Both due to transcription error onto EQA result forms
[2] - Two due to sample transposition and one to tick-box error
[3] - One due to transcription error and one to probable sample transposition error
[4] - Four due to transcription or sample transposition error
[5] - Probable sample transposition error

Errors (excluding transcription/transposition errors):

Antibody identification

- Three antibody identification errors:
  - One recorded the presence of anti-S, since they were unable to exclude it in the presence of anti-K and anti-Fy^a
  - One obtained a false negative result with a Fy(a-) K+ cell, and subsequently made an interpretation of anti-Fy^a+Le^b
  - One misinterpreted the reactions as anti-K+S

Crossmatching

- Three missed incompatibilities:
  - Two ABO incompatibilities, with no cause established
  - One laboratory recorded negative results for 'Patient' 1 *vs* 'Donor' Y and Z, due to use omission of the IAT crossmatch; contrary to laboratory policy they undertook an immediate spin crossmatch only.

Conclusions:

There was no particular problem with the detection of the A_2 to O ABO incompatibility.

4.12 06E3

Material

'Patient' 1: Inert
'Patient' 2: Anti-E (titre 8)
'Patient' 3: Anti-Fy[a] (titre 4)
'Patient' 4: Anti-D (titre 2)

Results

Table 13 – Summary of results for 06E3

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** - P3 – anti-D	**2/454**	**2** 2 [1]	**5/81**	**5** 2 3
Antibody ID	**All Samples** - Anti-D	**2/390**	**2** 2	**1/75**	**1** 1

[1]- One due to transcription error

Errors:

Antibody screening

- One participant (excluding the transcription error) missed the anti-D in the screen; this was not reproducible and no cause was identified.

Antibody identification

- Two participants recorded results of anti-C+D, due to false positive reactions. Neither error was reproducible on repeat.

4.13 06E4

Material

'Patient' 1: Inert
'Patient' 2: Anti-E+Fy^{a} (titres both 8)
'Patient' 3: Inert
'Patient' 4: Anti-E+s (titres both >32)

In-house testing showed weak non-specific reactions for 'patient' 3 plasma against some panel cells, using an enzyme technique. Antibody screening for 'patient' 3 was therefore withdrawn from scoring.

Results

Table 14 – Summary of results for 06E4

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**	**1/452**	**1**	**1/80**	**1**
	- P2 – anti-E+Fy^{a}		0		1
	- P4 – anti-E+s		1 [1]		
Antibody ID	**All Samples**	**8/388**	**9**	**4/75**	**4**
	- Anti-E+Fy^{a}		5 [2,3]		3
	- Anti-E+s		4 [2]		1

[1] - Tick-box error
[2] - One due to result transposition
[3] - One due to tick-box error

Errors (excluding the two errors due to result transposition and the tick-box error):

Antibody Identification
There were six antibody identification errors:

- Four laboratories did not identify the anti-E in one or other of the samples. In each case the anti-E was masked by the other specificity. Although two had a policy to systematically exclude further specificities, this was not followed. None used an enzyme panel, although two had access to one.
- One laboratory reported an additional specificity not present
- One reported anti-s+UI due to false positive reactions

This was the first exercise where UI was an allowable result for antibody identification, where supported by additional submitted worksheets.

4.14 06R5

Material

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

'Donor' W: O D negative, rr
'Donor' Y: O D positive, R_1R_1
'Donor' Z: O D negative, r'r

The majority of participants undertook this exercise as an emergency. The remainder completed the testing in the usual way (these were reference and other laboratories for whom the scenario was not applicable). All submitted results for the same tests, but crossmatching was not subject to penalty scoring since 'donor' cells were only provided to the small group undertaking this routinely.

Results

Table 15 – Summary of results for 06R5

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**	**2/455**	**3** [1]	**7/168**	**7**
D Grouping	**All Samples**	**0/455**	**0**	**3/168**	**3**
Antibody Screening	**All Samples** - P1 anti-c - P3 inert	**1/450**	**2**[1] 1 1	**4/166**	**4**
Antibody Identification	**All samples** - Anti-c - Anti-D	**4/391**	**6** 4 [2] 2 [2]	**4/122**	**4** 4
Incompatibilities	**All Samples** - P1DZ	**1/26** [3]	**1** 1	**N/A**	
Compatibilities	**All Samples**	**3/26**	**3**	**N/A**	

[1] - Two due to transposition error
[2] - One due to transposition error
[3] - Emergency exercise only 26 labs which did not perform the exercise in the emergency format

Errors:

ABO Grouping

Two laboratories made three ABO grouping errors, all of which reflected clinical practice:

- One laboratory reported an incorrect ABO group for 'Patient' 1 based on a single group undertaken manually in a DiaMed card. The error probably occurred at the reading or recording stage.
- Another transposed samples 1 and 3.

Antibody Identification (excluding transposition errors)

- Three participants recorded additional specificities not actually present.
- One participant recorded a result of anti-c+UI, stating that they were unable to exclude several additional specificities; their panels submitted did not support this interpretation.

See appendices X and XI for a summary of the instructions and supplementary report, respectively.

4.15 06E6

Material

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

Results

Table 10 – Summary of results for 06E6

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	**1/450**	**2** 2	**0/77**	**0**
Antibody ID	**All Samples** - Anti-c+K	**12/391**	**12** 12	**9/73**	**9** 9

Errors:

Antibody screening

- The false positive antibody screening results were not reproducible.

Antibody identification

- Of the 12 identification errors:
  - Four did not mention the possibility of the presence of anti-c; at least one of these involved false negative reactions.
  - Five did not conclusively identify anti-c because of weak reactions with the r'r cell; however all five included an enzyme panel which clearly showed the specificity to be anti-c.
  - Three reported anti-c plus additional specificities, but did not mention the possibility of the presence of anti-K.

5.10 06R7

Material

'Patient' 1: A D positive, anti-K (titre 4)
'Patient' 2: O D positive, inert
'Patient' 3: A D negative, anti-s (titre 4)

'Donor' W: A D negative, K-, Ss
'Donor' Y: A D negative, K-, SS
'Donor' Z: A D negative, K+, SS

Results

Table 11 – Summary of results for 06R7

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**	**0/463**	**0**	**0/169**	**0**
D Grouping	**All Samples**	**1/463**	**2**[1]	**1/169**	**1**
Antibody Screening	**All Samples**	**1/457**	**2**[2]	**7/168**	**7**
	- P1 – anti-K		1		5
	- P3 – anti-s		1		1
	- P2 - inert		0		1
Antibody Identification	**All Samples**	**10/393**	**10**	**3/125**	**3**
	- Anti-K		1 [3]		
	- Anti-s		9		
Incompatibilities	**All Samples**	**19/446**	**23**	**10/143**	**15**
	- P1DZ		2 [5]		4
	- P2DW (ABO)		2 [4]		2
	- P2DY (ABO)		2 [4]		2
	- P2DZ (ABO)		2 [4]		2
	- P3DW		15 [5]		5
Compatibilities	**All Samples**	**10/446**	**13** [6]	**5/143**	**9**

[1] - Due to transposition of results
[2] - Due to participant using whole blood sample instead of separate plasma sample
[3] - Due to probable 'tick-box' error
[4] - One due to the inappropriate use of 'electronic issue'
[5] - Two due to transcription/transposition error
[6] - Eight due to transcription/transposition error

Errors (excluding transcription/transposition errors):

Antibody screening

- One laboratory missed both antibodies in the screen, due to testing of the whole blood samples instead of the plasma samples.

Antibody identification

- Eight laboratories included an additional specificity(ies) with the anti-s, and one was unable to identify the anti-s due to false negative reactions.

Crossmatching

- Two laboratories missed all three ABO incompatibilities:
  - One used 'electronic issue', but did not book the 'donors' into the computer, so no computer check was undertaken. This does not reflect clinical practice.
  - The other recorded negative serological reactions in a manual system; the cause of this error is unknown.
- 13 laboratories recorded negative reactions by IAT for 'patient' 3 (anti-s) and 'donor' W (Ss).

4.17 06E8

Material

'Patient' 1: Anti-E+Fy[a] (titre 8 and 2, respectively)
'Patient' 2: Inert
'Patient' 3: Anti-D (titre 1 – UK NEQAS standard)
'Patient' 4: Inert

Results

Table 12 – Summary of results for 06E8

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**	**1/449**	**2[1]**	**2/82**	**2**
	- P2 - inert		1		
	- P3 - anti-D		1		2
Antibody ID	**All Samples**	**6/388**	**7**	**6/77**	**6**
	- Anti-E+Fya		3 [1]		3
	- Anti-D		4 [1]		3

[1] - Two due to transcription or result transposition error

Errors:

Antibody identification

- One participant recorded a result of anti-E+UI for 'Patient' 1, based on a false negative reaction.
- Two participants recorded an additional Rh specificity with the anti-D (one anti-C and one anti-E).

4.18 06R9

Material

'Patient' 1: A/O (50:50) D positive, inert
'Patient' 2: A D positive, inert
'Patient' 3: AB D positive, anti-E (titre 4)

'Donor' W: A D positive, R_2r, Fy(a-b+)
'Donor' Y: O D positive, R_2R_2, Fy(a+b+)
'Donor' Z: O D positive, R_1R_1, Fy(a+b+)

The main aim of this exercise was to assess the recognition of a mixed field reaction. For this purpose 'Patient' 1 was prepared using a mixture of group A and group O red cells.

Results

Table 19 – Summary of results for 06R9

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**	**2/454**	**2**	**2/177**	**3**
D Grouping	**All Samples**	**1/454**	**1**	**0/177**	**0**
Antibody Screening	**All Samples**	**4/447**	**5**	**1/175**	**1**
	- Anti-E		4 [1]		1
	- Inert		1 [2]		
Antibody Identification	**All Samples**	**1/383**	**1**	**0/135**	**0**
Incompatibilities	**All Samples**	**9/435**	**12** [3]	**5/149**	**7**
	- P3DW		7		3
	- P3DY		5		4
Compatibilities	**All Samples**	**8/435**	**10** [4]	**5/149**	**5**

[1] - Two due to transcription/transposition error, plus one used incorrect sample
[2] - One due to transcription/transposition error
[3] - Ten due to transcription/transposition error
[4] - Four due to transcription/transposition error

Acceptable results

'Patient' 1 represented a group A patient transfused with several units of group O red cells. Since no clinical information was supplied, ABO results of UI or A were deemed to be acceptable, as was UI for the D type. 'Patient' 1 was not subject to performance monitoring for crossmatching, since it was deemed to be quite acceptable to deselect the group A unit, where the patient's group was inconclusive.

Errors (excluding transcription/transposition errors):

ABO/D typing

- Two participants recorded a result of group O for 'Patient' 3. One made a manual edit to an automated group (edit required due to haemolysis in the reverse group), but did not follow their usual policy of also performing a 'rapid' group on first time patients. The cause of the other error was not established.
- One participant recorded a result of D negative for 'patient 3. This was not reproducible and the cause was not established.

Antibody Screening and Identification

- One participant missed the anti-E in the screen: cause unknown.
- One participant recorded a result of anti-E+UI, due to false positive reactions with an enzyme technique.

Crossmatching

- Two participants missed one incompatibility each: one versus 'donor' W and one versus 'donor' Y.

Mixed field reaction

Overall, 40% recorded the reaction for 'patient' 1 versus anti-A as mixed field (MF). This varied significantly by technology and use of automation. Full details can be found in the supplementary report in Appendix XII.

4.19 06E10

Material

'Patient' 1: Inert
'Patient' 2: Anti-D+C (titre 8 and 1, respectively)
'Patient' 3: Anti-Fy[a] (titre 2)
'Patient' 4: Inert

Results

Table 20 – Summary of results for 06E10

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** - P3 – anti-Fy[a]	**1/438**	**1** 1	**2/83**	**2** 2
Antibody ID	**All Samples** - Anti-C+D - Anti-Fy[a]	**10/379**	**10** 9 1 [1]	**6/78**	**6** 6

[1] - Probable 'tick-box' error

Errors:

Antibody screening

- No cause was established for the antibody screening error.

Antibody identification

- Nine participants did not detect/identify the anti-C in the mixture with anti-D:
  - Seven obtained negative reactions with r'r cells by IAT and did not utilize an enzyme technique, although four had an enzyme panel available
  - One overlooked the results of the enzyme panel
  - One stated that they were unable to exclude anti-C.

4.20 07E1

Material

'Patient' 1: Inert
'Patient' 2: Anti-c (titre 1)
'Patient' 3: Anti-D+s (titre 8 and 32 respectively)
'Patient' 4: Inert

Results

Table 21 – Summary of results for 07E1

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**	**0/439**	**0**	**0/84**	**0**
Antibody ID	**All Samples**	**7/385**	**7**	**5/79**	**5**
	- Anti-c		3		
	- Anti-D+s		4		5

Errors:

Antibody identification

- Three participants missed the anti-D in the mixture with anti-s:
  - One overlooked the results of an enzyme panel
  - One did not realise that anti-D could not be excluded
  - One stated that they were unable to exclude anti-E
- Three participants included additional specificities not actually present (three in 'Patient' 2 and one in 'Patient' 3.

4.21 07R2

Material

'Patient' 1: O D negative, r"r (cdE/cde) inert
'Patient' 2: B D positive, inert
'Patient' 3: O D positive, anti-K+Fy[a] (both titre 8)

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

Results

Table 22 – Summary of results for 07R2

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**	**2/449**	**3** [1]	**1/196**	**1**
D Grouping	**All Samples**	**4/449**	**4** [2]	**0/196**	**0**
Antibody Screening	**All Samples** - P3-anti-K+Fy[a]	**0/444**	**0**	**1/179**	**1** 1
Antibody Identification	**All Samples** - Anti-K+Fy[a]	**1/383**	**1** 1	**10/134**	**10** 10
Incompatibilities	**All Samples** - P1DZ (ABO) - P2DZ (ABO) - P3DW - P3DY	**6/431**	**9** 3 [3] 3 [3] 1 2	**12/160**	**24** 2 2 10 10
Compatibilities	**All Samples**	**3/431**	**6**	**2/160**	**4**

[1] - Two due to sample transposition and one due to 'tick-box' error
[2] - One due to transcription error and two due to 'tick-box' errors
[3] - All due to transcription/transposition error

Errors:

ABO/D grouping

- There were no errors due to the use of an anti-CDE reagent
- One participant transposed samples 2 and 3 whilst labeling them. Despite the sample ID being checked against the computer-generated worksheet later in the process, the error was not detected.
- The transcription error resulting in an incorrect D type, would not have occurred had the sample been booked into the computer and undergone routine checks.

Antibody identification

- One participant reported anti-Fy^a+Le^b for 'Patient' 3.

Crossmatching

- One participant missed both incompatibilities due to anti-Fy^a. These errors were not reproducible and procedural error was suspected (e.g. omission of the plasma).
- One participant missed the anti-Fy^a versus 'donor' Y; this was possibly due to the use of a higher than recommend red cell suspension in a LISS spin tube technique.

