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Report of titration exercise and questionnaire (17E7) – UK and ROI Distributed July 2017

Introduction

Antibody titration was included with exercise 17E7 as an optional, non-scoring element for laboratories undertaking titration of IgG alloantibodies as part of antenatal testing. The instructions were to titrate 17E7 Patient 4 plasma as if it were an antenatal booking sample, selecting appropriate red cells and using routine techniques. There was an accompanying on-line questionnaire for recording titration results and methods used as well as details of local policy regarding the management of antenatal cases with IgG alloantibodies.

Material

17E7 Patient 4 plasma: Anti-K (all participants returning titration results correctly identified anti-K).

Return Rate and data analysis

The questionnaire was completed by 130 UK and ROI participants, 44 of whom stated that they performed antenatal titrations in clinical practice. Two laboratories titrate in clinical practice but did not return a titration result and conversely three that do not titrate in clinical practice completed the exercise.

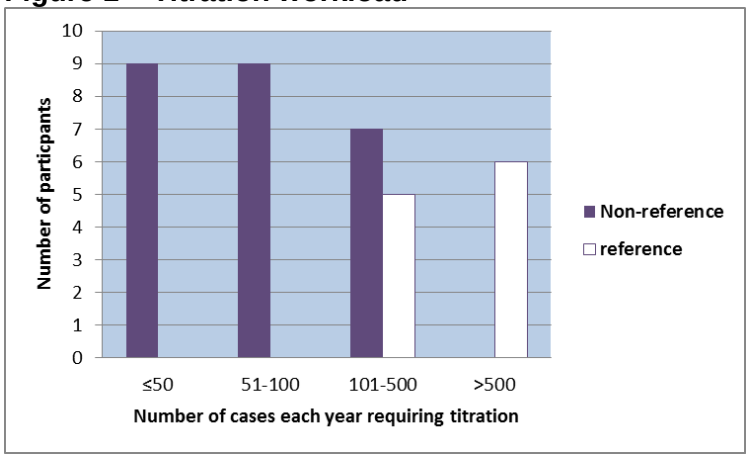
Forty-five laboratories performed the titration, five of which reported titration reaction grades but no titration value; in these cases an interpretation has been made based on the reaction grades recorded and the endpoint (weak, 1+ or 2+) stated to be used in answer to the questionnaire.

Where workload figures have been given as a range, the midpoint has been taken. As not all respondents completed all questions, the numbers in the result tables do not always equal 45; due to rounding, totals may not be exactly 100%. Reference laboratories were taken to be those within the blood services. Three non-reference laboratories reporting >1000 cases were excluded from workload figures, as it is possible that the question could have been misinterpreted to mean general antenatal cases.

Workload

Figure 1 shows the number of antenatal cases requiring titration of IgG alloantibodies per annum in the 36 laboratories responding to this question (11 reference and 25 non-reference).

Figure 1 – Titration workload



Titration policy

Forty four responses were analysed as one participant that undertook the titration did not provide any data for this section.

16/44 (36%) stated that the last weak reaction is taken as the end point of a titration, whilst 27/44 (62%) use a cut-off of the last 1+ reaction, and one (2%) the last 2+ reaction.

43/44 (98%) use cells with heterozygous expression of the relevant antigen for titration where possible, whilst one selects cells with apparent homozygous expression.

35/44 (80%) state that the previous sample (if available) is titrated in parallel with each new sample.

12/43 (28%) titrate the NIBSC standard anti-D in parallel with each titration performed.

