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UK NEQAS for Blood Transfusion Laboratory Practice (BTLP)
ABO titration pilot annual summary 2014/15

Introduction

The UK NEQAS (BTLP) ABO titration pilot has been running since an exploratory pilot exercise in 2009 revealed a wide variation of methodology in use, the titration results obtained and their use in the ABO incompatible (ABOi) transplant context. The main focus of the pilot is to look at ABO titration in laboratories supporting ABOi transplant, and by the end of this reporting period, 50 such laboratories were registered.

Since 2010, results obtained with 'standard' indirect antiglobulin (IAT) and direct room temperature (DRT) techniques based on DiaMed (as the most commonly used technology) have been requested alongside those using in-house techniques, in an attempt to allow a more direct comparison of results. The standard technique used in 2014/15 is attached as Appendix 1.

The reports issued for each pilot exercise during 2014/15 provided the median and range of results by method and included individual results for each laboratory. This report provides a more detailed analysis of the results.

Summary of exercises distributed in 2014-15

Table 1 shows a summary of the exercises distributed during the 2014/15 exercise cycle. All plasma samples issued were group O, and group A_1 cells were provided for titration. Shaded cells represent duplicate or replicate samples as defined within the table.

Table 1: Summary of ABOT exercises 2014/15

Data	14/15 ABOT1 June 2014	14/15 ABOT2 Sept 2014	14/15 ABOT3 Dec 2014	14/15 ABOT4 Mar 2015
Number of participants registered	87 (40 UK)	87 (39 UK)	91 (39 UK)	91 (39 UK)
Return rate	93%	94%	95%	90%
Number Std. results	59 DRT 71 IAT	62 DRT 68 IAT	63 DRT 71 IAT	62 DRT 71 IAT
Number in-house results	45 DRT 27 IAT, 10 DTT[2]	44 DRT 27 IAT, 10 DTT[2]	43 DRT 25 IAT, 11 DTT[2]	45 DRT 19 IAT, 13 DTT[2]
Plasma sample 1 Group and titre[1]	128 DRT 128 IAT	32 DRT 128 IAT	8 DRT 16 IAT	32 DRT 128 IAT
Plasma sample 2 Group and titre[1]	2 DRT 1 IAT	32 DRT 128 IAT	32 DRT 32 IAT	16 DRT 16 IAT
Plasma sample 3 Group and titre[1]	128 DRT 256 IAT	128 DRT 256 IAT	128 DRT 256 IAT	64 DRT 64 IAT
Cells provided for titration	A_1rr	A_1rr	A_1rr	A_1rr
Duplicate samples within exercise	None	P1 and P2	None	None
Replicate samples between exercises	Patient 3 14/15 ABOT1, T2 and T3	Patient 3 14/15 ABOT1, T2 and T3	Patient 3 14/15 ABOT1, T2 and T3	
Additional information collected	None	Reproducibility for duplicate samples	Workload and reasons for titration	None

[1] Titres shown are median results obtained with the standard technique [2] Plasma treated with DTT or equivalent

Participation

During the 2014/15 exercise cycle, a total of 98 laboratories were registered for the ABOT pilot Scheme for at least part of the year, with 85 laboratories remaining registered for all four exercises. However, not all laboratories returned results for all exercises.

Workload and clinical indications for undertaking titration

In December 2014, additional questions on clinical indications for titration and workload were included with exercise 14/15ABO T3, to which 82 laboratories responded. The percentage of laboratories reporting each titration workload range for each clinical category is shown for all laboratories, for UK laboratories (including Republic of Ireland) and for non-UK laboratories in figures 1 – 3 respectively.

Table 2 shows the % of laboratories overall, in the UK and outside the UK, performing titration for a range of clinical indications.

Table 2: Clinical indications for ABO titration

Clinical indication	% Laboratories		
	All (n=82)	UK (n=35)	Non-UK (n=47)
ABOi renal transplant (Tp)	51.2	62.9	42.6
ABOi other solid organ transplant (SO Tp)	23.2	25.7	21.3
Haemopoetic Stem Cell Transplant (HSCT)	62.2	48.6	72.3
ABO Haemolytic disease of the newborn (HDFN)	39.0	28.6	46.8
Other	20.7	17.1	23.4