4.22 07E3

Material

'Patient' 1: Anti-c+K (titre 4 and >32, respectively
'Patient' 2: Inert
'Patient' 3: Inert
'Patient' 4: Anti-E (titre 8)

Results

Table 12 – Summary of results for 07E3

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**	**2/444**	**4** [1]	**0/82**	**0**
	- P1 – anti-c+K		1		
	- P4 – anti-E		1		
	- P2/3 - inert		2		
Antibody ID	**All Samples**	**18/385**	**18**	**5/77**	**6**
	- Anti-c+K		18		5
	- Anti-E		0		1

[1] - All due to sample/result transposition errors

Errors:

Antibody identification (anti-c+K):

- Two participants reported anti-c only
- Seven reported anti-c plus an incorrect specificity
- Five reported UI (or anti-c+UI) but did not submit any panel sheets for assessment
- Four reported UI, stating that they were unable to exclude anti-c or other specificities; examination of the panel sheets submitted, did not support these interpretations.

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 (2005/2007)

- for 'E' exercises = 98.2%
- for 'R' exercises = 97.6%

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, but only eight during 2004.

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

Analyte	06E4 – 07E3		05E4 – 06E3		04E4 – 05E3		03R4 – 04E3	
	n	error rate (%Tx)	n	error rate (%Tx)	n	error rate (%Tx)	n	error rate (%Tx)
ABO	12	0.15 (63%)	12	0.11 (50%)	12	0.24 (100%)	8	0.11 (75%)
D	12	0.13 (71%)	12	0.25 (36%) [1]	10	0.15 (71%)	8	0.7 (22%) [1]
False Neg Ab Screen	17	0.16 (67%)	18	0.20 (75%)	17	0.14 (36%)	17	0.1 (40%)
False Pos Ab Screen	18	0.09 (71%)	18	0.28 (44%)	19	0.09 (88%)	15	0.1 (75%)
ABID (single)	9	0.7 (20%)	12	0.3 (31%)	8	0.6 (29%)	7	0.3 (44%)
ABID (dual)	8	1.8 (7%)	6	0.7 (50%)	8	1.2 (19%)	7	1.4 (25%)
Missed Incompatibility	12	0.8 (52%)	13	1.6 (30%)	10	1.0 (29%)	8	1.6 (12.5%)
Missed Compatibility	12	0.6 (41%)	20	0.6 (42%)	17	0.3% (87%)	14	0.3 (53%)

[1] - Includes one DAT positive cell. Adjusted figures for error rate, excluding the DAT positive cell are 0.2% (83% tx) for 2003/04, and 0.14 % (71% tx) for 2005/06

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

Analyte	06E4 – 07E3		05E4 – 06E3		04E4 – 05E3		03R4 – 04E3	
	n	error rate %	n	error rate %	n	error rate %	n	error rate %
ABO	12	0.61	12	0.58	12	0.25	11	0.3
D	12	0.19	12	0.67 [1]	10	0.24	11	0.6 [1]
False Neg Ab Screen	17	0.9	18	1.2	17	1.0	18	0.2
False Pos Ab Screen	18	0.1	18	0.3	19	0.4	17	0.1
ABID (single)	9	1.2	12	0.7	8	1.7	8	0.7
ABID (dual)	8	6.3	6	2.5	8	4.4	7	3.0
Missed Incompatibility	12	2.5	13	1.4	10	2.4	10	0.9
Missed Compatibility	12	1.0	20	0.9	17	1.2	21	0.9

[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

6. LEARNING POINTS FROM EXERCISE RESULTS

- Several exercises have highlighted the vulnerability of manual systems to transcription and transposition errors in all aspects of serological testing; this should be taken into account when establishing SOPs for manual procedures:
    - ABO errors occurred in 05R10, where results were incorrectly entered into the computer or onto a worksheet. These demonstrate the importance of inbuilt checks at critical points, however challenging this may be when work is undertaken by lone workers.
    - In 06R5, one laboratory reported an incorrect ABO group for 'Patient' 1 based on a single group undertaken manually in a DiaMed card. The error probably occurred at the reading or recording stage.
    - A false positive D type was the result of an error at some part of the setting up, reading or recording stage of a manual grouping procedure in 05R5.
    - An antibody identification error occurred in 05E7, made during the transcription step from the worksheet to the EQA result sheet. This type of error probably reflects clinical practice, as antibody identification results are usually input manually into the computer.
    - There were 17 errors in 06R2, and 15 in 06R7, due to transcription or transposition errors; these occurred in blood grouping, antibody screening, antibody identification and crossmatching. Although some were unlikely to reflect clinical practice they do serve to highlight the inherent risks in manual testing and result transcription procedures.
    - An ABO incompatible crossmatch was missed in 06R7 in a manual system.

- 05R5 demonstrated the risk of basing the interpretation of a D type on a single weak positive reaction.

- 06R9 demonstrated that mixed field reactions can be missed whatever technology is used. This should be taken into consideration before implementing abbreviated testing for ABO/D grouping of patients with historical groups on record.

- There are several learning points to be made with respect to antibody identification:
    - 06E4 demonstrated the need to systematically exclude the presence of additional antibodies, where one or more has already been positively identified. In this case anti-E was masked by the presence of a second specificity.
    - 06E4, 06E6 and 06E10 demonstrated the value of an enzyme panel, particularly when elucidating weak Rh antibodies or mixtures. Several laboratories had enzyme panels available but made the decision that they were not required.
    - 05E4 demonstrated that the results for a patient should be viewed as a whole, before assigning specificity: anti-K was missed when the reactions recorded in the initial antibody screen were overlooked.

- Automated systems are not infallible:
    - A strong anti-D was missed in 05E6 by a semi-automated system; a full investigation was not conclusive, but it was assumed that the probe aspirated an air bubble instead of plasma.
    - There were two cases of carryover of a strong anti-D (05E6) in automated systems.

- Several exercises demonstrate the need to treat EQA samples as much like clinical samples as possible. Errors which 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.

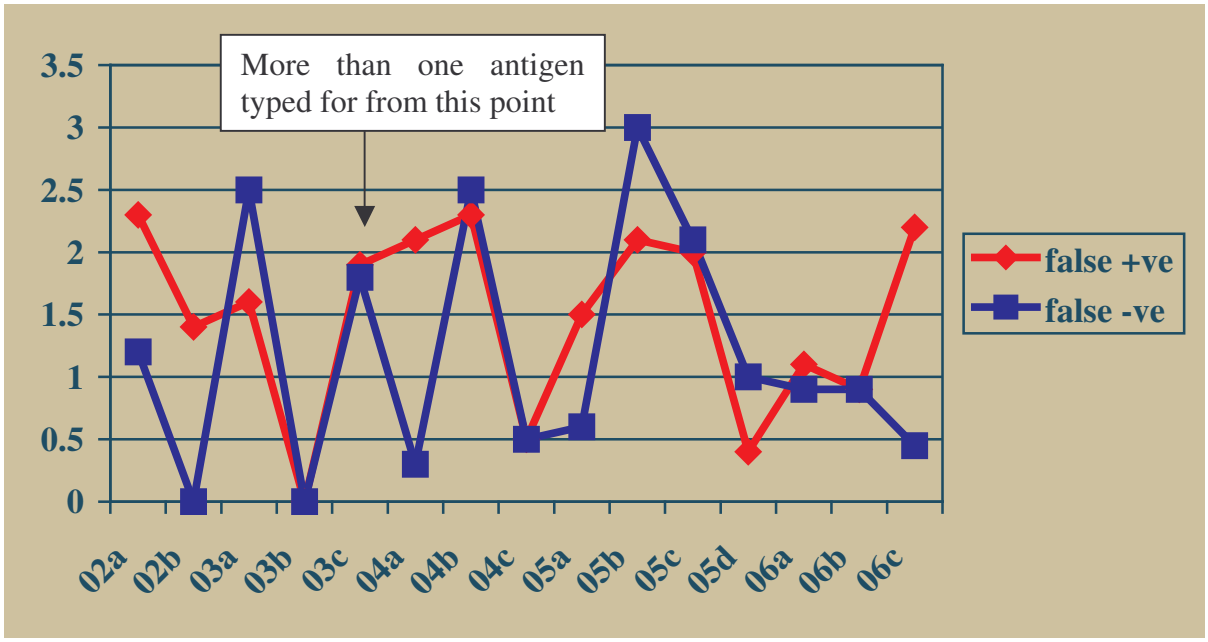
7. SCHEME DEVELOPMENT AND QUALITY INDICATORS

- Unconditional CPA accreditation of the Scheme has been maintained through a third cycle, including an inspection under the new standards in February 2007.

- The Unit at Watford, comprising schemes for BTLP, General Haematology and FMH, is in the process of validating a new computer system and developing an information web-site. This will facilitate web-based entry of results and return of reports.

- The Scheme has reported on six further phenotyping pilot exercises (see Section 8 for details). Figure 1 shows the error rate over time for red cell phenotyping, demonstrating a variable but significant problem. Red cell phenotyping will be offered as a substantive analyte in 2007, with full performance monitoring.

Figure 1 - Error Rates for Red Cell Phenotyping over Time



- Following three trials (see next section for details) the conditional acceptance of a result of UI for antibody identification was introduced in April 2006. A total of 102 participants returned 116 UI submissions during the first year. On review of the panel sheets and explanations, the Scheme agreed with 92 submissions (79.3%) and disagreed with 24 (20.7%). There have been six appeals (25%): two were upheld by the Scheme, and a further two were referred to IBGRL for arbitration; one of the latter was subsequently upheld. Appendix XIII lists all the UI submissions, and provides further details on the 24 where there was no agreement. This data will be reviewed by the Steering Committee during 2007.

- *Performance Targets* – All internal performance targets were met with the exception of reported sample quality for the whole blood and red cell samples. See footnote 2 to table 26 for details.

Table 26 – Performance targets from April 2005 to March 2007

Category	No. of Events	Target	Target Achievement Rate	Actual Achievement Rate
Exercise Distributions	20	On schedule	100%	**100%**
Report Distributions	20	Within 10 days of C/D [1]	90%	**100%**
Complaints	33	Dealt with in 4 weeks	70%	**85%**
New Unsatisfactory Performers	144	Make telephone contact	90%	**96%**
		Within 5 days of C/D [1, 2]	80%	**97%**
Borderline Performers	62	Make telephone contact	50%	**63%**
		Within 10 days of C/D [1,2]	80%	**95%**
Reported Sample Quality – Plasma	69	≤2% unsatisfactory	90% of samples	**99%**
Reported Sample Quality – Whole Blood Samples	24	≤2% unsatisfactory	90% of samples	**58%** [3]
Reported Sample Quality – Red cells in Alsever's	21	≤2% unsatisfactory	90% of samples	**86%** [3]
Integrity of Samples	55812	<0.5% unsuitable for testing per exercise	90% (i.e. 9/10 exercises)	**100%**

[1] - C/D = Closing Date
[2] - Of those contacted
[3] - There were problems with whole blood samples on three occasions and red cells in Alsever's on one occasion with significant haemolysis occurring during the bottling process.

8. QUESTIONNAIRES AND NON-SCORING ELEMENTS – SUMMARY OF DATA

8.1 Phenotyping Pilot

Seven exercises included a phenotyping element, with the following results:

05R5 (Jk^a and Jk^b antigens)

- Results were returned by 262 participants
- There were 39 incorrect results reported by 26 laboratories, giving an overall error rate of 2.7%:
  - 29 false negatives (3.0% error rate)
  - 10 false positives (2.1% error rate)
- Sixteen participants (6.1%) had no anti-Jk^a phenotyping reagent available, and 26 (9.9%) no anti-Jk^b.

05R8 (Jk^a and Jk^b antigens)

This exercise included supplementary questions relating to the use of controls. Results can be found in Appendix XIV

- Results were returned by 251 participants
- There were 29 incorrect results reported by 17 laboratories, giving an overall error rate of 2.0%
  - 19 false negatives (2.1% error rate)
  - 9 false positives (2.0% error rate)
- Fourteen participants (5.6%) had no anti-Jk^a phenotyping reagent available and 24 (9.6%) no anti-Jk^b.

05R10 (Fy^a and Fy^b antigens)

- Results were returned by 263 participants
- There were 12 incorrect results reported by 9 laboratories, giving an overall error rate of 0.8%
  - 10 false negatives (1.1% error rate)
  - 2 false positives (0.4% error rate)
- 14 participants (5.3%) had no anti-Fy^a phenotyping reagent available and 27 (10.3%) no anti-Fy^b.

06R2 (S and s antigens)

- Results were returned by 261 participants
- There were 13 incorrect results reported by 6 participants giving an overall error rate of 0.9%
  - 8 false negative (0.9% error rate)
  - 5 false positive (1.1% error rate)
- 12 participants (4.6%) had no anti-S phenotyping reagent available and 40 (15.3%) no anti-s.

06R7 (Jk^a and Jk^b antigens)

- Results were returned by 268 participants
- Nine laboratories recorded 14 errors, giving an overall error rate of 0.9%
  - 7 false negatives (1.0% error rate)
  - 7 false positives (0.9% error rate)
- 11 labs (4%) had no anti-Jk^a phenotyping reagent, and 25 labs (9%) had no anti-Jk^b.

06R9 (Fy^a and Fy^b antigens)

- Results were returned by 251 participants
- Seven laboratories recorded 10 errors, giving an overall error rate of 0.7%:
  - 5 false negatives (0.4% error rate)
  - 5 false positives (2.1% error rate)
- 14 participants (5.6%) had no anti-Fy^a phenotyping reagent available and 28 (11.2%) no anti-Fy^b

07R2 (Jk^a and Jk^b antigens)

- Results were returned by 253 participants
- 16 laboratories recorded 19 errors, giving an overall error rate of 1.4%
  - 15 false negatives (1.6% error rate)
  - 4 false positives (0.9% error rate)
- 13 laboratories (5.1%) had no anti-Jk^a phenotyping reagent available, and 26 labs (10.3%) had no anti-Jk^b.

8.2 Transfusion of Patients with SCD – distributed with 05R5

This questionnaire was distributed as a follow-up to the 04R10 'selecting phenotyped blood' questionnaire, to gather more detailed information on the transfusion of patients with sickle cell disease (SCD). The questionnaire was distributed to 222 laboratories, selected as those which provide blood transfusion support for patients with sickle cell disease. The data is based on 119 completed questionnaires:

- Approximately 90% of respondents undertake (in-house or through a reference laboratory) red cell phenotyping for at Rh and K prior to the first transfusion. The same proportion select blood matched for these antigens for transfusion.
- 77% select/request HbS negative red cells.

A summary of the questions can be found in Appendix II, and a full report in Appendix III.

8.3 ABO/D Typing Questionnaire – distributed with 05R8

This questionnaire was devised with the aim of establishing current practice for ABO and D typing of non-urgent routine patient samples (i.e. not donor samples or grouping anomalies). Summary data was compared with that obtained through a similar questionnaire distributed in 2002, with some significant changes noted during this time period:

- The use of automation has increased from 41% to 60% of laboratories
- 20% of laboratories are omitting the reverse group where a historical group result is available, compared with 13% in 2002
- Only 39% are now using an anti-A,B reagent compared with 64% previously
- 23% now use a single anti-D reagent (once) for new patients compared with only 5% in 2002
- The use of routine electronic issue has increased from 10% to 26%

A summary of the questions can be found in Appendix V, and a full report in Appendix VI.