Titration Results

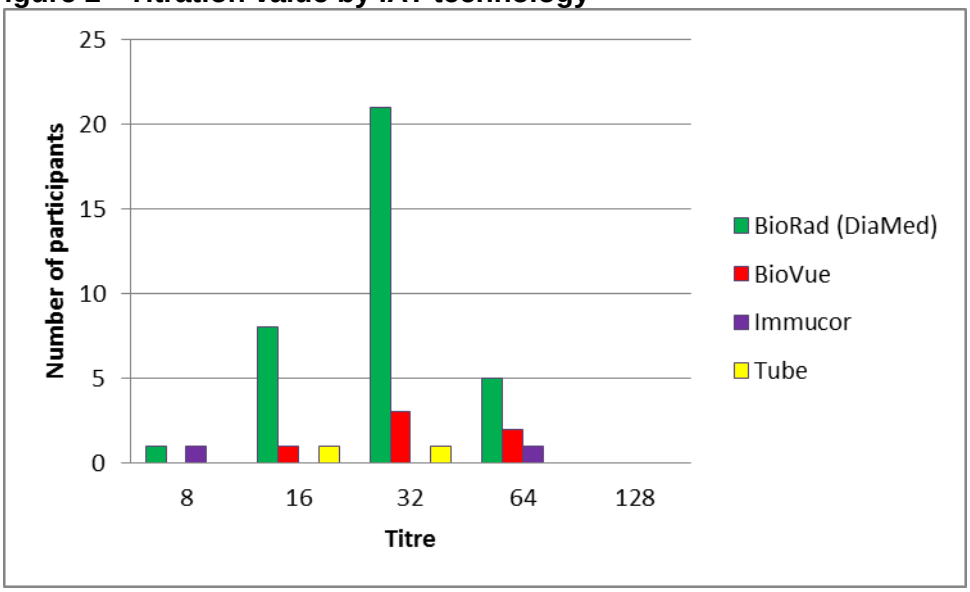
Table 1 shows the number of participants using each IAT technology and the overall titration values obtained. Figure 2 shows the titration value reported vs. % of participants using each technology.

Table 1 – Number (%) laboratories and titration results by IAT technology

Technology	Number (%)	Median titration result	Range
BioRad (DiaMed)	35 (78)	32	8 - 64
BioVue	6 (13)	32	16 - 64
Tube	2 (4)	24	16 - 32
Immucor	2 (4)	36	8 - 64
All technologies	45 (100)	32	8 - 64

Of the 45 laboratories using IAT technologies where a standard IAT method is provided by the manufacturer, 41 stated that they used the recommended IAT method, three did not answer this question, and another stated that they did not use the recommended method, indicating that it differed in terms of the incubation time used.

Figure 2 - Titration value by IAT technology



Details of Titration Method

Forty four responses were analysed as one participant that undertook the titration did not provide any data for this section.

Table 2 shows the plasma diluents used and Table 3 the use of red cell diluents (by technology).

Table 2 – Plasma diluent used

Plasma diluent	Number (%)
PBS	35 (82)
0.9% NaCl	4 (9)
BioRad (DiaMed) CellStab	1 (2)
BioRad (DiaMed) ID Diluent 2	2 (5)
Ortho 0.8% red cell diluent (RCD)	1 (2)
Total	43 (100)

Table 3 – Red cell diluent used (by technology)

Technology	Number of participants using each red cell diluent					
	BioRad (DiaMed) CellStab	BioRad (DiaMed) Dil-2	Ortho 0.8% RCD	LISS	PBS	Other
BioRad (DiaMed)	16	14	0	1	0	2
BioVue	0	0	4	0	1*	1
Tube	0	0	0	1	1	0
Immucor	0	0	1	0	0	1
All technologies	16	14	5	2	2	4

*possible data entry error as this participant also indicated that they followed the manufacturer's methodology.

Red cells heterozygous for the K antigen were selected for the titration by 43/44 (98%), whilst one selected 'homozygous' cells.

The stated end point of the titration varied between weak and 2+. The median titration result was the same for the 1+ and the weak end points.

Figure 3 shows a comparison of the reported titre vs. the reaction grade used as an end point, based on answers to the question on policy for determining the titration endpoint.