Fig. 1: Workload - all laboratories

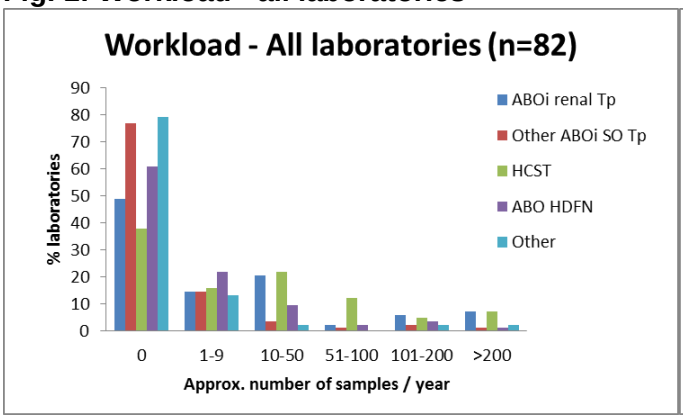


Fig. 2: Workload - UK laboratories

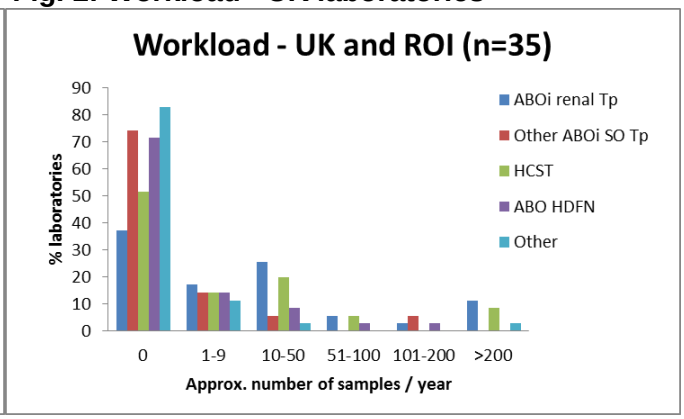
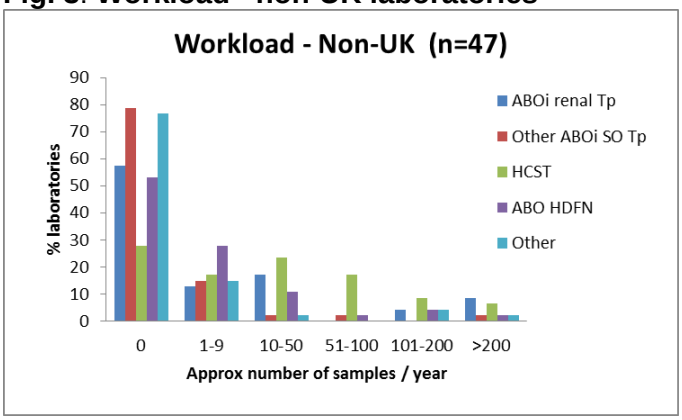


Fig. 3: Workload - non-UK laboratories

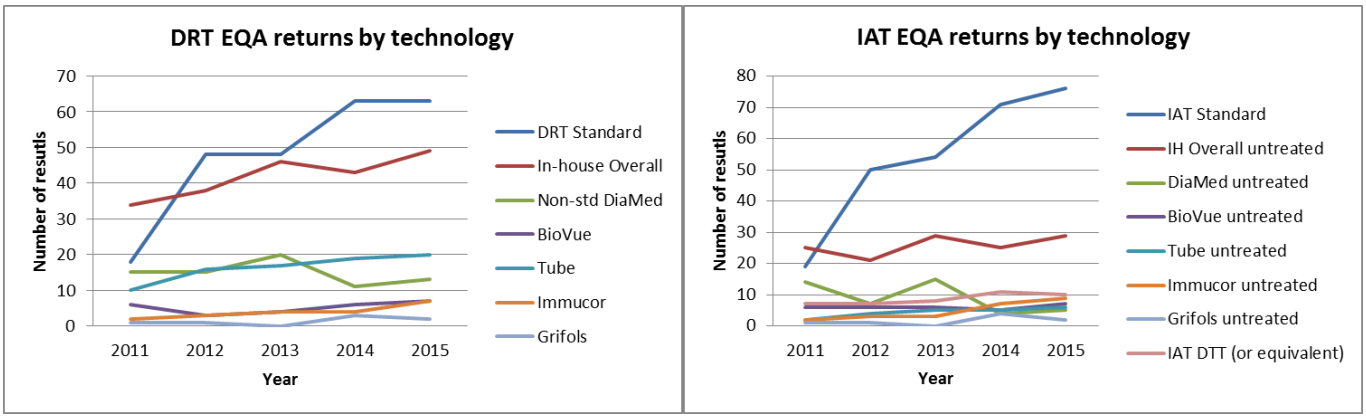


- 14 laboratories (5 UK and ROI, 9 non-UK) test fewer than 20 samples per year for any clinical reason, including 10 (5 UK and ROI, 5 non-UK) testing <10 samples per year, and two (non-UK) apparently not performing titrations on any clinical samples.