8.4 Additional Technique Questionnaire – distributed with 05E9

Following proposals to send separate samples for assessment of two different techniques (or procedures), two pairs of samples were distributed in 05E9, one for testing by the routine technique and the other for testing by a second technique (if available). A short questionnaire was supplied on which additional information relating to the second technique was logged. The majority of laboratories (52%) have a second technique for antibody screening, with the vast majority of these being a back-up to routine automation. A full report of the data received can be found in Appendix VII.

8.5 UI Trial with 'Rules' – 05R10

The third pilot exercise was distributed to trial the acceptance of UI (unable to identify) for antibody identification. Following feedback from those participating in the first two trials, this one included a set of 'rules' to be used in applying the full performance monitoring system. Appendix VIII outlines the 'Rules' and Appendix IX the results. It was subsequently agreed by the Steering committee to fully implement this as a routine, from April 2006.

8.6 Provision of Blood in an Emergency Situation – 06R5

An 'emergency' exercise is distributed approximately once every two years. On this occasion participants were requested to crossmatch units from stock rather than from 'donor' samples provided. A 'routine' exercise was provided for a few participating laboratories where the emergency format was inappropriate. A summary of the questions can be found in Appendix X and a full report in Appendix XI.

9. TRENDS IN USE OF TECHNIQUES IN UK NEQAS EXERCISES

The data is taken from one exercise in each calendar year and therefore only includes data from laboratories returning results. Questionnaire data has 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		2007	
	No.	% of total	No	% of total	No	% of total	No	% of total
LISS spin-tube	127	29%	29	6%	8	1%	5	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%	305	67%
BioVue	78	18%	105	23%	115	24%	117	26%
Microplate-LP[1]	21	5%	7	2%	0	0%	0	0%
Microplate-SPAT[2]	2	<1%	4	1%	1	<1%	0	0%
Capture RRS	6	1%	15	3%	25	4%	31	7%
Biotest Solid Screen	1	<1%	3	1%	5	1%	4	1%
More than one	33	8%	21	5	N/A	3%	N/A	N/A
Other/not stated	3	1%	3	1	2	0%	1	<1%
Total	436	100%	460	100	454	N/A [1]	452	N/A [2]

LP - liquid phase SPAT - solid phase antiglobulin test N/A - Not applicable

[1] - 15 recorded more than one technique (including seven Capture) and these appear in each of the relevant rows.

[2] - 12 recorded more than one technique (including eight Capture) and these appear in each of the relevant rows

9.2 IAT techniques used for crossmatching in the UK

Table 28 – IAT techniques used for crossmatching over time

Technique	1996		2001		2005		2007	
	No.	% of total	No.	% of total	No.	% of total	No.	% of total
LISS spin-tube	201	47%	68	15%	25	6%	21	5%
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%	297	69%
BioVue	72	17%	96	21%	109	24%	106	25%
More than one	17	4%	21	5%	7	2%	2	<1%
Other	2	<1%	6	1%	4	1%	4	1%
Total	426	100%	455	100%	451	100%	430	100%

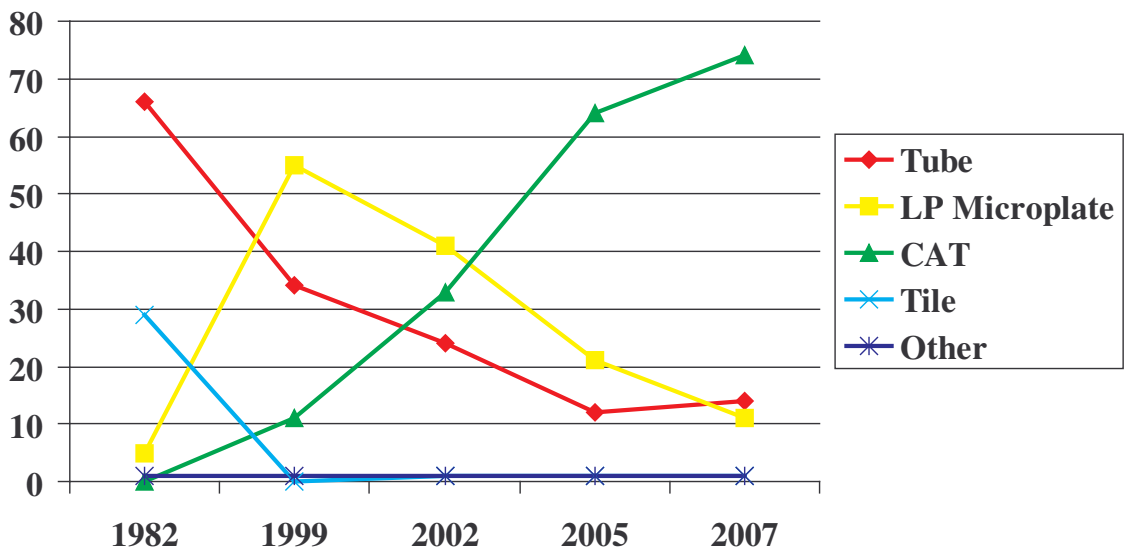
9.3 Use of enzyme techniques in the UK

Table 29 – Use of enzyme techniques over time

Procedure	% of participants using enzyme techniques			
	1996	2001	2005	2007
Screening	56%	30%	12%	10%
Crossmatching	20%	No data	No data	No data

9.4 ABO and D grouping techniques in the UK

Figure 2 – ABO/D grouping techniques over time



10. INFORMATION, EDUCATION AND PUBLICATIONS/PRESENTATIONS

Education

- Annual meeting November 2005: Are we doing the right thing?
- Annual meeting November 2006: Profile, Performance and Perspective – the Changing Face of Hospital Blood Transfusion Practice
- Annual Participants' Meeting for UK NEQAS General Haematology October 2005 – "From Microscope to Molecule" – review of BTLP Scheme over 20 years
- MRCPath teaching

Publications

- Abstract – Oral Presentation BBTS 2005, *Transfusion Medicine*, **15**, suppl 1, 4

Provision of Blood for Patients with Sickle Cell Disease (SCD)
J White, CE Milkins, D Benkhaled, M Rowley

- Abstract – Poster presentation ISBT 2005, *Vox Sanguinis*, **89**, Suppl 1, 73

Selection of phenotyped blood for pre-menopausal females in the UK and mainland Europe
J White, CE Milkins, D Benkhaled, M Rowley

- Abstract – Poster Presentation ISBTS 2006, *Vox Sang*, **91**, suppl 3, P162

Are UK Guidelines for Provision of Blood for Patients with Sickle Cell Disease Followed?
JLWhite, CE Milkins, D Benkhaled, M Rowley

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
- BCSH Blood Transfusion Task Force
- BBTS SIG for Blood Bank Technology
- Transfusion Training Committee of the London and South East Technical Advisory Groups
- Writing group for the BCSH IT guidelines and FMH guidelines
- National Occupational Standards
- Healthcare Science Career Pathways workshops
- Specialist Advisory Committee for Immunohaematology (SACIH)
- CMOs National Transfusion Committee IT Working Group (Joint with the NPSA)
- IBMS working group on laboratory errors based on SHOT/SABRE returns

11. FINANCIAL STATEMENT

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

Income:

Participant Type	2005/07
UK Clinical Laboratories:	549,500
Non-UK Clinical Laboratories:	195,000
Non-Clinical Laboratories:	11,500
Grand Total	**£756,000**

Expenditure:

Category	2005/07
Capital Expenditure	0
Salaries:	390,500
Revenue:	215,500
Overheads:	139,500
Education/R&D (inc. books meetings etc.)	10,500
Grand Total	**£756,000**

Appendix I

Composition of Steering Committee at March 2007

Mrs Helen Barber (Chair), Barnet General Hospital
Mrs Clare Milkins (Secretary), Scheme Manager, UK NEQAS
Dr Megan Rowley, Scheme Director, UK NEQAS
Ms Jenny White, Deputy Manager, UK NEQAS
Mr Mark Williams, National Blood Service, Leeds
Mr Alan Howie, North of Scotland Blood Transfusion Service, Raigmore Hospital, Inverness
Mrs Jane Leftley, Maidstone Hospital
Dr Edwin Massey, NBS Bristol/United Bristol Healthcare Trust
Dr A Benton, Morriston Hospital, Swansea/Welsh Blood Service
Mrs H Cawley, BUPA Murrayfield, Wirral
Mr S Bates (NQAAP representative), Cheltenham General Hospital
Mr R Knight (co-opted), National Blood Service, Colindale

Appendix II

Transfusion of Patients with SCD – distributed with 05R5 – Summary of Questions

This questionnaire is intended as a follow-up to the 04R10 'selecting phenotyped blood' questionnaire, to gather more detailed information on the transfusion of patients with sickle cell disease (SCD). Please complete this questionnaire based on the **majority of transfusion episodes** and detail any variation from this practice in the comments box with question 10. We would be very grateful if you could return this questionnaire with the results of Exercise 05R5.

1. Approximately how many patients with SCD are registered with your Haematology clinic
   i) none ii) <10 iii) 10 – 50 iv) >50

2. Approximately how many patients with SCD are seen per year that are ***not*** registered at your Haematology clinic
   i) none ii) <10 iii) 10 – 50 iv) >50

3. Approximately how many episodes of red cell transfusion
   to patients with SCD do you see per year

4. When a sickle cell patient presents at your hospital for the first time, do you attempt to contact any
   previous treatment centre to obtain a) Transfusion history b) Red cell phenotype

5. The first time you see patient with SCD (even if previously tested elsewhere, but not transfused in the last 3 months) which of the following red cell phenotypes are tested for (in-house or referred)
   ABO/D; K; C; c; E; e; Other (specify additional antigens)

6. For transfusion of patients with SCD, **with no atypical antibodies**, is blood provided that is 'matched' (i.e. antigen negative blood given where the patient lacks the antigen) for the following:
   ABO/D; K; C; c; E; e; Other (specify additional antigens)

7. For routine transfusion of patients with SCD, that have **existing atypical antibodies**, do you do anything different to the answer given to Q6 above (apart from selecting blood negative for antigen(s) corresponding to the antibody(ies) present). If 'Yes' please specify.

8. Approximately what % of your patients with SCD have red cell antibodies?

9. Is HbS negative blood selected for patients with SCD?

10. Is your policy for 'very urgent' / 'out of hours' transfusion of patients with SCD is different to the answers above? If 'Yes' please specify and include any other comments regarding circumstances where practice.

Appendix III

Report of 'Transfusion of patients with sickle cell disease (SCD)' questionnaire distributed with exercise 05R5 - May 2005

Introduction

This questionnaire was distributed to all laboratories stating (in a previous questionnaire - 04R10) that patients with sickle cell disease (SCD) are included in their case mix. The aim was to gather more detailed information about the selection of blood for patients with SCD, and the level of compliance with BCSH guidelines. However, since it is possible that any hospital, even those with no registered patients with SCD, may need to treat such a patient in an emergency situation this report should be of interest to all participants.

Unlike patients with other haemoglobinopathies, such as β thalassaemia major, where patients often receive their first blood transfusion before one year of age, and therefore may be less likely to produce red cell antibodies[1, 2], patients with SCD are not often multiply transfused at such an early age, and up 60%[4,8,9] will produce antibodies, often to multiple red cell antigens. UK BCSH guidelines for pre-transfusion compatibility testing[3] state that it is desirable that patients with SCD are phenotyped 'as fully as possible' pre-transfusion, that blood is matched for D, K, C, c, E and e, so long as this 'does not impede the delivery of effective transfusion support'. BCSH guidelines for transfusing neonates and older children[4] state that children with SCD should be phenotyped for 'Rh, K, Fy, Jk and MNS before the first transfusion', that all S-s- patients be typed for U, and that blood for transfusion is matched for Rh and K. Both guidelines state that HbS negative blood should be selected for patients with SCD.

Return Rate

137/222 (61.7%) completed questionnaires were returned, however, only 119 were analysed since the remaining 11 were returned by laboratories that stated that they in fact do not transfuse patients with SCD, even on an occasional basis. Since some respondents did not complete all questions, the numbers in the result tables do not always match 119, and due to rounding, totals may not be exactly 100%. All results refer to patients with SCD unless otherwise stated.

Results

Questions 1, 2 and 3 - Number of patients with SCD registered, number seen per year that are not registered, and annual number of transfusion episodes.
Table 1 shows the number (%) of laboratories that have patients with SCD registered with their haematology clinic, and Table 2 the annual number of episodes of transfusion to patients with SCD.

Appendix III

Table 1 – Number of laboratories with registered patients

Number of patients with SCD registered	Number (%) of laboratories
None	23 (19.3%)
<10	61 (51.3%)
10 - 50	22 (18.5%)
>50	12 (10.1%)
Not applicable (blood service)	1 (0.8%)
Total	**119 (100%)**

Table 2 – Annual number of transfusion episodes

Number of episodes of Tx to patients with SCD	Number (%) of laboratories
None	21 (17.8%)
<5	41 (34.8%)
5 - 10	30 (25.4%)
11 - 50	18 (15.3%)
51 - 100	3 (2.5%)
>100	5 (4.2%)
Total	**118 (100%)**

- The majority (71%) of laboratories that have no registered patients with SCD see <10 such patients per year, and 55% did not record any episodes of transfusion to patients in this group.

- Approximately 75% of transfusion episodes per year are handled by laboratories with >10 registered patients (n=34), and 35% by those with >50 registered patients (n=12). The midpoint of the reported range of registered patients was taken to estimate the number of transfusion episodes per hospital / institution.

- The 12 laboratories with >50 registered patients follow BCSH guidelines for transfusing patients with SCD, except that one only provides HbS negative blood for specific cases, e.g. exchange transfusion.

- 32/34 institutions with >10 patients registered, follow BCSH guidelines with respect to providing Rh and K matched blood. The two that do not follow guidelines accounted for 15 episodes of Tx.

- 30/34 provide HbS negative blood, with the four that do not accounting for approximately 90 episodes of Tx.

Q4 - Requesting transfusion records and historical phenotype from previous treatment centres, for patients with SCD seen for the first time

Table 3 shows the number of laboratories requesting historical data when a patient with SCD is admitted for treatment for the first time.

Table 3 – Laboratories requesting historical data from previous treatment centres

Patient history requested of previous treatment centres	Number (%) of laboratories
Transfusion history and phenotype	75 (63%)
Transfusion history only	14 (11.8%)
Phenotype only	6 (5.1%)
Often not informed of sickle status	1 (0.8%)
Neither	23 (19.3%)
Total	**119 (100%)**

Appendix III

Q5 / Q6 - Phenotyping of patients with SCD and 'matching' blood for transfusion (i.e. selecting antigen negative blood where the patient lacks the antigen).