Figure 3 – Comparison of titration end point used vs. titre obtained

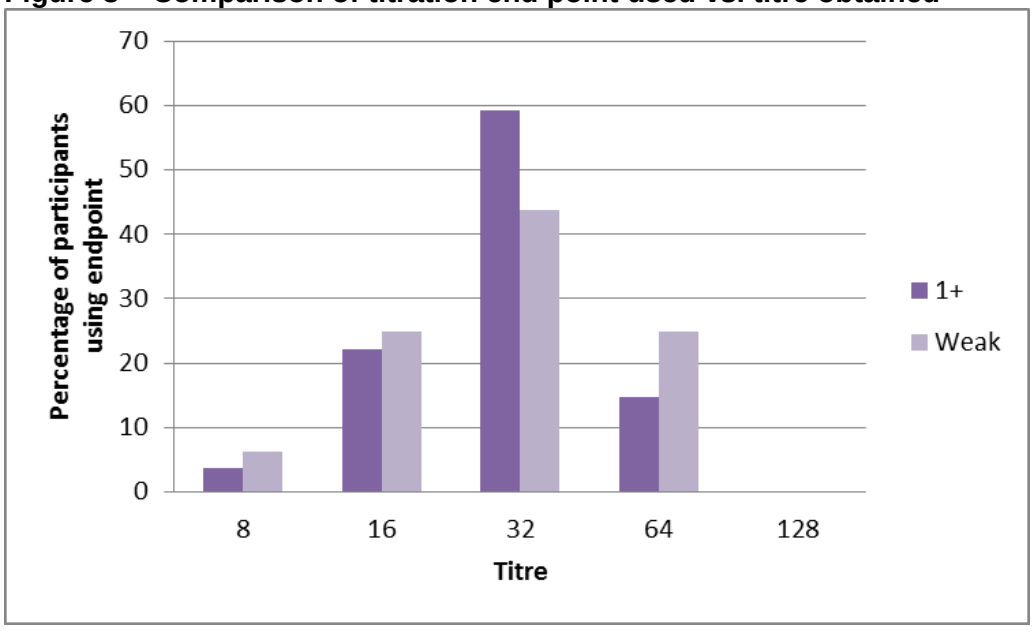


Table 4 shows the actions that would be taken by laboratories if the titration result for Patient 4 was obtained in a 12-week antenatal booking sample (assuming discussion locally with haematologist/obstetricians).

Table 4 – Actions triggered by the titration result for Patient 4

Action	Number (%)
Ascertain obstetric history	28 (64)
Refer to a fetal medicine specialist regardless of history	27 (61)
Refer to blood services for advice	7 (16)[2]
Request a paternal sample	35 (80)
Request cffDNA	7 (16)
Request a repeat sample	29 (66)
Other[1]	8 (18)

[1] Includes: discuss with obstetric team (3), ascertain transfusion history (2), proceed depending on paternal K type (2) and refer to medic (1)
[2] May reflect the number of blood service reference laboratories responding

Table 5 shows the timing of the first repeat sample requested (assuming no further information is available).

Table 5 – Repeat sample timing

First repeat sample	Number (%)
In 2 weeks	0 (0)
In 4 weeks	37 (93)
Other[1]	3 (7)
Total	40 (100)

[1] Includes: at 20 weeks (2) and depends on paternal K type (1)

Referral policy for anti-K

Table 6 shows the titration values at which a referral to a fetal medicine specialist would be triggered, were anti-K to be detected in a clinical sample from a pregnant woman at booking.

Table 6 –Titration values triggering referral to a fetal medicine specialist

Titre for referral	Number of participants (%)
Not referred	8 (19)
8	1 (2)
16	0 (0)
32	3 (7)
Any titre – all referred	30 (70)
Decision not made in laboratory	1 (2)
Total	43 (100)

Discussion

Workload

There were 36 responses to this question; 11 (31%) were from reference centres and 25 (69%) from other laboratories. Taking into account the higher estimated workload of the reference laboratories, approximately 80% of testing appears to be undertaken by reference services. It is also likely that some of the work undertaken in hospitals is also referred for confirmation.

Titration policy

The 2016 BSH guidelines for blood grouping and antibody testing in pregnancy[1], recommend titration of antibodies by IAT using cells heterozygous for the corresponding antigen, titration of the National Institute for Biological Standards and Controls (NIBSC) anti-D standard in parallel with each test and where possible testing in parallel with the previous sample. In this exercise, 27% stated that the NIBSC standard is used as an internal control and 80% stated that they would titrate the previous sample in parallel, if it were available. The majority (98%) stated that they select red cells heterozygous for corresponding antigen, in line with BSH guidance.