Technology used for titration in participating laboratories

Figures 4 and 5 show the technologies used to return EQA results from 2011 – 2015, using data from returns for the last exercise in each calendar year.

Figs. 4 and 5: EQA returns by technology (2011 – 2015)



There has been an increasing use of the Standard techniques compared to that for other technologies. In 2014/15, the Standard technique was used for approximately 70% results returned by DRT and 80% by IAT.

Standard techniques (IAT and DRT)

The numbers returning results for the Standard DRT and IAT for each exercise can be seen in Table 1. The number of laboratories returning 'Standard' results showed little variation throughout the year (59-63 for DRT and 68-71 for IAT), as did the number using these results in clinical practice (23-28 DRT and 41-46 IAT). The picture is similar for laboratories using results obtained by the Standard technique to support renal transplant programmes (16-19 DRT and 25-29 IAT), where approximately half of these are in the UK.

Table 3 shows the number (%) of these laboratories with one or more result that is >1 doubling dilution from the IAT or DRT method median in each of the four exercises, and Tables 4 and 5 show the same information, but include only laboratories using their Standard results in clinical practice (for any application) and those using their results to support a renal transplant programme, respectively.

Table 3: All laboratories' Standard results in relation to Standard method median

Category	n (%) laboratories >1 doubling dilution from the median result			
	14/15 ABOT1	14/15 ABOT2	14/15 ABOT3	14/15 ABOT4
>1 dilution from DRT standard median	11/59 (18.6%)	9/62 (14.5%)	5/63 (7.9%)	3/62 (4.8%)
>1 dilution from IAT standard median	8/71 (11.3%)	3/68 (4.4%)	6/71 (8.5%)	7/71 (9.9%)

Table 4: Standard results used in clinical practice in relation to Standard method median

Category	n (%) laboratories >1 doubling dilution from the median			
	14/15 ABOT1	14/15 ABOT2	14/15 ABOT3	14/15 ABOT4
>1 dilution from DRT standard median	3/26 (11.5%)	4/24 (16.7%)	2/31 (6.5%)	1/30 (3.3%)
>1 dilution from IAT standard median	5/46 (10.9%)	1/42 (2.4%)	6/46 (13.0%)	6/46 (13.0%)

Table 5: Standard results used to support renal transplant in relation to Standard method median

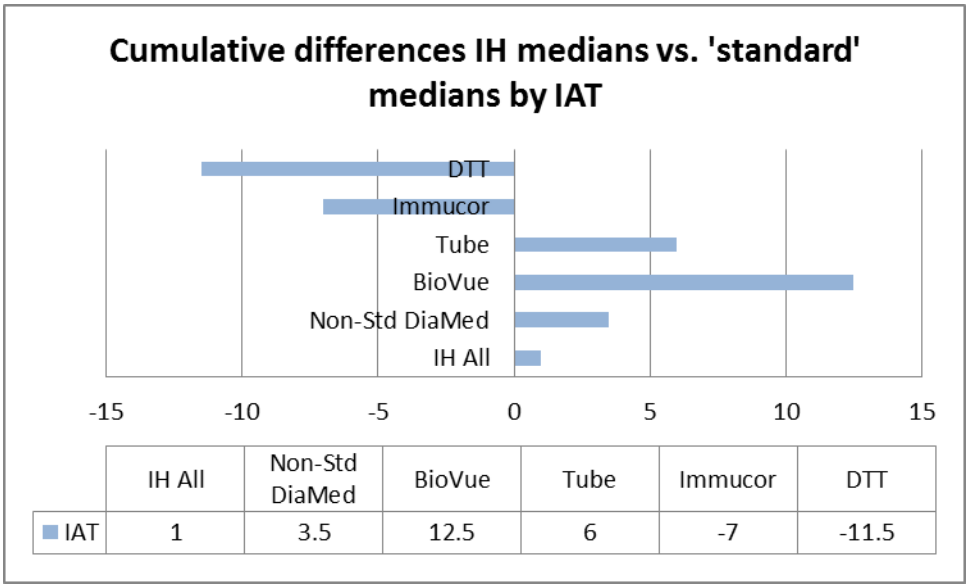
Category	n (%) laboratories >1 doubling dilution from the median			
	14/15 ABOT1	14/15 ABOT2	14/15 ABOT3	14/15 ABOT4
>1 dilution from DRT standard median	2/18 (11.1%)	3/16 (18.8%)	1/19 (5.2%)	1/21 (4.8%)
>1 dilution from IAT standard median	2/27 (7.4%)	1/28 (3.6%)	3/27 (11.1%)	4/32 (12.5%)