Table 4 shows the antigens selected for phenotyping and Table 5 the 'matched' blood provided for patients with SCD.

Table 4 – Antigens phenotyped for:

Antigen(s) included in phenotype	Number (%) of laboratories
Rh, K and additional specificities[1, 2]	39 (32.8%)
Rh and K only	64 (53.8%)
Rh but not K (+/-) others	4 (3.4%)
K only	1 (0.8%)
None	10 (8.4%)
Not applicable (NBS)	1 (0.8%)
Total	**119 (100%)**

[1] One laboratory only phenotypes if transfusion is require

[2] Additional specificities:

- 9/39 phenotyped for D, K and some additional specificities but not all antigens to which the most common 'clinically significant' antibodies are made (Jk^a, Jk^b, Fy^a, Fy^b, S and s)
- 29/39 phenotyped for D, K and all antigens to which the most common 'clinically significant' antibodies are made (Jk^a, Jk^b, Fy^a, Fy^b, S and s). Some of these also phenotyped for low frequency antigens, and / or antigens where the corresponding antibodies are of unlikely clinical significance.

Table 5 – Antigens 'matched' for:

Antigen(s) included in phenotype	Number (%) of laboratories
Rh (CcEe), K and additional specificities	17 (14.3%)
Rh (CcEe) and K only	86 (72.3%)
Rh (CcEe) only	1 (0.8%)
K only	3 (2.5%)
Rh C, E and K	2 (1.7%)
None	9 (7.6%)
Not applicable (NBS)	1 (0.8%)
Total	**119 (100%)**

Q7 - Difference in protocol for patients with SCD with pre-existing red cell antibodies

- 102/117 did not record any changes in protocol for patients with red cell antibodies
- 15 did state that their protocol was different, including 5 that would undertake 'matching' for further antigens.

Q8 – Percentage of patients with SCD having red cell antibodies

In larger centres (>10 registered patients), the estimated proportion of SCD patients with red cell antibodies ranges from 0% – 40%.

Q9 – Selection of HbS negative blood

- 87/113 laboratories select HbS negative blood for transfusion to patients with SCD.
- Of the 26/113 that do not select HbS negative blood:
  - 5 recorded no transfusion episodes
  - One has >50 registered patients

Appendix III

Q10 – Differences in policy for 'urgent' and out of hours working

- 19/117 stated that their policy changes for 'urgent' situations and 23 made comments including:
  - Suspend policy on 'matched' blood in an emergency, rather than delay transfusion (n=14)
  - May not be able to provide HbS negative blood in an emergency (n=9)
    - All were in England and 7/9 within one NBS zone
  - Give rr K- HbS negative blood until the patient's phenotype can be established (n=1)
  - Issue Rh and K matched, until extended phenotype is known (n=1)
  - Give rr blood until Rh phenotype is known (n=3)
  - HbS negative blood is used only in specific clinical scenarios, where Hb <4 g/dl, for 'sickle lung' and for exchange transfusion (n=1)
  - Blood <7 days or 10 days would be selected (n=1)

Discussion

Phenotyping and antigen matching

Patients with SCD should be phenotyped at registration or at first contact, since transfusion may be required in an emergency situation. It is also possible that transfusion may be necessary in a situation where the patient is not able to reach the hospital at which they are registered, and for this reason it is useful if a 'card' can be issued with details of blood groups, phenotype and any red cell antibodies. The rationale behind phenotyping prior to transfusion is to be able to provide blood matched for the antigens most likely to stimulate alloantibodies (i.e. Rh and K), and to aid identification and further selection of blood in the event of the patient producing clinically significant antibodies to other blood group antigens (e.g. Fy and Jk), or autoantibodies. Matching is undertaken to prevent the stimulation of alloantibodies, and subsequent difficulties in providing compatible blood where the patient has multiple antibodies to common antigens. There is also some evidence of increased susceptibility to autoantibodies[5] once alloantibodies are produced. In some highly alloimmunized patients, a syndrome of autoimmune haemolytic anaemia can follow alloimmunisation or a delayed haemolytic transfusion reaction[5]. The condition may persist from several weeks to 2-3 months before disappearing, complicating the detection and identification of further alloantibodies that may have been stimulated by the transfusion.

Although routine transfusion for anaemia is not advised in SCD[6], up to 90% of patients with SCD will require transfusion support at some stage, to prevent or treat complications of their disease[7]. It is likely that the level of transfusion to SCD patients will be maintained or even increase in the future, at least for paediatric cases, as evidence accumulates from trials, such as the American multicentre trials for stroke prevention in sickle cell anaemia (STOP)[8] and STOP 2. The presence of alloantibodies causes increasing problems in providing blood, with many SCD patients producing complex mixtures of antibodies. It is known that the risk of alloimmunisation to red cell antigens increases with each unit transfused[9]. In patients with SCD the incidence of alloimmunisation has been shown to increase exponentially with increasing number of transfusions; the incidence was calculated in one study to be 3.1% per unit[10].

There is good evidence that a high proportion of antibodies produced in response to transfusion are of Rh and K specificity, and that matching for these antigens significantly reduces the risk of immunisation. A UK study of patients with SCD[11] showed that 17.6% had alloantibodies and that 66% of these antibodies were of Rh or K specificity; these findings are similar to those in many other trials, with some studies quoting an immunisation rate of up to 60%[9]. More recently, it has been shown in a randomised prospective trial[7] that if blood for transfusion is matched for Rh C, E, c, e and K, then the incidence of alloimmunisation can be reduced to 0.5% (or 0.25% for clinically significant antibodies).

The same study showed that the rate of DHTR was also low at 0.11% *cf.* 11% quoted in previous trials. A retrospective study[12] of alloimmunised patients with SCD over a 12 year period showed that if transfused blood had been matched for Rh C, E, c, e and K then 53.5% of alloimmunisations could have

been avoided. There is also anecdotal evidence that if patients can be prevented from producing alloantibodies to the most antigenic red cell antigens, that they will be less likely to produce antibodies to other blood group systems[2].

Providing HbS negative blood

The rationale behind providing HbS negative blood is to prevent interference with monitoring of HbS levels during exchange transfusions, or other procedures where the endpoint of the transfusion therapy or effectiveness of the treatment is measured by the level of HbS, e.g. in exchange transfusion. In situations where it is not essential to monitor HbS levels in this way, e.g. top-up transfusion for stroke prevention, it may not be essential to use blood found negative for HbS, and in most regions in the UK the risk of randomly selecting an HbS positive unit will be small. Furthermore, where multiple alloantibodies are present, blood of compatible phenotypes is more likely to be from a racial group with a higher prevalence of HbS.

Questionnaire data

In the 119 responding hospitals, the number of annual transfusion episodes per hospital ranged from 0 to 560, with 79% recording <10 per year. In larger centres (>10 registered patients), the estimated proportion of SCD patients with red cell antibodies ranges from 0% – 40%. There was insufficient data (with the small number of centres without a policy to match, and for those that do, lack of information regarding the length of time the policy had been in place), to be able to correlate the policy for matching for Rh and K and level of alloimmunisation.

Where patients have been treated elsewhere, 81% of laboratories contact previous treatment centres for a transfusion history and / or red cell phenotype, when seeing a patient for the first time. Where the patient is seen for the first time, 33% undertake extended phenotyping, a further 58% type for Rh and K only, whilst 8% ABO/D type only. Red cells transfusions are matched for at least Rh and K in 90% of laboratories overall, and in 97% of the larger centres (>10 registered patients n=34).

Overall 22% stated that they do not select HbS negative blood; this varies by region and centre size: 86% in Scotland (n=7) compared with 13% in the South Thames region (n=23); 26% of small centres (<10 patients; n=85) compared with 12% of larger centres. The regional variation may be influenced by the fact that UK Blood Services do not always provide HbS negative blood for all patients with SCD, and by the prevalence of HbS in the patient and donor populations.

There is variation in practice in the UK, with a tendency for the larger centres to be more likely to follow guidelines and recommendations on selection of blood both matched for Rh and K, and negative for HbS. Based on the number of transfusion episodes recorded, overall, approximately 85% of transfusions to patients with SCD in the responding hospitals are of HbS negative blood matched for Rh and K.

Conclusions

There is no standard NBS policy on the provision of HbS negative blood, except for where blood is required for neonates and exchange transfusion. Not all laboratories are following BCSH recommendations on matching for Rh and K. This is despite clear evidence in the literature that matching for these antigens reduces alloimmunisation and consequent difficulty in obtaining compatible blood if further antibodies are made. Matching may also protect to some degree against complications with HTR and autoantibodies. Future guidelines and policies may need to consider the relative risk of inadvertently transfusing a unit of HbS positive blood.

There is evidence from this questionnaire that those institutions that rarely see patients with SCD are less likely to follow BCSH guidelines. Also, that over 90% of those transfusing sickle patients will on occasions, transfuse patients that are not registered at their hospital. Therefore, it is important that all

blood transfusion departments have a policy based on BCSH guidelines for transfusing patients with SCD, even if no such patients are registered at their institution.

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Appendix IV

Supplementary Report – D typing
Exercise 05R8 –Distributed 26 September 2005

Introduction

The primary aim of exercise 05R8 was to assess proficiency in D typing a rr DAT positive sample, and this report details the results of D typing for 'Patient' 1 - A RhD Negative, DAT 3+. The red cells in 'Patient' 1 whole blood sample were sensitised with IgG anti-c.

Results

Overall Results

This analysis includes results received both before and after the closing date for exercise 05R8. Unable to interpret (UI) was considered acceptable for both ABO and D typing. 422/461 (91.5%) reported D negative for 'Patient' 1. Twenty laboratories reported A UI, and a further 12 reported UI for both ABO and D. Overall, 184/456 (40.4%) reported a DAT result, with 173/184 (94%) recording it as positive.

Results of laboratories making D typing errors

Three laboratories incorrectly reported 'Patient' 1 as D positive, (excluding one due to transcription error), and a further four reported D^{VAR} (defined on the UK NEQAS result sheets as weak or partial D), giving an overall error rate of 7/461 (1.5%). Six of these seven used Ortho BioVue cassettes containing a potentiated anti-D reagent(s) and a reagent control. Individual results and interpretations for the seven laboratories are detailed in Table 1.

BioVue cassettes were used for initial testing by 6 laboratories: 5 Combo (anti-A, anti-B, anti-D, control and reverse group), and one ABO Rh-D (anti-A, anti-B anti-A,B, anti-D, anti-CDE, control).

- 1/6 recorded a strong positive reaction with anti-D and a weak reaction with the reagent control
- 2/6 recorded the same grade of reaction reactions with two anti-D reagents and a reagent control
- 3/6 did not record results of the reagent control.

In two cases where a positive control was recorded, a fully automated system was used for ABO/D typing, and the automation appropriately alerted the user to the discrepant result. In both cases the result was authorised manually as D positive. However, one of these went on to do additional testing with IgM saline reacting anti-D, but due to problems with communication, the original result, already transmitted to the laboratory computer system, was reported in error.

Two laboratories reported results of D^{VAR} based on reactions obtained with blended IgG/IgM reagents by IAT.

- One of these (not testing patient samples) obtained negative results with two anti-D reagents by direct agglutination, but continued to perform an IAT with the blended reagent. They reported D^{VAR} based on a weak positive result by IAT, combined with a false negative result for the DAT used as a control.
- The other found a weak positive result with the anti-D in a BioVue Combo cassette, a positive DAT and a positive reagent control (not recorded). This laboratory then proceeded to test with a blended IgG/IgM by IAT, found a weakly positive result and reported a result of D^{VAR}.

Appendix IV

Table 1 – Results of those reporting 'Patient' 1 as D^{VAR} or D positive

Lab.	Reagent(s)	Reaction grades recorded				Interpretation
		Anti-D 1	Anti-D 2	Reagent Control	DAT	
1	BioVue Combo cassette	S	Not used	W	Pos	D Pos
2	BioVue Combo cassette + BioVue ABD	MF	MF	MF	NT	D^{VAR}
3	BioVue Combo cassette + IgG / IgM blend (tube IAT)	W	W	Not rec[1]	Pos	D^{VAR}
4	BioVue ABO Rh-D cassette	S	S	S	NT	D Pos
5	BioVue Combo cassette + Additional anti-D reagent[2]	W	W	Not rec[1]	Pos[3]	D^{VAR}
6	BioVue Combo cassette	W	Not used	Not rec[1]	NT	D Pos
7	IgM saline monoclonal + IgG/IgM blend (tube IAT)	N	W	Not rec[1]	Neg	D^{VAR}

[1] not recorded on result forms, but stated as positive, during discussion regarding errors.
[2] unknown second anti-D reagent
[3] recorded as 'auto' rather than DAT.

Results of laboratories with no D typing errors

Table 2 includes data from laboratories that correctly interpreted 'Patient' 1 as D negative or reported UI. The numbers recording combinations of reaction grades for a reagent control and anti-D reagent are shown (where more than one anti-D was used, the results were identical except where stated). The expected results are shaded, i.e. where the anti-D reagent and reagent control gave the same reaction grade, or where a control was not used and a negative reaction was recorded with one or more anti-D reagents. A few laboratories apparently based their interpretation on results identical to those recorded by those making incorrect interpretations. However, it may be that results submitted were, in some cases, a composite of initial and follow-up testing

Table 2 – Results of laboratories reporting 'Patient' 1 as D negative or UI

Control Reaction Grade	Anti-D Reaction Grade			
	Strong Pos	Weak Pos	Mixed Field	Negative
Strong Positive	4	2[1]	1	4
Weak Positive	1	28[2]	5	8
Mixed Field	0	0	10	0
Negative	1	1	0	155[3]
No Control Used	2	4	0	231[3]

[1] both recorded a negative reaction with a second anti-D reagent
[2] 4 recorded a negative reaction with a second anti-D reagent
[3] 1 recorded a weak reaction with a second anti-D reagent

- 71 recorded results for one anti-D reagent only
- 7 included no D typing reaction grades

The 64 laboratories reporting a positive reaction with a reagent control (either weak, strong or mixed field) made the following D typing interpretations:

- 33 D negative
- 28 unable to interpret (UI)
- 2 D positive
- 1 D^{VAR}

Appendix IV

Discussion

As in the last exercise including a D negative (rr) DAT positive 'Patient' sample (04R2), the majority of the errors made in this exercise were due to misinterpretation of results. However on this occasion, there were encouragingly fewer D typing errors (7 *cf.* 17 in 04R2), and none were due to the omission of a reagent control for a potentiated anti-D reagent.

Where cells are sufficiently coated with IgG to cause a false positive reaction, a reagent control should react to the same degree as the reagent it is designed to control. A positive reaction with the reagent control invalidates the test result, even where the reaction with the control is weaker than that obtained with the anti-D reagent. Even where the reagent control is found to be negative, or the reagents in use do require a control, it is still not safe practice to assign a D type of D positive or DVAR based on a weak (<3) reaction with a single anti-D reagent1.

In three of the laboratories contacted, not all staff were not aware that a positive reaction with the reagent control invalidates the test results for that patient.

