

Steering Committee
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Pilot exercise for titration of anti-A and anti-B 14/15ABO T2 - distributed 9 September 2014

PRN

Introduction

This was the second exercise in the 2014-15 cycle of the ABOT pilot scheme. Participants were requested to titrate anti-A in three plasma samples against the A₁ 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.

Material

The following material was provided:

- Three group O samples for anti-A titration, prepared from fresh frozen plasma.
- The plasma samples for Patients 1 and 2 were prepared from the same pool.
- One Group A₁ D negative (rr) red cell sample.

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 pilot was distributed to 87 laboratories, 39 in the UK and Republic of Ireland (ROI) and 48 outside of the UK. Results were returned by 82/87 (94.2%) laboratories by the closing date, with two returning two sets of results (one for IAT with untreated and DTT treated plasma, and the other for two different in-house IAT techniques with untreated plasma). 36 (44%) laboratories returned results for both standard and in-house techniques, 13 (16%) for an in-house technique only, and 33 (40%) for the standard technique only. Not all laboratories tested by both IAT and DRT – the numbers of results analysed for each method are shown in Table 1.

ABO titration is undertaken to support renal transplant programme(s) in 45/82 (55%) laboratories. 34/45 (76%) returned results obtained with the 'standard' DRT technique and 16/34 (47%) stated that they use this technique in clinical practice. 39/45 (87%) returned results using the 'standard' IAT technique and 28/39 (72%) stated that they use this technique in clinical practice.

Reported sample quality

All laboratories reported satisfactory sample quality for the group A red cells and for Patient 1 and 2 plasma samples. One reported unsatisfactory sample quality for Patient 3 plasma due to the presence of fibrin.

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	Number of results	Titration result (range) vs. A cells		
		P1 (Group O)	P2 (Group O)	P3 (Group O)
DRT Standard	62	32 (8-256)	32 (8-256)	128 (64-512)
DRT In-house overall	44	16 (8-512)	32 (8-512)	64 (16-512)
DRT In-house DiaMed	14	16 (16-64)	16 (16-64)	64 (32-128)
DRT In-house BioVue	5	64 (16-128)	64 (16-128)	128 (64-256)
DRT In-house tube	18	16 (8-512)	24 (8-512)	64 (16-512)
DRT In-house Immucor	4	24 (8-128)	32 (8-128)	64 (32-512)
DRT In-house Grifols	2	128 (128)	128 (128)	256 (256)
DRT in-house Other	1	128	256	512
IAT Standard	68	128 (64-512)	128 (32-1024)	256 (64-1024)
IAT In-house overall untreated	27	128 (8-512)	128 (8-512)	256 (32-512)
IAT In-house DiaMed untreated	8	128 (32-512)	128 (64-512)	256 (64-512)
IAT In-house BioVue untreated	4	512 (128-512)	256 (128-512)	384 (256-512)
IAT In-house tube untreated	7	128 (16-512)	128 (16-512)	128 (64-512)
IAT In-house Immucor untreated	4	96 (8-128)	128 (8-128)	192 (32-256)
IAT In-house Grifols untreated	3	256 (256-512)	512 (256-512)	512 (512)
IAT in-house Other	1	64	32	64
IAT DTT (or equivalent) overall	10	64 (16-128)	96 (16-128)	128 (32-256)

Table 2 – Your results (PRN 26018) No results expected

Technique	Titration result		
	P1 anti-A	P2 Anti-A	P3 anti-A
DRT Standard	no result	no result	no result
IAT Standard	no result	no result	no result
DRT In-house (not applicable)	no result	no result	no result
IAT In-house (not applicable)	no result	no result	no result

Additional information

Samples for Patients 1 and 2 were prepared from the same pool, and Table 3 shows the intra-laboratory reproducibility of results for these samples.

Table 3 Reproducibility of testing duplicate samples (Patients 1 and 2) by technique used

Technique (number of results)	Results P2 vs. P3		
	Number (%) Identical	Number (%) 1 dilution apart	Number (%) >1 dilution apart
DRT Standard (62)	52 (84%)	10 (16%)	0 (0%)
IAT Standard (68)	47 (69%)	20 (29%)	1 (2%)
DRT In-house (44)	32 (73%)	11 (25%)	1 (2%)
IAT In-house untreated (27)	15 (56%)	12 (44%)	0 (0%)
IAT In-house DTT treated (10)	7 (70%)	3 (30%)	0 (0%)

Annual report for 2013/14 to follow.

Appendix

1. – The ‘Standard’ techniques for DRT and IAT issued with this exercise.

Appendix 1

'Standard' techniques 14/15ABO T1

- 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 37°C for 15'
- d) Centrifuge 10' in DiaMed centrifuge

Direct agglutination 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