Cumulative difference between standard medians and medians using 'in-house' (IH) techniques

The median IAT results obtained for the 12 samples distributed in 2014/15 using in house techniques (Tube, BioVue, DiaMed and Immucor technology) and the standard technique were examined for distance from the Standard median. Each result was assigned a score of 1 for each dilution above the median and of -1 for each dilution below the median.

Where the median fell between two doubling dilutions the results either side of were assigned a value of 0.5. The scores were totalled to give a cumulative score as shown in figure 6, where '0' represents the IAT median using the standard technique. The numbers for other in-house methods were too small for analysis.

Fig. 6: Cumulative difference IH IAT medians vs. 'standard' IAT median



Replicate samples (over three exercises)

Exercises 14/15ABOT1, 14/15ABOT2 and 14/15ABOT3 contained a replicate sample from a pool of plasma that was frozen in aliquots, with one thawed for each exercise. Results from laboratories who completed all three exercises using the same technology for each are included in Table 6, which shows the number (%) of results obtained with each method that were the same for all three replicates, and the number (%) where one or more result differed by one, two or more than two doubling dilutions.

Table 6: Reproducibility of titration results for the three replicate samples, by method

Method (number)	Same each time	Maximum difference		
		1 dilution	2 dilutions	>2 dilutions
Std DRT (51)	11 (22%)	28 (55%)	11 (22%)	1 (2%)
All IH DRT (25)	4 (16%)	13 (52%)	6 (24%)	2 (8%)
IH DiaMed DRT (9)	1 (11%)	8 (89%)	0 (0%)	0 (0%)
IH Tube DRT (11)	3 (27%)	3 (27%)	3 (27%)	2 (18%)
Std IAT (63)	25 (40%)	33 (52%)	2 (3%)	3 (5%)
All IH IAT (14)	3 (21%)	8 (57%)	1 (7%)	2 (14%)
IH IAT DTT (9)	1 (11%)	4 (44%)	2 (22%)	2 (22%)

Results within 1 dilution over the three replicates:

- 56/76 (74%) all DRT results
- 68/77 (88%) all IAT results using non-DTT treated plasma
- 5/9 (56%) all IAT results using DTT treated plasma

- 39/51 (76%) Standard DRT results
- 17/25 (68%) In-house DRT results

- 57/63 (90%) Standard IAT results
- 11/14 (79%) In-house IAT results (non-DTT)

Duplicate samples (in same exercise)

Exercise 14/15ABOT2 included two samples from the same pool (P1 and P2). Table 7 shows the difference between results for P1 vs. P2 in individual laboratories, displayed by technology for DRT and IAT (technologies with <5 results have not been included).

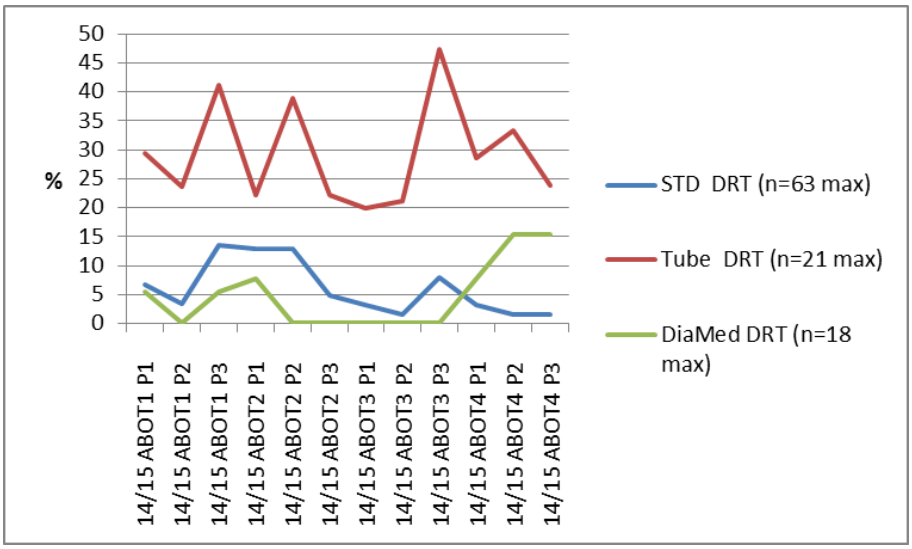
Table 7: Reproducibility in testing of duplicate samples by technique