In both cases where automation was used, the analyser highlighted the anomalous results and did not offer an interpretation for the D type. However, the warnings were overridden and an incorrect interpretation made by the operator. When a positive result is obtained with the reagent control, testing should be repeated, where possible, with non-potentiated IgM monoclonal anti-D reagents by direct agglutination. If such reagents are not available, patients should be regarded as D negative until a referral has been made to confirm the D type. In the context of UK NEQAS exercises, it is advisable to record the interpretation as 'unable to interpret' (UI).

In 04R2 no errors were made due to testing patient cells vs. anti-D reagents by IAT, however, this time two such errors were made. BCSH guidelines recommend that D typing is not performed by IAT when testing patient samples for transfusion purposes.1

In one case, an anomalous D typing result was noted and additional manual testing undertaken, but a result of D positive had already been transmitted to the laboratory computer system, and was reported without comparison with the additional testing. It is advisable not to transfer results until all testing is complete, since a breakdown in communication can lead to incomplete results being reported.

Appendix IV

Conclusions

It is vitally important to understand the characteristics and limitations of the anti-D reagents in use[1], even when they are sold as part of a 'testing system'. The principle that a discrepant result for a control invalidates the test results applies to all blood group serology testing, and should be understood by all staff performing tests and interpreting results. Warnings given by automated systems should be acted upon and not overridden without careful consideration of the consequences, and test results should not be verified until they are complete. The implications of mistyping a D negative patient as D positive are potentially very serious, especially for women of childbearing age as highlighted in the SHOT annual reports[2].

References

[1] BCSH (2004) Guidelines for compatibility procedures in blood transfusion laboratories, sections 5.3.2, 5.3.4 and 5.10.3. *Transfusion Medicine*, 2004. www.bcshguidelines.com

[2] D Stainsby et al (2004) Serious Hazards of Transfusion Annual report 2002/2003.

Appendix V

ABO/D Typing Questionnaire distributed with 05R8 – Summary of Questions

Unless otherwise stated, please answer the following questions with respect to non-urgent ABO/D typing of **routine patient** samples only (i.e. not donor samples or grouping anomalies). Blood centres should not complete this questionnaire unless they perform routine patient ABO/D typing, e.g. on antenatal patients.
Unless otherwise stated, detail **only techniques and reagents that are used for the majority of routine patient samples**.

SECTION 1 – Pre-transfusion / ante-natal patient testing

1. Which technique is primarily used for ABO grouping in the following situations: Routine grouping (new patient); rapid grouping; this UK NEQAS exercise?:

   a) Microplate (liquid phase) b) DiaMed ID system c) Ortho BioVue system
   d) Solid Phase e) Tube (spin or sedimentation) e) Tile/slide f) Other

   If different for patients with a historical group or those requiring crossmatching, or for D typing, please specify

2. Is your ABO/D typing automated for a) Routine Group? b) This UK NEQAS exercise?

3. Do you use an A-B-D-A-B-D card/cassette for any purpose?

If Yes, please specify circumstances in which it is used:

   a) Confirmatory test on a sample that has already been grouped
   b) Confirmatory test on sample not previously tested, but with historical group
   c) Other (specify other).

4. Which reagents are included in your ABO/D group in the following situations: Routine (no historical group); routine (with historical group), Rapid (if applicable); confirmatory testing (if applicable)?.

It is assumed that anti-A, anti-B and anti-D are always used – please tick all others that apply:

   a) Anti-A,B (or A+B) b) Anti-CDE c) Diluent/reagent Control
   d) A cells e) B cells f) O cells g) Auto control h) None of these

5. Which of the following combination of anti-D reagents (including anti-CDE reagents) do you use in the following situations: Routine (No historical group); routine (with historical group); rapid testing; confirmatory testing?
   a) 2 or more different anti-Ds b) Single anti-D, once only c) Single anti-D – twice
   d) 2 or more in one column e) None

6. Are your anti-D reagents for routine grouping (including anti-CDE reagents):
   a) IgM monoclonal b) Not sure c) Other (specify)

7. Please specify the anti-D reagent(s) (including anti-CDE reagents) used for testing routine samples (as determined in Q1), either by card/cassette name and profile, or reagent manufacturer and clone.

8. Do you routinely test all apparent D negative pre-transfusion samples with an anti-D by indirect antiglobulin test (IAT)?

   If yes, do you include an appropriate negative control or a direct antiglobulin test (DAT)

Appendix V

SECTION 2 – Weak and partial D

1. Briefly, what criteria do you have for identifying patients that are possibly weak or partial D e.g. a positive reaction with one anti-D reagent and negative with another, or weak reaction(s) <2+?

2. Once a sample has been identified as possible weak or partial D, how do you proceed?
   a) Investigate using a panel of anti-D reagents designed to classify weak or partial D
   b) Send to a reference laboratory for confirmation
   c) Use an anti-D by IAT (with a negative reagent control or DAT)
   d) Use an anti-D by IAT (with **no** negative reagent control or DAT)
   e) Assign a D type with no further investigation
   f) Other (specify)

3. Do you select D positive or D negative for the following patients in a routine situation?: - if you distinguish (in-house or by ref lab) between weak and partial D answer a-d only, otherwise answer e and f only:

   a. Female aged 30 - weak D
   b. Female aged 30 - partial D
   c. Male aged 60 - weak D
   d. Male aged 60 - partial D
   e. Female aged 30 - weak or partial D (do not distinguish)
   f. Male aged 60 - weak or partial D (do not distinguish)

4. How do you report a weak or partial D to the clinician (whether classified in-house or by ref lab), e.g. D positive, *OR* weak D *OR* D positive (weak D) etc?

5. How do you store the D type of a weak or partial D patient on your laboratory computer system (whether classified in-house or by ref lab), e.g. D positive, *OR* weak D *OR* D positive (weak D) etc?

6. If a pregnant woman is identified as weak or partial D, do you give routine postnatal anti-D prophylaxis

Appendix V

SECTION 3 – Cord / newborn testing

1. Does your laboratory ABO/D type neonatal or cord samples for the purposes of
   a) Anti-D administration b) A transfusion (or transfusion record)?

2. If tested for anti-D administration, do you use any of the following **different** or **additional** reagent?
   a) 'Newborn/cord' grouping card/cassette detecting D^{VI}
   b) 'Newborn/cord' grouping card/cassette **not** detecting D^{VI}
   c) Other monoclonal IgM anti-D that detects D^{VI}
   d) IgG reagent by antiglobulin technique
   e) Other

3. If tested for transfusion purposes, please specify card profile / reagents used if different from those used for adults
4. If a cord is found to be D^{VI} positive, is a maternal sample also tested for D^{VI}?

SECTION 4 - Miscellaneous

1. Do you include batch controls for ABO/D forward grouping, e.g. by testing anti-A, anti-B, anti-D etc. vs. reagent red cells or previously tested samples

   a) With each routine ***manual*** batch (one or more sample)
   b) With each ***manual*** rapid group
   c) Twice daily for ***automated*** grouping
   d) When reagents are changed during ***automated*** grouping
   e) Other (specify)

2. Do you dilute any of the following reagents (as specified in previous sections) in house?
   a) Anti-A b) Anti-B c) Anti-D

3. Which best represents your crossmatching process for patients with no atypical antibodies:
   a) Electronic Issue b) 'Immediate spin' (IS) only c) IAT only d) IAT and IS

Please record any comments relating to additional or alternative techniques for different patient groups or situations not covered in the main questionnaire.

Appendix VI

Routine ABO and D Typing Questionnaire Distributed with Exercise 05R8

This questionnaire was devised with the aim of establishing current practice for ABO and D typing of non-urgent **routine patient** samples (i.e. not donor samples or grouping anomalies). Blood centres were asked not to respond unless they perform routine patient ABO/D typing, e.g. on antenatal patients. Information was requested relating to techniques and reagents used primarily for the majority of routine patient samples, and not those used for 'back-up', urgent procedures or special cases (except where specified). There was a section at the end of the questionnaire where details of any such additional procedures could be included as free text. A summary of the questions is attached as appendix 1. Completed questionnaires were returned by 426/469 (90.8%) laboratories; however, since some respondents did not answer all questions the totals in each table are not necessarily 426.

Summary of Data

Table 1 displays an overall summary of the data. Where available, data from a similar questionnaire distributed in 2002 has been given for comparison. Full details are given in the body of the report.

Table 1 – Summary of data

Routine ABO/D Grouping	**2005 (n=426)**	**2002 (n=446)**
Automation	60%	41%
Liquid phase microplates	21%	41%
Column Agglutination Technology (CAT)	65%	33%
No reverse group on patients with historical groups	20%	13%
No reverse group on patients without historical group	1%	1%
ABO grouping reagents		
Dilute ABO reagents	<1%	7%
Include anti-A,B reagent	39%	64%
D typing reagents		
At least one monoclonal anti-D reagent used for routine grouping	>99%	>99%
Single anti-D used once for patients with a historical group	41% [1]	15%
Single anti-D reagent used once for patients with no historical group	23% [1]	5%
Dilute anti-D reagent	<1%	5%
Routinely include IAT for D typing on apparent D negatives (appropriate control used)	5% (87%)	3% (100%)
Include and anti-CDE reagent	6%	≥ 10%
Routine Crossmatching (no atypical antibodies)		
Electronic issue	26%	10%
'Immediate' spin	11%	15%
IAT (± other technique(s))	63%	75%

[1] – includes a pool of anti-D reagents in a single card or cassette column

Appendix VI

Technology and Automation

Technology

The numbers and % using each technology for ABO and D typing of routine and urgent samples are shown in Table 2.

Table 2 – Techniques used for routine and rapid grouping

Technique used for ABO/D grouping	Number (%)	
	Routine (primary technique)	Rapid Group (where applicable)
Liquid Phase Microplate	88 (21%)	35 (10%)
Tube	53 (12%)	226 (65%)
DiaMed	189 (44%)	30 (9%)
BioVue	87 (20%)	24 (7%)
Tile/Slide	5 (1%)	34 (10%)
Solid Phase Microplate	3 (1%)	0 (0%)
WHO tray	1 (<1%)	1 (<1%)
Total	**426**	**350**

- Participants are asked to undertake the EQA exercises using their routine techniques; however, 32 participants used a different ABO technique for UK NEQAS exercise 05R8 compared with that used routinely.
- At least 11 laboratories group all new patients by a second technique.
- At least ten laboratories set up a confirmatory group with crossmatches, using a different technique.
- Several laboratories commented that they use different techniques in emergency situations, on-call, or for antenatal patients.

Automation

- Overall 254/426 (60%) laboratories use automation for routine patient ABO/D typing, but only 76% of these used their automation to ABO/D type 05R8 samples.
- 21% use liquid phase microplates, 78% use CAT and 1% solid phase.
- 71% of those who use CAT for routine ABO grouping are using automation, compared to 60% overall for routine grouping.

Controls

Tables 3 and 4 show the frequency of use of ABO grouping controls by automated and manual methods respectively. Thirteen laboratories using automation did not answer this question.

Table 3 – Controls undertaken by users of automated systems

Control	Number (%) of laboratories (n=241)
Twice daily	117 (49%)
Once daily	68 (28%)
Only when reagent batch changes	18 (7%)
None	43 (18%)
Other [1]	8 (3%)

[1] – weekly (n=2); every batch (n=5); x4 daily (n=1)

Appendix VI

Table 4 – Controls undertaken by users of manual systems

Control	Number (%) of laboratories (n=172)
At least every batch	99 (56%)
Once daily	37 (22%)
Twice daily	2 (1%)
Only when reagent batch changes	6 (3%)
None	21 (12%)
Other [1]	7 (4%)

[1] – weekly (n=4); twice weekly (n=2); monthly (n=1)

ABO Grouping Reagents

Anti-A,B (or anti-A+B)

Overall, 167/426 (39%) stated that they routinely include an anti-A,B reagent (or anti-A+B), but this figure reduces to 62/276 (22%) of those using CAT. However, of the 62 CAT users who stated that they routinely include anti-A,B (or anti-A+B), approximately one third named card/cassette profiles that do not include this reagent.

Reverse Group

- A reverse group is performed on new patients by 422/426 (99%), and on patients with a historical group by 320/401 (80%).
- All four participants that do not perform a reverse group on new patients undertake blood transfusions within their establishment:
    - Three include a specific reagent diluent control for each patient sample.
    - All four are using column agglutination technology; one uses automation, whilst the other three use manual systems.
    - The laboratory using automation includes twice-daily ABO controls.
    - Of the three using manual systems, only one includes ABO controls with each batch; one includes weekly controls and the other apparently uses no batch controls at all (apart from the diluent control).
    - Three use an IAT crossmatch, whilst the fourth uses electronic issue; the latter includes a tube cell group prior to issue of blood.

- Of the 81 who do not perform a reverse group in the presence of a historical record, 74 (91%) use automation and 7 (9%) use manual systems. Thirty-five of the former and none of the latter routinely use electronic issue (although one of the latter did not answer the question relating to electronic issue).

ABD/ABD cards/cassettes

These cards/cassettes do not carry a control well and are intended by the manufacturer to be used to confirm the group of a sample that has already been grouped.

- 131/425 (31%) use ABD/ABD cards or cassettes for some purpose:
    - 69/131 (53%) use them for the main purpose of grouping patients who already have a historical group on record. In addition, two use them only if there are two historical groups on record, one only if the automation (using solid phase) is down, and one for urgent crossmatching.
    - 40 (31%) use them solely for their intended purpose, including the confirmation of the donor packs (n=2), and checking anomalies (n=3).
    - 25 (19%) use them for other purposes (+ or – the above two reasons), including grouping high-risk samples (n=5) and cord samples (n=10).

- Of the 69 that use these cards/cassettes for grouping patients in the presence of an historical group, 42 use DiaMed and 27 BioVue.

Appendix VI

D Typing Reagents

Number of anti-D reagents used

The number of anti-D reagents used for typing new patients, those with a historical group and EQA samples are shown in Table 5.

Table 5 – Number of anti-D reagents used for routine and rapid testing

Number of anti-D reagents	Number (%)		
	First time (new) patient (n=420)	Patient with historical group (n=409)	Rapid (n=335)
Single reagent, once only	97 [1] (23%)	167 [2] (41%)	114 (34%)
Single reagent, twice	11 (3%)	3 (1%)	7 (2%)
Two or more different reagents	312 (74%)	239 (58%)	214 (64%)

[1] - 40 of these use a blended reagent in a single column

[2] - 49 of these use a blended reagent in a single column

Use of two reagents (for patients with no historical group; n=312)

- 274 (88%) use two IgM monoclonal anti-D reagents.
- 23 (7%) use one IgM monoclonal and one IgM/IgG blend.
- 10 (3%) use one IgM monoclonal, but gave no answer for the 2nd reagent.
- 1 (<1%) reference laboratory uses two monoclonal IgG reagents that are produced in house.
- 3 were not sure whether or not their reagents were monoclonal and gave insufficient detail for a judgement to be made.

