Introduction

This was the final exercise in the 2017-18 cycle of the ABOT scheme. Participants were requested to titrate anti-A in three plasma samples against the A_1 red cells provided. The titrations were to be undertaken with routine methods and techniques (using those for assessing patient suitability for ABO incompatible living organ transplantation where appropriate to clinical practice), and also using a standard DiaMed technique provided, where the required resources were available.

Scores are presented on page 7 of this report along with the basis for scoring.

Three patient red cell samples were included for A_1 typing, to be undertaken by laboratories in which this test is performed in clinical practice.

Material

The following material was provided:

- Samples for Patients 1, 2 and 3, prepared from filtered fresh frozen plasma (Patient 1 and 2 group O, and patient 3 group B).
- One group A_1 red cell sample for titration.
- Three red cell samples in Alsever's (Patients W, Y and Z) for A_1 typing.

Standard DiaMed techniques for DRT and IAT were provided with the exercise instructions (see Appendix 1), and these are referred to as 'standard' techniques in this report.

Return rate / data analysis

The exercise was distributed to 98 laboratories, 41 in the UK and Republic of Ireland (ROI) and 57 outside of the UK. Results were returned by 93/98 (94.9%) laboratories by the closing date.

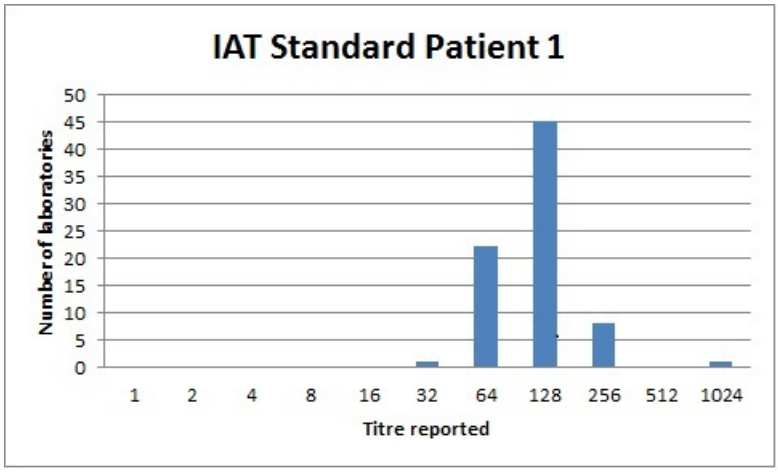
ABO titration is undertaken to support ABO incompatible transplant programmes in 67/90 (74%) laboratories responding to the question.

Results obtained using the 'standard' techniques(s) for DRT and / or IAT were returned by 79/93 (85%) laboratories. Forty six of these also returned results for an in-house DRT and/or IAT method. Nineteen laboratories returned results for in-house methods only.

Not all laboratories tested by both IAT and DRT – the numbers of results analysed for each method are shown in Table 1.

All participants recorded satisfactory sample quality for all samples.

Below are graphical representations of the distribution of results for IAT standard, DRT standard and DRT In-house Tube.