Technique (number of results)	Results P1 vs. P2		
	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%)

Inter-laboratory comparison by technology

For IAT, the numbers using each technology, other than the standard DiaMed method, are too small for a comparison of results to their own median to look at inter-laboratory variation by technology. However, for DRT, the Standard technique was compared with non-standard DiaMed and tube, as more than ten results were available for these technologies. Figure 7 shows the percentage of results returned by each technology that are >1 dilution away from the technology median; the numbers in brackets show the number using each technology in 14/15 ABOT4. Example histograms showing data by technology for a range of different titres (high, medium and low) are shown in Appendix 2.

Fig. 7: Percentage of DRT results >1 dilution from technology median



Shadow scoring for 2014

Shadow scoring has been undertaken on the data collected in the 14/15 series of exercises, and will continue in 2015/16 using the following criteria:

- Only results obtained using the Standard method or with any method used by > 20 participants are scored.
- One doubling dilution lower/higher than the method median is acceptable.
- Outlying results due to obvious transposition errors are not included when calculating medians.
- IAT and DRT results are scored separately.
- Points are allocated according to how far the result was from the method median, with an increment of one point per dilution.
- There is a separate score for non-return of results.

These scores will be displayed on individual reports during 2016 for information only, and do not constitute a formal scoring system.

Progress with anti-A and anti-B reference preparations (in collaboration with NIBSC)

UK NEQAS (BTLP) has been working with the National Institute for Biological Standards and Control (NIBSC) to develop a reference preparation for high titre anti-A and anti-B. This preparation has been accepted as a WHO reference reagent and is now available from NIBSC as 'High titre anti-A and anti-B in serum 14/300' https://www.nibsc.org/documents/ifu/14-300.pdf. Many thanks to those participants who took part in the international validation process.

Discussion

Titration techniques are likely to include manual steps in dilution and / or reading and therefore some variation in replicate results is to be expected. However, it would be reasonable to expect results of replicate samples to be within one doubling dilution.

Replicate samples distributed in 14/15 ABOT1, T2 and T3

IAT results appear to be more reproducible than DRT results. Overall, 74% of intra laboratory results obtained by DRT and 88% by IAT (untreated plasma) were within one doubling dilution for the three replicate samples. This was examined by technique, and 90% of IAT results obtained using the standard technique were within one doubling dilution cf. 79% in-house IAT results (using untreated plasma). The DRT results were not significantly different, with 76% standard DRT results being within one doubling dilution cf. 68% in-house DRT results. It is not clear how technology affects reproducibility, apart perhaps from the method for reading the titration endpoint. These finding concur with those reported previously (annual report 2012-13), except for the DRT, where the in-house techniques (overall) appeared more reproducible based on the 2012-13 data. Only 55% of IAT results obtained using DTT treated plasma were within one dilution over the three exercises.

Duplicate samples distributed in exercise 14/15 ABOT2

Two replicate samples were distributed in exercise 14/15 ABOT2, and 99% of all paired results for individual laboratories were within one dilution. The proportion of results within one doubling dilution was higher than that seen for the replicate samples sent over three exercises (14/15 ABOT2, T3 and T4). This might be because fewer replicates (2 cf. 3) were compared, or because fewer variables impacted on results of tests performed on the same day, e.g. they were less likely to have been undertaken by different individuals.