Use of single anti-D reagent once, for patients with no historical group

Of the 97 respondents using a single anti-D once for patients with no historical group:

- 97 (100%) use an IgM monoclonal reagent.
- 74 (76%) routinely use automation for their ABO/D typing.
- 96 (99%) use CAT (35 BioVue and 62 DiaMed).
- 1 (1%) uses a tube technique.

Anti-CDE Reagents

24 laboratories (6%) stated that they include an anti-CDE reagent in routine testing (including two blood service reference laboratories), ten using BioVue cassettes, nine DiaMed cards and five liquid phase microplates or tube.

D^{VI}

At least nine hospital laboratories select one of their two anti-D reagents that detect D^{VI} (four Totem plus at least a further five D^{VI+} DiaMed cards). Only 5/9 laboratories are within the UK, the remainder being in Eire.

Use of IAT anti-D reagents

- 23/420 (5%) laboratories routinely test apparent D negative pre-transfusion samples with an IAT anti-D reagent; 20 include an appropriate control or DAT.
- One reference laboratory tests r' and r'' samples by IAT but not rr samples.

Monoclonal reagents

There are several combinations of two different reagents in use, with RUM 1 being the most commonly used (n=216), either singly or in combination with a second reagent.

Appendix VI

Weak and Partial D

The question asking what criteria are used for identifying patients that may be weak or partial D, in order that appropriate confirmatory testing can be undertaken, was clearly misunderstood by many, since 158 laboratories did not provide an answer. Of those that did answer:

- 184 cited discrepant results between the two anti-D reagents (or with a historical result) with or without other criteria.
- 202 cited a reaction grade of less than a value:
    - <1+ - 8
    - <2+ - 179
    - <3+ - 11
    - <4+ - 4
- 5 cited a mixed field result plus other criteria.

Once a sample has been identified as a potential weak or partial D:

- 318/422 (75%) seek confirmation from a reference centre (two refer antenatal samples only).
- 51 investigate using a panel of anti-D reagents designed to classify weak and partial D.
    - 12 of these are reference centres.
    - 16 also send to a reference centre for confirmation.
- Overall, 71 respondents neither use a panel of anti-D reagents, nor refer.
    - 28 did not state how they report such results to the clinician.
    - 18 would report as D positive (one with a comment).
    - 13 would report as 'D positive (weak D)' or equivalent.
    - 12 stated that they would report weak D as D positive and partial D as D negative; however, it is not clear how they distinguish between weak and partial D.

Selection of blood for female patient aged 30 years and male patient aged 60 years.
Table 6 shows the number and percentages of participants that would give D positive or D negative blood to male and female patients of known weak or partial D types.

Table 6 – Selection of D positive or negative blood for patients with weak and partial D

Patient type	D type of blood selected for transfusion	
	D positive	D negative
Female aged 30, weak D	235 (81%)	56 (19%)
Female aged 30, partial D	15 (5%)	277 (95%)
Male aged 60, weak D	259 (91%)	27 (9%)
Male aged 60, partial D	77 (27%)	209 (73%)
OR		
Female aged 30, weak/partial D (no distinction made)	70 (38%)	114 (62%)
Male aged 60, weak/partial D (no distinction made)	115 (62%)	70 (38%)

Appendix VI

Reporting to the Clinician

Again this question was widely misunderstood, with 107 giving no analysable answer. Table 7 summarises the responses. Many laboratories qualified their response by indicating that they include a specific comment in their report to the clinician; this number is shown in brackets where appropriate.

Table 7 – Clinical reporting of weak and partial D (numbers in brackets relate to the number who include a comment on the reports to the clinician)

D type as reported to the clinician	D type as defined by testing procedures in-house or by reference laboratory		
	Weak D	Partial D	Weak/Partial D (no distinction made)
D positive	141 [2] (14)	38 [4] (26)	28 (3)
D negative	2	108 (35)	0
D weak [1]	67 [3] (4)	1	21 (3)
D variant [1]	0	30 (20)	1
As reference report	10	26	3

[1] – Or equivalent description
[2] – Four are reference laboratories
[3] – Nine are reference laboratories
[4] – Five responded that it depends on the D category (probably only reported as D negative if D^{VI})

28/141 reporting weak D as D positive, record a different result on the computer or include an additional comment.

Post-natal anti-D prophylaxis

Table 8 shows the number of laboratories issuing routine post-natal anti-D prophylaxis to women of weak and partial D status. Ninety-three laboratories stated that pregnant women are not in their case-mix and eight did not answer. A further three answered with respect to weak but not partial D.

Table 8 – Issue of postnatal anti-D prophylaxis to women with weak and partial D

D type	Give postnatal anti-D prophylaxis?		
	Yes	No	As advised by ref lab
Weak D (n=279)	5 (2%)	194 (69%)	80 (29%)
Partial D (n=276)	92 (33%)	26 (9%)	158 (57%)
No distinction made (n=46)	8 (17%)	23 (50%)	15 (33%)

D Typing of Cord Samples

- 265/426 (62%) laboratories D type cord samples for the purpose of anti-D administration.
    - 193 (73%) state that they use different or additional typing reagents.
- 244/426 (57%) laboratories D type cord samples for the purpose of transfusion.

Table 9 shows the number of laboratories who employ additional reagents for D typing cord samples.

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Table 9 – Additional reagents used for D typing of cord samples

Reagents	Number [1]
a) D^{VI+} 'Newborn/cord' grouping cards/cassettes	46
b) Other monoclonal IgM anti-D to detect D^{VI}	19
c) D^{VI-} 'Newborn/cord' grouping cards/cassettes	122
d) IgG reagent by antiglobulin technique	20
e) Other	7 [2]

[1] – Where more than one reagent is used, each category has been incremented, except for a) and b) where only one D^{VI+} reagent has been logged.

[2] – Includes IgM monoclonal not detecting D^{VI} (n=2); 'adult' DiaMed card that detects D^{VI} (n=1), anti-CDE (n=1); unspecified anti-D by tube (n=1); refer to NBS (n=1)

If a cord sample is found to be DVI positive, 33/66 (50%) stated that they also test the maternal sample for D^{VI}; this data excludes 14 laboratories who did not answer the question or who answered 'Not Applicable'.

Routine Crossmatching (no atypical red cell antibodies)

The IAT (± immediate spin) is still the most commonly used crossmatching procedure, used by 260 (63%) respondents for all crossmatching. Where patient samples meet the appropriate requirements, 108 (26%) use electronic issue, and 44 (11%) 'Immediate Spin' only.

Discussion

Testing protocols and techniques

Liquid-phase microplate technology is no longer the most commonly used method for routine ABO/D typing, with the use of Column Agglutination Technology (CAT) doubling since 2002, and now representing the routine technique used by two-thirds of laboratories. This is mainly due to the increasing use of automation, although 14% of all respondents are using CAT in a manual system.

Where manual systems are used, current BCSH guidelines[1] recommend duplicate D typing, a reverse group with a negative control, and a reagent diluent control (where recommended by the manufacturer). Where there is full automation, with drastically reduced associated risk of procedural error, it is acceptable to rely on a single anti-D test. Similarly there is scope to abbreviate the ABO group by omitting reverse grouping in the presence of a matching historical record, full automation and a risk assessment. The risk assessment should include the possibility that the first sample tested may have been taken from the wrong patient. An international study calculated that 'wrong blood in tube' occurred at a median rate of 1 in 2000 samples[2]. The 2004 SHOT report[3] included a case of ABO incompatible transfusion, where the BMS omitted grouping the current sample, relying on a historical record, which turned out to be incorrect. It is interesting to note that two laboratories will only omit the reverse group in the presence of two matching historical groups, significantly reducing the aforementioned risk. Some laboratories have a different protocol for grouping if a crossmatch is also required (as opposed to a group and save), with at least ten respondents reporting that they set up a confirmatory group (using a different technique) with the crossmatch.

Unfortunately many of those laboratories that are abbreviating their ABO/D typing procedures are using manual systems, which are prone to procedural error, cross-contamination of reagents and transcription error or transposition of samples and results, whatever the technology used. For example, 24% of those not performing duplicate D typing are using manual CAT systems, and 9% laboratories omitting the reverse group (on patients with a historical group) are using manual systems.

More worryingly, four hospital laboratories do not perform a reverse group even on patients without a historical record (and three of these are using manual systems). This is potentially dangerous, even with automated systems, since grouping anomalies can occur and could be missed without a reverse group and

procedural errors in manual systems could certainly be overlooked, with serious consequences. There are several reports to SHOT every year of laboratory errors involving ABO and D typing. All of the former have been made in manual systems (where data has been available).

ABD/ABD cards and cassettes are intended for use as confirmatory tests on samples that have already been grouped (patient and donor). However, their use for other purposes is increasing, with 16% of laboratories now using them to group patients for whom a historical record is available. This is outwith guidelines and it should also be remembered that the BioVue cassettes contain 3% PEG in the anti-D column, requiring a control reagent. Previous UK NEQAS exercises have demonstrated that these reagents can cause false positive D typing in the presence of IgG coated D negative red cells (04R2, 05R8).

An IAT anti-D reagent is being used by 5% of participants for routine confirmation of apparently D negative pre-transfusion samples, with all but three using an appropriate control. This has increased from 3% in 2002. This practice is recommended *against* in the UK guidelines[1,4], due to the chance of a false positive result in the presence of a positive DAT; we therefore recommend that UK hospital laboratories update their practice to comply with UK guidelines. Current monoclonal IgM reagents negate any need to undertake further testing for weak D in routine patient testing, since all but the weakest examples will be detected by direct agglutination.

Specific reagent selection

6% of participants are using anti-CDE reagents, a reduction since 2002. Two UK NEQAS surveys have demonstrated the potential for D typing errors due to mis-interpretation of positive results with this reagent (99R2 and 02R2) and the BCSH guidelines[1,4] recommend that it is not used in routine typing of patient samples.

At least nine hospital laboratories include one anti-D reagent that detects D^{VI} for routine patient typing. The guidelines[1] clearly recommend that routine patient typing reagents should not detect D^{VI} because of the potential for sensitisation following a transfusion of a D^{VI} individual with D positive blood. This would be of particular concern for young females of child bearing potential.

Weak and partial D

Conventional theory has been that individuals with a partial D type can make anti-D and those with a weak D type cannot. The BCSH guidelines for compatibility procedures and those for grouping and screening in pregnancy make it clear that D typing reagents for patient samples should not detect D^{VI}, since this is the category with the fewest D epitopes and is therefore the most likely to make anti-D [1,4]. These guidelines also state that patients *known* to be partial D, should be treated as D negative. It is of concern that 15 laboratories indicated that they would transfuse D positive blood to a 30 year old woman with known partial D status, and a further 70 would transfuse D positive blood where there is no distinction made between weak and partial D. Similarly, 26 laboratories indicated that they would not give postnatal anti-D prophylaxis to a woman with known partial D status, and a further 23 would not give anti-D where no distinction is made. It is recognised that current IgM monoclonal anti-D reagents will detect many partial D types (other than D^{VI}), and patients may only be recognised as having partial D after they have made anti-D. However, many partial Ds are also weak, making it impossible to distinguish the two serologically without an appropriate panel of anti-D reagents; for most hospital laboratories this will require referral to a reference laboratory, from which guidance should be sought about how these patients should be managed with respect to anti-D prophylaxis and transfusion.

There is now evidence that some individuals who are defined as weak D can also make anti-D [2]; this has even occurred in the most common weak D types (J Poole, personal communication), further blurring the distinction between weak and partial D. When considering the implications for blood transfusion and anti-D prophylaxis, there are several considerations to be made: Can we identify which weak D types may become immunised? What are the chances that the individual will become sensitised and what are the relative risks of sensitisation compared with other antigens, e.g. K or c? Will the antibody be as clinically significant as anti-D

made by a D negative individual? What is the cost-effectiveness of undertaking serological and molecular investigations, and what impact would transfusing D negative, rather than D positive red cells have on supply and demand? Should we distinguish between females of childbearing potential and other patient groups? Should we err on the side of caution and give anti-D prophylaxis to all recognised weak and partial D women? Although there is evidence that anti-D made by partial D types is capable of causing severe HDN, there are currently no easy answers to these other questions.

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Appendix VII

Report of Additional Technique Questionnaire Distributed with 05E9

Introduction

Last year we proposed some changes to exercise design, in collaboration with Steering Committee members. These proposals were discussed at several regional meetings (e.g. TADG meetings) around the UK and input sought from attendees, via questionnaires. One proposal was to send separate samples for assessment of two different techniques (or procedures), e.g. routine and urgent, or automated and manual. This received overwhelming support from those canvassed and 05E9 was used to pilot the idea. Two pairs of samples were distributed, one for testing by the routine technique and the other for testing by a second technique (if available). A short questionnaire was supplied on which additional information relating to the second technique was logged.

The return rate for the questionnaire was 98% (442/451).

Results of Questionnaire Analysis

Q1 – Do you have more than one technique/procedure for antibody screening and/or identification?

- 229 (52%) have a second technique for antibody screening
- 126 (29%) have a second technique for antibody identification

The following data applies only to those who answered YES to either category above. One respondent did not answer any of the subsequent questions; the totals are therefore 228 and 125 for screening and identification respectively.

Q2 – How would you describe your 'Method 2' as used in this exercise?

Description of 'Method 2'	Number (%) [1]	
	Screen (n=228)	ID (n=125)
Back-up to routine automated method	191 (84%)	69 (55%)
Back-up to routine manual method	14 (6%)	34 (27%)
On-call	89 (39%)	37 (16%)
Urgent	130 (57%)	42 (18%)
Manual checking of anomalies	126 (55%)	55 (24%)
Other [2]	9 (4%)	14 (6%)

[1] - The majority of respondents ticked more than one category
[2] - Mostly for testing small samples or to set-up with a crossmatch

Q3 – Is your 'Method 2' manual or automated?

- 217/228 (95%) used manual techniques for screening and 112/122 (92%) for identification (three did not provide an answer).

Appendix VII

Q4 – Indicate which technique or techniques were used for 'Method 2' IAT screen.

- 207/228 (91%) used column agglutination technology (CAT) for their 2nd method (includes 5 laboratories who also used tubes and one who also used Capture R)
- 23/228 (10%) used a tube technique (includes 5 laboratories who also used CAT)
- 5/228 (2%) used Capture R as their 2nd technique.
- 21/190 (11%) who primarily use CAT, used a different IAT technique for their 2nd method
    - 6 used a different CAT
    - 12 used a tube technique
    - 3 used Capture R.
- 17/17 (100%) who primarily use solid phase used a different IAT technique for their 2nd method.

Figures given for the primary technique exclude 26 cases where multiple methods were stated.

Conclusions

The data shows that the majority of participating laboratories utilise a second IAT technique, requiring external quality assessment. This project will be progressed following completion of the IT development, currently being undertaken by this Scheme.