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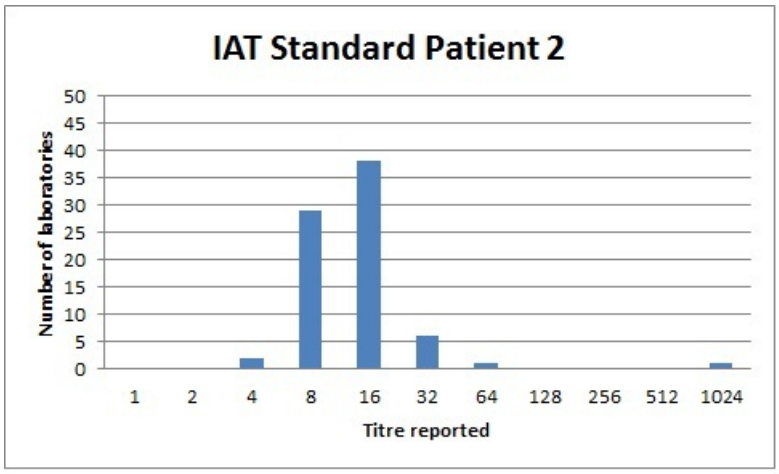
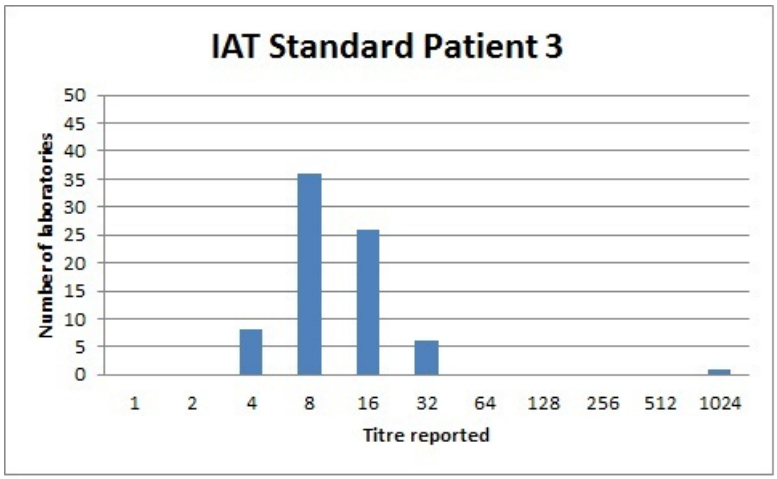
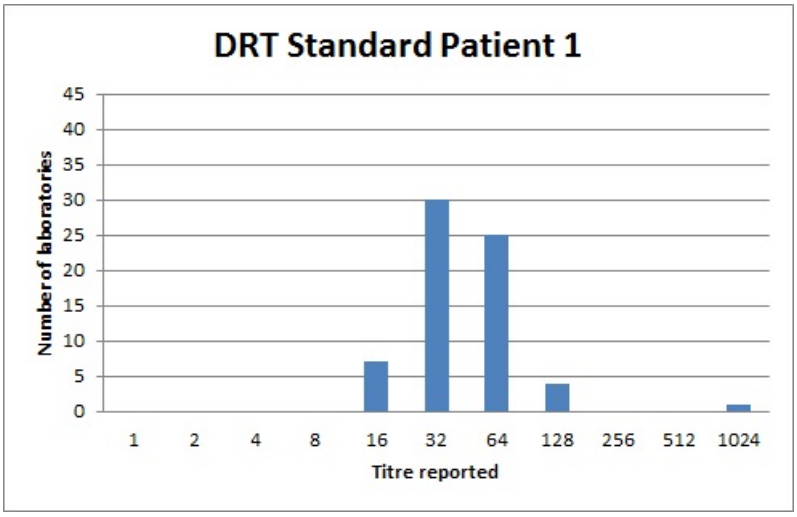
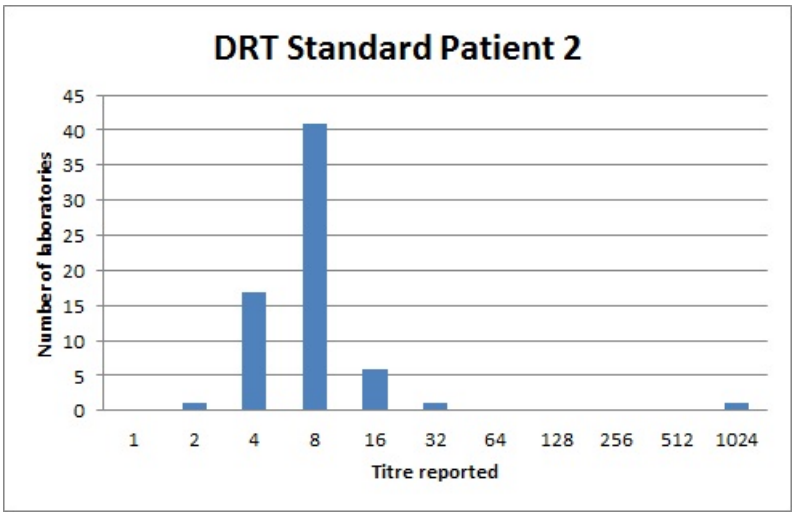
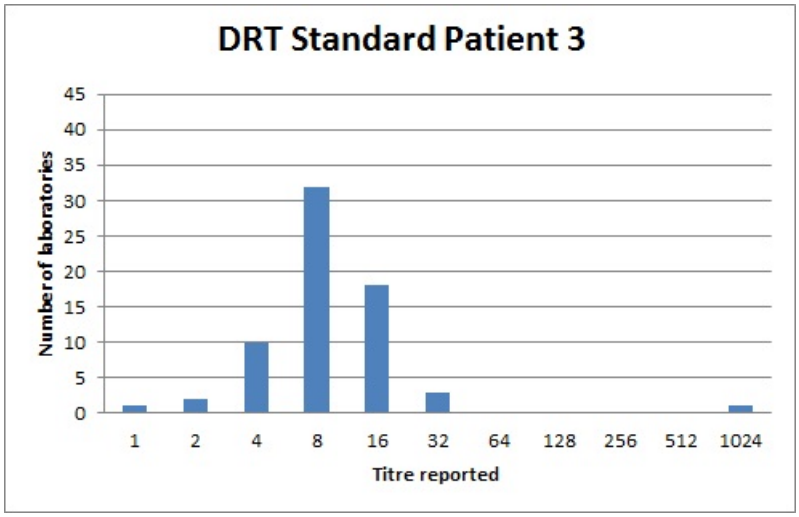
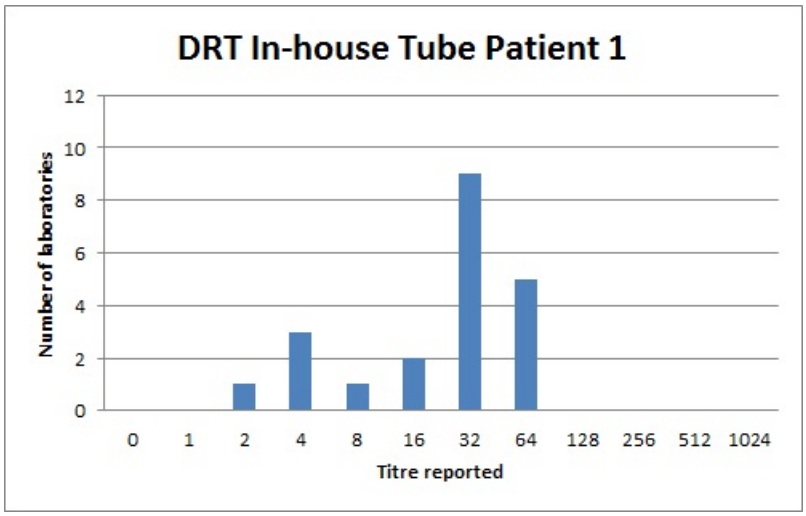