Cumulative difference in-house method medians vs. Standard method medians

As expected, DDT treated plasma gave a lower cumulative median than the 'Standard' (DiaMed) technique which uses untreated plasma. The overall in-house cumulative median was very close to that for the standard technique; however, this varied by individual in-house technology. The Immucor cumulative median was also lower, presumably since only IgG is detected using Capture technology. The other techniques had a higher cumulative median, with BioVue being the highest (see Figure 4). The position of the BioVue cumulative median remains unchanged since 2012/13, whilst tube and DiaMed (non-standard) cumulative medians have shifted from slightly negative to slightly positive relative the Standard. However, the numbers using these technologies are small and have reduced further since 2012-13.

Comparisons to method medians for each technology

For IAT, the numbers using each technology, other than the standard DiaMed method, are too small for a comparison of results to their own median to look at inter-laboratory variation by technology. However, for DRT, the Standard technique was compared with non-standard DiaMed and tube, as more than ten results were available for these technologies. This showed both the Standard and non-standard DiaMed techniques to have a tighter range than that for tube.

Conclusions

There is still considerable variation in results obtained between techniques, and the introduction of a standard technique would facilitate the transfer of results and transplant protocols (i.e. acceptable titration values for admission to ABOi renal transplant programs and suitability for transplant) across centres.

For DRT, use of the Standard technique appears to reduce the inter laboratory range of results, compared to a tube technique, but it is not possible to establish from the 2014 data whether this is also true for IAT. Where any single technology is used, reproducibility on the day is good within one doubling dilution, but becomes more variable in a significant proportion of laboratories when replicate samples are tested on separate occasions.

It should be possible to use the NIBSC WHO reference reagent ‘High titre anti-A and anti-B in serum 14/300’ for standardising titration testing methodology for anti-A and anti-B, and for establishing consistent cut-off values in the transplant context, where appropriate.

Appendices

1. Standard technique in use 2014/15.
2. Example histograms showing Standard and in-house results for individual samples distributed in 2014/15.

Appendix 1

‘Standard’ techniques 14/15ABO T4

- 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

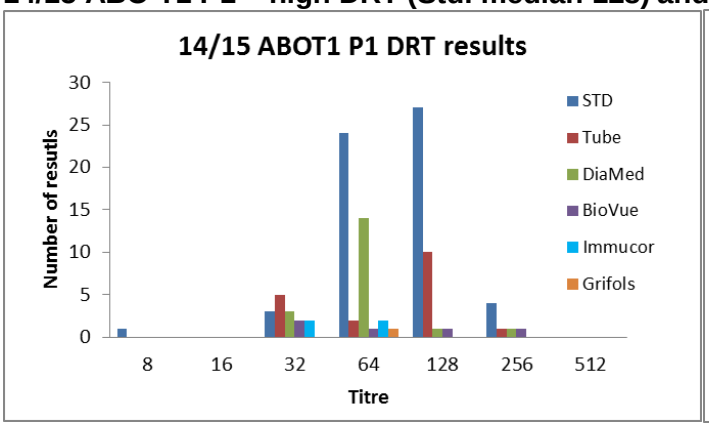
a) Add 50ul of cells suspended in CellStab or ID-diluent 2 to each microtube
b) Add 25ul of each plasma dilution to the corresponding microtube
c) Incubate at 37^{o}C for 15’
d) Centrifuge 10’ in DiaMed centrifuge

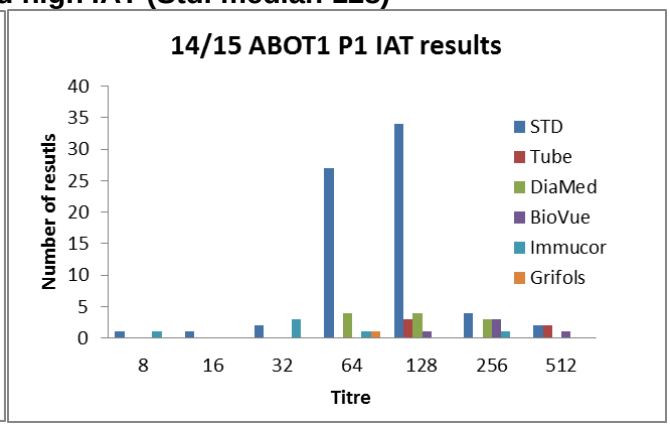
Direct agglutination at room temperature (**DRT**) using NaCl cards