Appendix VIII

05R10 UI Trial – Rules

a. the following <u>will</u> incur penalties

- Misinterpretations contributed to by false negative or false positive reactions
- If a specificity (actually present) is not entered in the 'can positively identify' section 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.
- If a specificity not actually present is entered in the 'can positively identify' section.
- If a specificity is entered in the 'cannot exclude' box, but we feel that it can be excluded with an appropriate antigen positive cell or method. E.g. Non-exclusion of an Rh antibody in the presence of a negative enzyme result.
- If a specificity is entered in the 'cannot exclude' box, but the patient phenotype provided, shows that the patient is positive for the corresponding antigen.

b. the following <u>will not</u> incur penalties

- Being unable to exclude a specificity in line with BCSH guidelines. E.g. 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 in the 'not excluded' box, rather than in the 'can positively identify' box. However, this would only apply if our in-house testing also found non-reactivity with heterozygous cells by the same; otherwise, this would be classed as a false negative result.

Appendix IX

05R10 UI Trial – Results

Material: 'Patient' 2 – anti-c (titre 8)

- 14 returns of UI (or anti-c+UI), plus two from laboratories not registered for antibody identification.
  - 13 were supported by their panel returns
    - 2 were unable to exclude anti-Fy^a
    - 11 were unable to exclude anti-E or antibodies to low frequency antigens/low clinical significance antigens
  - One recorded anti-Jk^b as 'not excluded'; however, this interpretation was based on a false positive reaction with a Jk(a-b+) cell (R_1R_1), and the interpretation was not upheld.

Outcome:

It was agreed by the Steering Committee that this should be introduced as a routine from April 2006, with an annual review.

Appendix X

06R5 Emergency Exercise - Summary of instructions and questions

SCENARIO

You're on your own at midnight (00.00). Three family members arrive in A&E at the same time following an RTA. The request forms give patient and request details.

- Clark (D.O.B 1/1/68) has internal injuries and requires 2 units of blood for theatre in 10 - 15 minutes (00.10 – 00.15) of sample receipt, and may need more later.
- Jenny (D.O.B 25/2/66) has leg fractures and requires 2 units for theatre in 1 hour from receipt of sample (01.00).
- Dalila (D.O.B 29/4/92) requires a group and save but may need blood for theatre in the morning

INSTRUCTIONS

- Book specimens into computer (or treat as usual).
- Prepare, label and issue 2 units for Clark within 10 - 15 minutes and 2 units for Jenny within 1 hour, ***using your own blood supplies***.
- Document results in the same way as you would normally document emergency testing.
- Complete the additional and routine EQA forms later.

QUESTIONS ON ADDITIONAL RESULT SHEETS

P1 – Clark

1. Initial group:

a. Was an initial group performed?
b. If yes, enter reactions for each reagent used
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. Other

3 .Confirmatory group

a. Enter reactions for each reagent used in the confirmatory group
b. 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. How were the units labelled (tick the closest match or specify other)

i. Compatible with
ii. Suitable for
iii. Uncrossmatched
iv. Other

Appendix X

6. *What other tests did you start within the 10-15 minutes*

i. Further ABO/D group – specify manual or automated
ii. IAT crossmatch - specify manual or automated
iii. Antibody screen - specify manual or automated
iv. Other

7. What further serological tests did you undertake (started after issue of blood) before the start of the next day?

i. Repeat group - specify manual or automated
ii. Antibody screen - specify manual or automated
iii. IAT crossmatch on units already issued - specify manual or automated
iv. Antibody panel - specify manual or automated
v. Other

P2 – Jenny

1. Initial group:

a. Was an initial (rapid) group performed?
b. If yes, enter reactions for each reagent used
c. Enter blood group interpretation
d. Which technology was used?

2. Further tests completed within an hour

i. Group (repeat or first) - specify manual or automated
ii. Antibody screen - specify manual or automated
iii. IAT crossmatch - specify manual or automated
iv. Immediate spin - specify manual or automated
v. Other

3 . Group details

a. If you entered 'Yes' to 2.i, enter reactions for each reagent used
b. Did you resample the primary sample or use an aliquot already made?

4. Did you start any further tests (not already mentioned) within the hour? If so specify

5. What ABO/D group units did you elect for transfusion with the hour available and where they selected for any further phenotypes?

6. ***How were the units labelled (tick the closest match or specify other)***

i. Compatible with
ii. Suitable for
iii. Uncrossmatched
iv. Other

7. What further serological tests did you undertake (started after issue of blood) before the start of the next day?

i. Repeat group - specify manual or automated
ii. Antibody screen - specify manual or automated
iii. IAT crossmatch on units already issued - specify manual or automated
iv. Antibody panel - specify manual or automated
v. Other

Appendix X

P3 – Dalila

1. Group and save sample

a. Was any work performed on this patient before 9 am next day?
b. If yes, what was done
    i. Sample ID checked against form
    ii. Booked in
    iii. ABO/D group
    iv. Antibody screen

c. If a group was performed, enter the reactions obtained for the reagents used.

General questions

1. Did you test for any of the following (either 1st time or repeat of on-call testing for a) Clark, and b) Jenny:

i. Blood group
ii. Antibody screen
iii. IAT crossmatch
iv. Antibody panel

2. Dealing with the emergency scenario

a. What was your sequence of testing for the three samples?
    i. Issued blood for P1 (Clark) before starting testing on P2 (Jenny) and/or P3 (Dalila)
    ii. Began testing P1 and loaded P2 and/or P3 for automated G+S within 10-15 mins
    iii. Began manual testing P1 and P2 within 10-15 mins
    iv. None of these

b. Would it be your laboratory's policy to call in another member of staff if this scenario occurred 'out of hours'?

Appendix XI

EMERGENCY ISSUE EXERCISE (06R5)
QUESTIONNAIRE ANALYSIS

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. Participants returned 414 questionnaires in time for analysis. Three sets of data were withdrawn from analysis for Clark (Patient 1) as follows: one reference laboratory undertook the work for both patients 1 and 2 within one hour; one participant did not satisfactorily complete section 1; one participant did not return page 1 of the questionnaire. One set of data was withdrawn for Jenny and a different set for Dalila, due to missing data sheets. The total number of questionnaires analysed for Clark (Patient 1) is therefore 411, and for Jenny (Patient 2) and Dalila (Patient 3) is 413. Throughout the questionnaire not all respondents answered all the questions, therefore, the total number in the tables do not always equal the number of questionnaires analysed. A summary of the questions is attached as Appendix 1.

ISSUE OF BLOOD FOR CLARK (O D POSITIVE) WITHIN 10-15 MINUTES (n=411)

'Rapid' group procedures and techniques

- 395 (96%) participants performed a 'rapid' group, whilst 16 (4%) did not.
- For 182 (46%) of laboratories the 'rapid' group comprised a cell group only and for 213 (54%) both a cell and reverse group. Table 1 shows the use of controls and Table 2 the technology used for 'rapid' grouping.
- 182 (46%) included a test with an anti-A,B (or A+B) reagent.
- 239 (60%) undertook D typing in duplicate.

Table 1: Use of controls for 'rapid' grouping

Criteria	Negative control included [1]
Cell group only (n=182)	54 (29.7%)
Cell and reverse group (n=213)	109 (51.2%)
Overall (n=395)	**163 (41.3%)**

[1] Diluent control or auto

Table 2: Technology used for 'rapid' grouping (n=395)

Technology used	Number of laboratories [1]
Card/cassette	57 (14%)
Microplate	27 (7%)
Tube	278 (68%)
Tile/slide	46 (11%)
Gallileo	1 (<1%)

[1] 14 laboratories used two techniques and these have been counted in both categories

Appendix XI

Rapid group results

One participant recorded an incorrect result of A D positive, due to sample transposition with sample 3, whilst the remaining 394 recorded the correct result of O D positive.

Further tests completed prior to labelling blood for collection

- Of the 16 (4%) laboratories that did not perform a blood group, one checked the ABO groups of the donations prior to issue.

- Of the laboratories that performed a rapid group, 232/395 (58.7%) completed a 2nd group prior to issue. Table 3 shows details of how this second group was performed and Table 4 combinations of rapid and confirmatory groups recorded at 10 minutes.

- 176 (44.6%) performed an immediate spin crossmatch.

- 45 (11.4%) completed other tests; including:
    - group check of the donations (n=30), including 4 where this is routinely performed
    - computer group check (n=2)
    - reduced incubation antibody screen (n=7)
    - Rh and/or K typing (n=4)
    - DAT (n=2)
    - IAT crossmatch (n=2)
    - first stage of manual polybrene crossmatch (n=1).

- 63 (15.9%) did not perform a 2nd group or an immediate spin crossmatch; of these:
    - 25 did not include a reverse group; however, one of these performed a reduced incubation IAT crossmatch
    - three checked the donor groups
    - one did the first stage of a manual polybrene crossmatch.

Table 3: Details of second group (n=232)

Criteria	Number of laboratories
Cell group only	81 (35.1%)
Full group	149 (64.2%)
Reverse group only	2 (0.9%)
Same aliquot as initial group [1]	26 (11.5%)
New aliquot removed from sample [1]	201 (88.6%)

[1] five gave no answer

Table 4: Details of combination of rapid and confirmatory groups (n=395)

Rapid	'Confirmatory' group within 10-15 minutes			
	Cell only	**Full**	**Reverse only**	**None**
Cell only	31 (7.8%)	93 (23.5%)	2 (0.5%)	56 (14.2%)
Full group	50 (12.7%)	56 (14.2%)	0 (0%)	107 (27.1%)

Appendix XI

Selection of donor units (data summarised in Table 5)

- The 16 laboratories that did not perform an ABO/D group within 10 minutes all selected the O RhD negative units for issue at 10-15 minutes.
- Of the 395 that did complete a group:
    - 355 (89.9%) selected O D positive units (2 stated that they also selected O D negative units)
    - 39 (9.9%) selected O D negative units
    - 1 selected A D positive units (based on an incorrect group result of A D positive).

- In total 55 (13.4%) selected O D negative units
    - 31 (56.4%) used blood designated as 'flying squad' or equivalent
    - 40 (72.7%)stated that it was K negative
    - 18 (32.7%) stated that it was rr (CDE negative).

- Of those not selecting the group O D negative units:
    - 46 did not perform a 2nd group or an immediate spin crossmatch
    - at least 18 performed a single cell group, without a reverse group, control, or immediate spin XM, including the participant who selected the A D positive units.

Table 5 – Summary of donor units selected vs. level of grouping undertaken on Clark

Tests undertaken	Donor units selected		
	O D negative	O D positive	A D positive
No group	16	0	0
Rapid group only	25	137	1
Rapid + confirmatory group	14	218	0
Total	55 (13.4%)	355 (86.4%)	1 (0.2%)

Labelling of donor units

Table 6 shows the labelling used on the donor units, compared with the group of the units selected and whether or not a blood group was performed on the patient. The questionnaire requested that the closest match be ticked.

Table 6 – Labelling of donor units vs. group of units selected

Units labelled (or similar phrase)	Group of units selected		
	O D negative (no group done)	O D negative (group done)	O D positive (group done)
'Compatible with'	1	2	21
'Suitable or selected for'	2	4	64
'Uncrossmatched for'	9	23	207
Other [1]	4	10	63

[1] Including 'Emergency blood', 'Flying Squad', 'Group Compatible', and no label

Appendix XI

Further testing started but not completed before issue of blood

361 (87.8%) started further testing within the 10-15 minutes, with details of manual versus automated testing shown in table 7. Further testing included:

- Blood group - 201 (55.7%); however 50% of these had previously stated that they had completed two groups prior to issue.
- Antibody screen – 349 (96.7%)
- IAT crossmatch – 274 (75.9%)
- Other testing - 14 (3.8%); this included room temperature crossmatching, red cell phenotyping and antibody identification

Table 7 – Further testing started but not completed prior to issue of blood

Further testing started within 15 minutes	Manual testing	Automated testing
Blood group (n=201)	114 (56.7%)	87 (43.3%)
Antibody screen (n=349)	256 (73.3%)	93 (26.6%)
IAT crossmatch (n=274)	248 (90.5%)	26 (9.5%)

Retrospective testing – started after units issued but before next 'working day'

The data for grouping and antibody screening was not suitable for analysis, since many respondents said that they started these tests both before and after issue of blood. However a least 10 participants did start an automated group, and 30 an automated antibody screen *after* blood was issued, having started manual tests *prior* to issue. At least 86 laboratories undertook retrospective IAT crossmatching on the issued units. 356 (86.6%) undertook antibody identification (88% manually). Nearly 30% of laboratories undertook additional testing before the next day, the majority of which comprised patient and/or donor phenotyping and a DAT.

Overall, 411 (100%) of laboratories completed a blood group and 407 (99.0%) an antibody screen (or ID panel) before the next working day.

ISSUE OF BLOOD FOR JENNY (A D NEGATIVE) WITHIN ONE HOUR (n=413)

ABO/D group

- 347 (84.0%) laboratories stated that they performed an initial or rapid ABO/D group, using similar technology to that used for Clark.
- 100% completed either a rapid and/or a full group within the hour, with 126 (30.5%) using automation.
- 15 (3.6%) did not complete a reverse group within the hour, but one of these did perform an immediate spin crossmatch.
- One laboratory mistakenly used anti-A,B instead of anti-D, and consequently recorded an incorrect result of A D positive. The confirmatory group revealed the error, and the units were withdrawn prior to issue.
- 410 laboratories (99.3%), completed an antibody screen within the hour; the three that did not, all completed an IAT crossmatch
    - 128 (31.2%) used automation for antibody screening

- 377 (91.3%) completed an IAT crossmatch
    - 38 (10.1%) used automation

- 54 (13.1%) undertook an immediate spin crossmatch

- 52 (12.6%) stated that they completed an antibody identification panel and 11 (2.7%) some red cell phenotyping.

Appendix XI

Further testing started but not completed within the hour

- 258 (62.5%) started further testing, the majority of which was antibody identification (87%)

- In total, 283 laboratories (68.5%) started or completed antibody identification before the next working day.

Units selected for transfusion

- 39 (9.4%) selected O D negative blood; all of these completed blood grouping, antibody screening and IAT crossmatching; three used automation.
- One selected A D positive blood (misgrouped the patient as D positive)
- The remaining laboratories (90.3%) selected group specific (A D negative) blood

Table 8 shows the labelling used on the donor units, compared with the group of the units selected

Table 8 – Labelling of donor units vs. group of units selected

Units labelled (or similar phrase)	Blood group of units selected	
	O D negative	A D negative
'Compatible with'	33	273
'Suitable or selected for'	4	44
'Uncrossmatched for'	1	15
Other [1]	1	29

1 – Many of these included a statement that the units were compatible, but that the patient had an unidentified antibody.

Further testing started after blood was issued but before next working day

As for Clark, the data for grouping and antibody screening for Jenny was not suitable for analysis, since many respondents said that they started or completed these tests both before and after issue of blood.

Twelve laboratories undertook retrospective IAT crossmatching. At least 58 undertook antibody identification, and a further 18 stated that they would have referred elsewhere for antibody identification. Thirty-six performed some red cell phenotyping.