IAT Standard Patient 2
Number of laboratories
50
45
40
35
30
25
20
15
10
5
0
1
2
4
8
16
32
64
128
256
512
1024
Titre reported

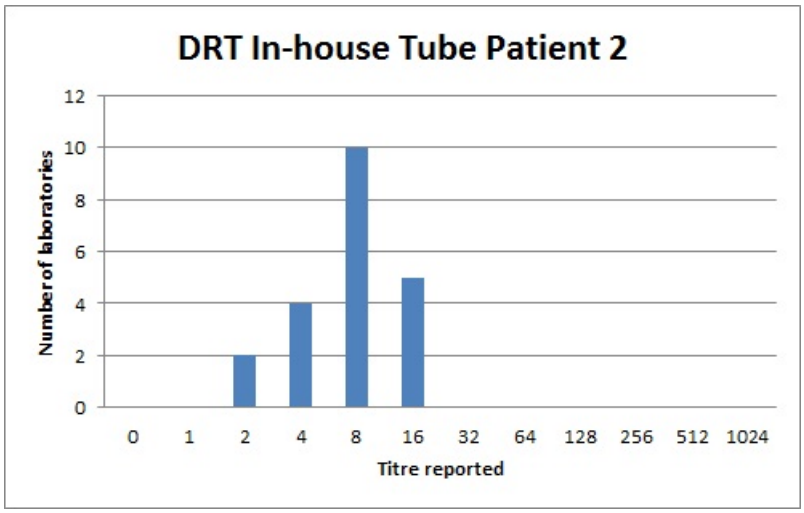

IAT Standard Patient 3
Number of laboratories
50
45
40
35
30
25
20
15
10
5
0
1
2
4
8
16
32
64
128
256
512
1024
Titre reported