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

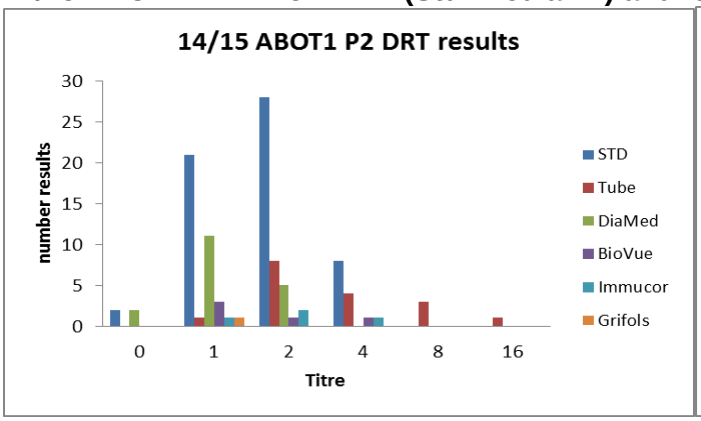
Appendix 2: Example histograms showing Standard and in-house results for samples 2014/15.

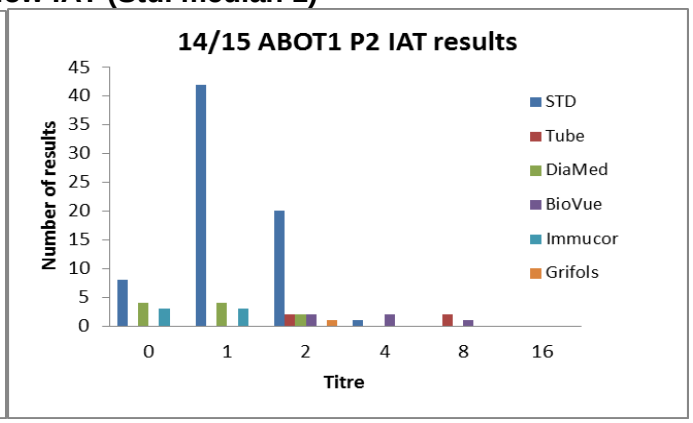
14/15 ABO T1 P1 – high DRT (Std. median 128) and high IAT (Std. median 128)



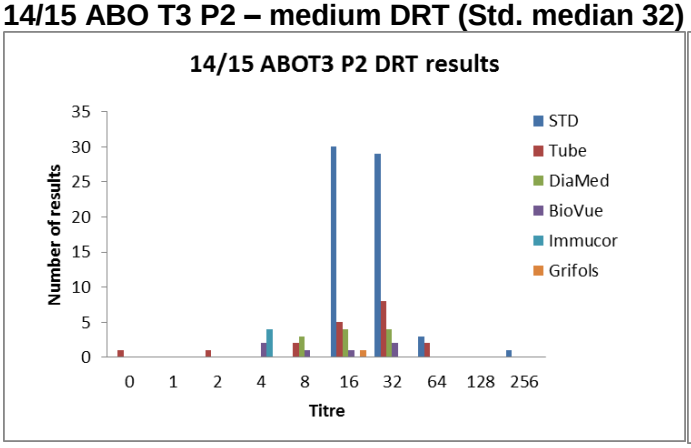


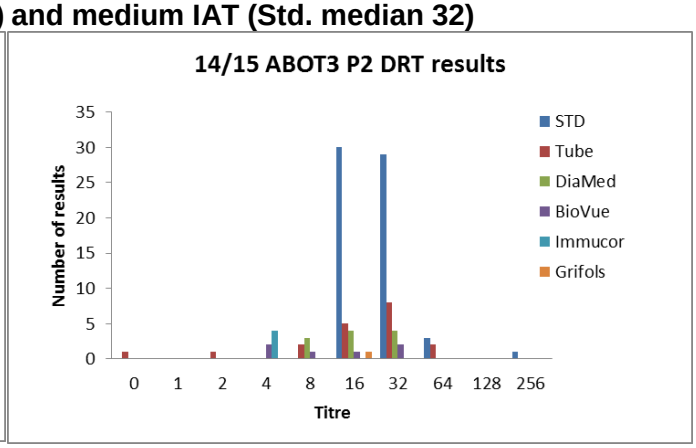
14/15 ABO T1 P2 – Low DRT (Std. median 2) and low IAT (Std. median 1)





14/15 ABO T3 P2 – medium DRT (Std. median 32) and medium IAT (Std. median 32)





14/15 ABO T3 P3 – High DRT (Std. median 128) and high IAT (Std. median 256)