WORK UNDERTAKEN ON DALILA (A D POSITIVE) BEFORE NEXT DAY (n=413)

- 370 (89.6%) performed some work on this sample before 9a.m. next day:
    - 16 (4.3%) of these simply checked the sample ID against the request form
    - 22 (6.0%) also 'booked in' the request
    - 319 (86.4%) performed a group and screen
    - 12 (3.3%) performed a group, but no screen
    - 1 (<1%) performed a screen but no group
    - There was an approximate 50/50 split between manual and automated testing

- Of the 331 performing a blood group, 18 (5.4%) did not include a reverse group.
- Two laboratories (0.6%) incorrectly recorded blood grouping results of O D positive. One was due to sample transposition with the sample from Clark, whilst the cause of the other error is unknown.

Appendix XI

GENERAL QUESTIONS

Table 9 shows how many laboratories undertook routine testing the next day. Since virtually all laboratories completed at least a group and screen during the emergency period, this is repeat testing. However, analysis of the blood grouping data for Jenny shows that a lower proportion of laboratories repeated the testing where automation had been used during the emergency period: approximately 12% compared with 33% where prior work was undertaken manually.

Table 9 - Routine testing next day

Test undertaken	Clark (n=411)	Jenny (n=413)
Blood Group	117 (28.5%)	110 (26.6%)
Antibody Screen	88 (21.4%)	77 (18.6%)
IAT Crossmatch	29 (7.1%)	21 (5.1%)
Antibody Panel	65 (15.8%)	54 (13.1%)

Dealing with the emergency scenario (n=407)

Three choices were given, for the order in which the patient samples were tested (see Appendix 1). Several laboratories ticked multiple answers, some of which have been rationalised to give the following overall picture:

- 51% issued blood for Clark before starting any testing on Jenny.
- 14% began testing Clark and loaded the sample for Jenny (and/or Dalila) for automated group and screen within the 10-15 minutes.
- A further 3% started loading the samples for automated testing within the 10-15 minutes, but issued blood for Clark first.
- 33% began manual testing of both Clark and Jenny within the 10-15 minutes.
- A further 10% stated that they began manual testing on Jenny within the 10-15 minutes but issued blood for Clark first.
- 2% said that none of the options reflected their practice.

Out of hours policy

48/405 (11.9%) laboratories stated that it would be their laboratory's policy to call in another member of staff if this scenario did occur out of hours. Nine laboratories did not answer this question.

DISCUSSION

Issue of group specific blood within 10 – 15 minutes

A small minority (4%) issued O D positive blood having performed a single cell group only, with no control and no immediate spin crossmatch. In this exercise the patient (Clark) was group O, and there was therefore little or no risk involved, however this would be of concern if the same policy were applied to patients of other blood groups. Indeed of major concern was the participant who transposed samples 1 and 3, incorrectly grouping Clark as A D positive, and issuing group specific blood, on the basis of a single rapid group (no reverse group or control) and no immediate spin crossmatch. The BCSH guidelines [1] state that group specific blood may be issued following a rapid group, plus either a confirmatory group or an immediate spin crossmatch, undertaken using a new aliquot.

Appendix XI

Repeat ABO/D grouping

A 2nd group was performed within the 10 minutes by 59% of respondents. However, 26 of these (12%) performed the second test on the same aliquot of cells as the first group; had the incorrect specimen been selected for the first group, the error would have be perpetuated. This compares with 18% using the same aliquot in the last emergency exercise (03R9). BCSH guidelines recommend returning to the original specimen when repeating tests [1].

Issue of O D negative blood

The NBS Clinical Policies Group recommend 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, the O D negative units were selected by 55 (13%) laboratories; this compares with 15% in 03R9. Of these 55, 14 (25%) had performed a rapid and confirmatory group and a further six (11%) 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]. However, SHOT data has demonstrated that more laboratory errors occur out of hours and with manual testing [3], probably accounting for some of the caution noted in this exercise. To complicate matters further, in this exercise the patient had anti-c, demonstrating that O D negative blood is not necessarily the safest option. Even with a hour available for testing, 9% of laboratories stated that they selected group O (D negative) rather then A red cells for Jenny, despite completing grouping, screening and crossmatching, even in some cases (8%), where automated (presumably routine) techniques were employed.
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 vast majority of laboratories undertook some work on the group and save sample (Patient 3 – Dalila) before 'the next working day', with 86% performing a full group and screen; approximately 50% utilised automation. Of great concern were the two laboratories that incorrectly grouped this sample. One transposed the sample with that for Clark, but presumably did not check the details again after the samples had been allocated laboratory accession numbers. Although the samples were inadequately labelled for this exercise (i.e. patient 1, 2 etc. rather than full patient details), this is still a serious omission. The cause of the other grouping error is unclear, but is likely to have occurred at the reading or recording stage of the manual CAT test. This supports the SHOT data by demonstrating the potential for error in manual testing, particularly in non-routine situations, and adds weight to the argument for retesting samples that have been tested manually out of hours. However, in this exercise, the minority of laboratories stated that they undertook repeat testing routinely next day. The 10% of laboratories who did nothing with this sample until the next day, took the risk of delaying transfusion had the patient's status changed requiring urgent transfusion.

Use of automation

Although not helpful for issuing blood in an extreme emergency (e.g. within 10 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 30% of laboratories utilised automation to provide blood for Jenny within the hour, and 50% to undertake the group and screen on Dalila.

Appendix XI

REFERENCES

1. BCSH (2004) Guidelines for compatibility procedures in blood transfusion laboratories. *Transfusion Medicine,* 2004, **14,** 59-73 and www.bcshguidelines.co.uk
2. NBS Clinical Policies: www.blood.co.uk/hospitals/guidelines/index.htm
3. SHOT reports, 2004 and 2005. www.shotuk.og

Appendix XII

Supplementary Report for Exercise 06R9 - Distributed 16 October 2006

Introduction

The sample provided for ABO/D grouping for Patient 1 was prepared to simulate a dual population of group O D positive and group A D positive cells. For the purposes of this EQA exercise, the expected result for ABO was 'UI' (unable to interpret), since no clinical or transfusion history was provided to elucidate the cause of the mixed field (MF) reaction. This analysis includes data from 457 participating laboratories, including those returning results after the closing date, and excluding two not recording a reaction grade for Patient 1 cells vs. anti-A.

Material

'Patient' 1 A/O D positive MF – 50:50 A/O, prepared with group A D positive red cells, group O D positive red cells and group A plasma.

Results

Detection of the MF reaction

Overall 186/457 (40.7%) detected the mixed field (MF) reaction between 'Patient' 1 cells and anti-A. Table 1 shows the detection of the MF reaction by technology. Table 2 shows the use of automation by technology (excluding those using both manual and automated techniques, and those using multiple methods), and Table 3 the number (%) of each of these groups detecting the MF reaction.

Table 1 - Number detecting MF reaction by technology (% of those using each technology)

Technology	Number*	Recording MF (%)
BioVue	82	46 (56%)
DiaMed	191	93 (49%)
Tube	88	20 (23%)
Microplate	46	7 (15%)
Other	11	2 (18%)
Multiple methods	35	16 (46%)
Total	**453**	**184 (41%)**

* Four did not state technology used, and two of these recorded MF.

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

Technology	Number	Manual (%)	Automated (%)
DiaMed	172	79 (46%)	93 (54%)
BioVue	79	28 (35%)	51 (65%)
LPMP	41	16 (39%)	25 (61%)

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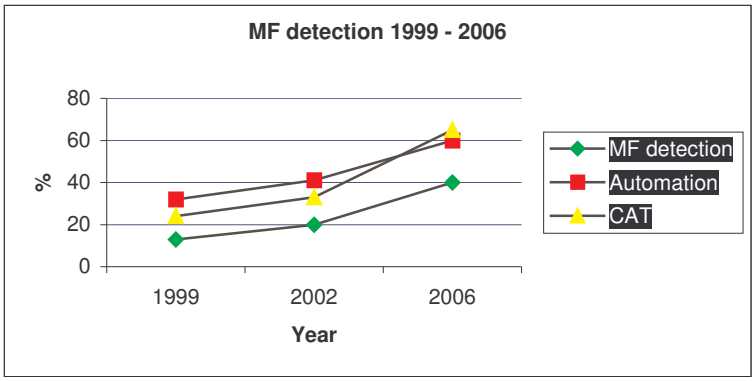
Table 3 – Number (%) detecting MF by technology and manual/automated testing

Technology	Manual detecting MF(%)	Automated detecting MF(%)
DiaMed	21 (27%)	66 (71%)
BioVue	8 (29%)	35 (69%)
LPMP	3 (19%)	3 (11%)

Trends in detection of a 50:50 MF reaction

Figure 1 shows the increasing detection rate of a 50:50 mixed field reaction (1999 and 2002 examples were MF D positive / D negative), use of automation, and of column agglutination technology.

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



Interpretation of ABO grouping results – Patient 1

Table 4 shows the reaction grades reported and ABO grouping results reported for 'Patient' 1.

Table 4 - Reaction grades and interpretations for 'Patient' 1

Reaction grade 'Patient' 1	Interpretation		
	UI	A	Total (%)
MF	75	111	**186 (41%)**
Strong positive	0	256	**256 (56%)**
Weak positive	0	9	**9 (2%)**
Negative	1	5	**6 (1%)**
Total (%)	**76 (17%)**	**381 (84%)**	**457 (100%)**

Direct Antiglobulin Test (DAT)

Table 5 shows the number performing a DAT on 'Patients' 1 and 2. 20/186 (11%) of those recording the MF reaction performed a DAT on 'Patient'1 compared to 19/271 (7%) not recording the MF.

Table 5 – DAT performed on 'Patients' 1 and 2

Sample	Performed DAT
'Patient' 1 (Haemolysed)	39
'Patient' 2 (Not haemolysed)	36

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Issue of group A blood for 'Patient' 1

Table 6 shows the results of the crossmatch between 'Patient' 1 vs. 'Donor' W (group A), and whether the unit would be issued for transfusion vs. the blood group recorded for 'Patient' 1.

Table 6 – Crossmatch /issue 'Donor' W vs. blood group recorded for 'Patient' 1

Crossmatch Interpretation 'Patient' 1 vs. 'Donor ' W	'Patient' 1 reported as group A		'Patient' 1 reported as UI	
	Number	Number issuing 'Donor' W (%)	Number	Number issuing 'Donor' W (%)
Compatible	105	99 (94%)	46	31 (67%)
Deselected	4	0 (0%)	25	0 (0%)
Incompatible	1	0 (0%)	2	0 (0%)

Discussion

The overall detection rate of a 50:50 MF reaction has improved in successive exercises, from 13% in exercise 99R2, 20% in 02R2 to the current rate of 41% in 06R9. It can be seen from the figures in Tables 1 and 3 that laboratories using CAT and automation are significantly more likely to detect the MF reaction ($p = <0.001$ for each factor independently). Increasing use of automation and CAT appears to account for the improvement, but it is worth noting, that in 1999 and 2002 the MF reactions were due to the presence of D positive and D negative cells rather than an 'ABO MF', so performance is not directly comparable. The overall detection rate by technology seen in Table 1 is skewed by the use of automation, and when data from those using manual and automated systems is analysed separately, the detection rate for the two column agglutination technologies is similar (as shown in Table 3).

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 20% of laboratories (05R8 ABO/D grouping questionnaire data). In a clinical situation it might be expected that more laboratories would perform a DAT, especially if there is also significant haemolysis. Not unexpectedly in this EQA exercise (where the cause of the haemolysis was given at the outset), there was no significant difference in the recording of a DAT between those recognising and those not recognising the MF reaction, or between the number performing a DAT on 'Patient' 1 and on 'Patient' 2.

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It is interesting to note that the majority (60%) of those detecting the MF reaction reported 'Patient' 1 as blood group A. This may have been due to the lack of any trace of anti-A in the reverse group combined with the negative DAT (it is possible that in some cases a DAT may have been performed but not recorded, since DAT is a non-scoring optional part of the EQA exercise) being indicative of a group A transfused with O 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, 130/186 (70%) of those detecting the MF reaction stated that they would issue 'Donor' W (group A) for 'Patient' 1, including 31/46 (67%) of those recording the group as UI.

Appendix

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

Appendix XII

Appendix 1 to Supplementary Report for Exercise 06R9

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. A to O [1])	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. O to A)	Donor group (plasma depleted until recipient red cells are no longer detectable).
BMT (Combined mismatch e.g. A to B)	Group O until ABO antibodies to the donor ABO antigen(s) are not detectable by IAT, and DAT is negative. Then donor group.

[1] – i.e.group A donor to group O recipient

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.

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

Appendix XIII

Summary of Data for UI submissions April 2006 to March 2007

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 VII

Table 1 – details by exercise

Exercise Code	Antibodies	No. UI returns	No. agreed	No. disagreed	No. appeals
06E4	E+s	5	2	3	1 [1]
06R5	C	12	11	1	0
06E6	c+K	24	15	9	0
06R7	S	15	12	3	3
06E8	E+Fy[a]	1	0	1	0
06R9	E	1	0	1	0
06E10	C+D	1	1	0	0
07E1	D+s	12	12	0	0
07R2	K+Fy[a]	3	3	0	0
07E3	c+K	42	36	6	2 [1]
Total		**116**	**92**	**24**	**6**

[1] – Appeals upheld (one following arbitration by Joyce Poole, IBGRL)

Table 2 – Reasons for disagreeing with the UI submissions

Category	No. submissions
Could have identified the antibody with the enzyme panel results submitted	7
Could have identified the antibody with the IAT panel results submitted	6
False positive or false negative reactions recorded	5
Wrong antibody identified	2
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 a masked antibody (actually present)	1
Total	**24**

Appendix XIV

Controls questionnaire distributed with red cell phenotyping – 05R8

Controls - overall

- Four laboratories stated that they did not use a positive control, for either their anti-Jk^a or anti-Jk^b reagent, and three of these did not use a negative control for either reagent
- A further eight stated that they used a positive control but not a negative controls.

Table 13 shows the number (%) using cells with heterozygous or apparent homozygous expression of Jk^a and Jk^b antigens, as positive control cells.

Table 13 – Zygosity of positive control cells

Phenotype of positive control	Reagent	
	Anti-Jk^a	Anti-Jk^b
Heterozygous Jk(a+b+)	162 (72%)	154 (72%)
Homozygous*	63 (28%)	61 (28%)
Total	225 (100%)	215 (100%)

* Jk(a+b-) for anti-Jk^a or Jk(a-b+) for anti-Jk^b

- All but two laboratories, used heterozygous positive control cells for controlling both anti-Jk^a and anti-Jk^b reagents, or else used 'homozygous' cells for both.

- A reaction grade of <3 was recorded for the positive control by 23/62 (37.0%) of those using homozygous control cells (and recording a reaction grade), and 60/158 (37.8%) of those using heterozygous control cells.

Reagents

Reagents from up to 15 different manufacturers were in use.

- Five laboratories used out of date anti-Jk^a and /or anti-Jk^b reagents, and two of these used homozygous positive control cells
- One further laboratory used out of date reagents, kept only for teaching purposes
- All of those making errors used reagents that were in date
- There was no apparent correlation between reagent used and incorrect results.