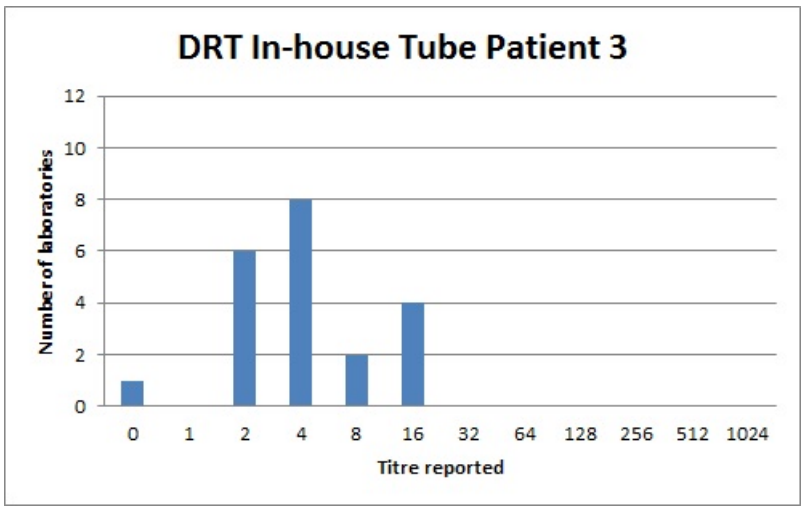

DRT Standard Patient 1
Number of laboratories
45
40
35
30
25
20
15
10
5
0
1
2
4
8
16
32
64
128
256
512
1024
Titre reported


DRT Standard Patient 2
Number of laboratories
45
40
35
30
25
20
15
10
5
0
1
2
4
8
16
32
64
128
256
512
1024
Titre reported


DRT Standard Patient 3
Number of laboratories
45
40
35
30
25
20
15
10
5
0
1
2
4
8
16
32
64
128
256
512
1024
Titre reported


DRT In-house Tube Patient 1
Number of laboratories
12
10
8
6
4
2
0
0
1
2
4
8
16
32
64
128
256
512
1024
Titre reported


DRT In-house Tube Patient 2
Number of laboratories
0
2
4
6
8
10
12
0 1 2 4 8 16 32 64 128 256 512 1024
Titre reported


DRT In-house Tube Patient 3
Number of laboratories
0
2
4
6
8
10
12
0 1 2 4 8 16 32 64 128 256 512 1024
Titre reported

Titration results

Table 1 shows the method median titration results by DRT, IAT using untreated plasma and IAT using pre-treated plasma, and Table 2 shows your results.

Table 1 - Titration median result and range, by method and technology

Technique	Titration result (range)					
	Patient 1 number of results	Patient 1 median (range)	Patient 2 number of results	Patient 2 median (range)	Patient 3 number of results	Patient 3 median (range)
DRT Standard	67	32 (16-1024)	67	8 (2-1024)	67	8 (1-1024)
DRT In-house DiaMed	14	32 (16-64)	14	4 (2-16)	14	8 (2-16)
DRT In-house BioVue	9	32 (8-64)	9	8 (2-64)	9	8 (2-32)
DRT In-house Grifols	2	24 (16-32)	2	6 (4-8)	2	6 (4-8)
DRT In-house Tube	21	32 (2-64)	21	8 (2-16)	21	4 (0-16)
DRT In-house Immucor	8	8 (8-16)	8	4 (2-8)	8	2 (2-4)
IAT Standard	77	128 (32-1024)	77	16 (4-1024)	77	8 (4-1024)
IAT In-house (untreated) DiaMed	5	128 (128-512)	5	16 (8-32)	5	8 (4-16)
IAT In-house (untreated) BioVue	6	192 (8-512)	6	48 (8-128)	6	12 (8-64)
IAT In-house (untreated) Grifols	2	96 (64-128)	2	16 (16-16)	2	16 (16-16)
IAT In-house (untreated) Tube	10	128 (16-256)	10	32 (8-64)	10	12 (8-32)
IAT In-house (untreated) Immucor	9	64 (8-64)	9	4 (1-8)	9	2 (1-4)
IAT In-house DTT Treated (or equivalent) DiaMed	8	96 (16-128)	8	8 (2-16)	8	8 (2-8)
IAT In-house DTT Treated (or equivalent) BioVue	3	256 (256-256)	3	32 (32-32)	3	32 (16-32)
IAT In-house DTT Treated (or equivalent) Tube	3	128 (64-128)	3	8 (4-16)	3	4 (2-8)

Table 2 - Your results

Technique	Titration Result		
	Patient 1 (Anti-A)	Patient 2 (Anti-A)	Patient 3 (Anti-A)

Discussion

One laboratory reported a titre of 1024 for all three patients by both IAT Standard and DRT Standard techniques; aside from these outlying results, the results for both standard techniques demonstrate a normal distribution. The results of the DRT In-house tube technique appears to show a greater variation and the results do not form a normal distribution. This variability may be a result of a number of factors (e.g. incubation time, dilution medium, diluent used for red cells, cell to plasma ratio etc.); UK NEQAS BTLP will send out a questionnaire to the users of this technique to obtain information about variation in practice.

A_1 typing

76/93 (82%) laboratories reported results of A_1 typing, including 57 that use these results to support an ABO incompatible solid organ transplant programme. One laboratory only recorded the A_1 type for Patient W.

Two laboratories recorded a total of three errors in A_1 typing. The first laboratory appears to have transposed either results or samples for Patients Y and Z, the other laboratory recorded a false positive result for Patient Z.

Your result and the expected and overall results are shown in Table 3.

Table 3 - Results of A_1 typing

Sample	Your result	Expected result	Overall results	
			A_1 positive	A_1 negative
Patient W		Positive	76 (100%)	0 (0%)
Patient Y		Positive	74 (99%)	1 (1%)
Patient Z		Negative	2 (3%)	73 (97%)

Scoring for ABO titration

Categories of testing scored
Difference from median result for results obtained by:

1. Standard IAT
2. Standard DRT
3. Any other in-house technology with >20 laboratories testing by IAT or DRT

Definition of satisfactory results
Titration value within 1 doubling dilution of 'target', i.e. method median.

'Scores' for 'outlying' results

- One point for each doubling dilution >1 away from 'target', e.g. if the target were 32, then one point would be incurred for results of 8 or 128, two points for 4 or 256, three points for 2 or 512 etc.
- Points will be accumulated within each category, within each exercise
- Points will be accumulated between exercises, also by category

Table 3 - Your scores

Technique	Score for this exercise[1]	Performance this exercise	Cumulative score[2]	Cumulative performance

[1]includes all three current samples
[2]includes nine most recent samples where results were returned (including the current samples)

Table 4 - Non-return scores

Non-return score for this exercise	Cumulative non-return score[1]	Your performace

[1]includes three most recent exercises

Performance Monitoring (UK Laboratories only)

Definition of unsatisfactory performance (UP)

- A total of three points within a category of testing in a single survey.
- A total of three points within a category of testing over three surveys (current and two previous for which results were returned).
- Non-return of results in two of the three most recent surveys.

Definition of persistent unsatisfactory performance (PUP)

- More than one episode of unsatisfactory performance in any category of testing, within 12 months.
- Two episodes of UP due to non-return of results in a 12 month period.
- One episode of UP from each of the above within a 12 month period.

Appendix 1

'Standard' techniques 1718ABOT4

- Prepare dilutions of plasma in saline (PBS or NaCl) using a doubling dilution method. Make the dilutions with a minimum volume of 200µl, using an automatic pipette. Use a new tip to dispense each dilution.
- Prepare a 0.8 - 1% red cell suspension in CellStab (use ID-diluent 2 if CellStab is not available).
- Read the endpoint of the titration as the last weak reaction.

LISS indirect antiglobulin test (**IAT**) using IgG or polyspecific cards

a. Add 50ul of cells suspended in CellStab or ID-diluent 2 to each microtube
b. Add 25ul of each plasma dilution to the corresponding microtube
c. Incubate at 37oC for 15'
d. Centrifuge 10' in DiaMed centrifuge

Direct agglugtination at room temperature (**DRT**) using NaCl cards

a. Add 50ul of cells suspended in CellStab or ID-diluent 2 to each microtube
b. Add 50ul of each plasma dilution to the corresponding microtube
c. Incubate at RT for 15'
d. Centrifuge 10' in DiaMed centrifuge