Results for Patient 4

There was some variation in the titration values reported in this exercise (from 8 to 64); 43/45 (96%) were within one dilution of the median. Although the majority (98%) selected red cells heterozygous for the K antigen, since cells for titration were not provided, some variation would have been introduced by the use of different examples of Kk cells. There was no change in the median result for those using a weak or 1+ reaction to denote the endpoint of the titration.

Testing protocols and referral to a Fetal Medicine Unit

The 2016 BSH guidance[1] on the frequency of testing and referral to a fetal medicine specialist of women with clinically significant red cell antibodies in pregnancy depends on antibody specificity, quantification or titration value and whether or not there is a history of Haemolytic Disease of the Fetus and Newborn (HDFN) in a previous pregnancy. All women who have previously had an infant affected by HDFN should be referred before 20 weeks to a specialist unit, irrespective of antibody level.

The 2016 BSH guidance[1] also indicates that pregnant women with anti-K or other Kell system antibodies (unless the father is confirmed to be negative for the corresponding antigen) should be assessed serologically at monthly intervals to 28 weeks gestation and at fortnightly intervals thereafter until delivery and referred to a fetal medicine specialist when the antibody is first identified. In this exercise only 60% of participants indicated they would refer to a fetal medicine specialist and only 78% would request a paternal sample. Non-invasive fetal genotyping using cell free fetal DNA (cffDNA) should be performed in pregnant women who have a history of HDFN or where quantification values or titres suggest that the pregnancy is at risk of HDFN. In this case, only 16% of participants would have referred for cffDNA, this may be because laboratories would wait for paternal K typing or because cffDNA for K (*KEL*01*) is generally only offered after 20 weeks gestation, due to the increased risk of a false negative result before this time[2].

Results for the UK NEQAS ABO Titration Scheme are scored as satisfactory if they are within one dilution of the median (by technology and where there are ≥20 results). In this exercise 96% of results were within one dilution of the median value for all results obtained by any technology. This may be a reflection of the probable lower IgM component of an anti-K compared to that found in ABO antibodies, and the variability of technologies in detecting IgM by IAT. However, the numbers using some technologies are very low and further investigation would be required to confirm this hypothesis.

As a final question participants were asked if they would be interested in participating in a UK NEQAS IgG titration scheme; 73/176 respondents worldwide including 45/130 in the UK and ROI indicated they would be interested.

Conclusions

Since the last UK NEQAS IgG titration exercise in 2011, there is an increase in the use of the NIBSC anti-D standard, with 27% (*cf.* 9% in 2011) titrating it in parallel with clinical samples as a control.

The results for exercise showed less variation in IAT results between different technologies than is seen in the UK NEQAS ABO Titration scheme.

Given the level of interest expressed, a UK NEQAS (BTLP) IgG titration pilot scheme is planned for 2018; participants will be invited to register once the details have been finalised.

References

[1]Guideline for blood grouping and red cell antibody testing in pregnancy http://www.b-s-h.org.uk/guidelines (accessed 10/10/2017)

[2]International Blood Group Reference Laboratory User guide for referring samples to IBGRL Molecular Diagnostics: Fetal RhD, Kell, Rhc, RhE, RhC and sex genotyping from maternal blood Effective 19/06/2017 http://ibgrl.blood.co.uk (accessed 10/10/2017)

Appendices

Appendix 1 – Summary of questions

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General

- In which country is your laboratory based?
- Do you undertake titration of IgG antibodies as part of antenatal testing?
- If Yes, how many antenatal women per year does this apply to?

Titration – 17E7 Patient 4

- Enter the IAT reaction grades (4+, 3+, 2+, 1+, weak, first 0), your titration score if routinely used and endpoint titre that you would report (e.g. 128 or 32).
- Which IAT technology did you use for the titration?
- Did you perform an IAT method exactly as described in the product insert for your technology?
- If your IAT method varied from the manufacturer's instructions, please give details below
- Which diluent did you use to make the plasma dilutions?
- Which red cell diluent did you use?
- What was the zygosity (for the relevant antigen), of the red cells used for this titration?
- If this were a 12-week antenatal booking sample, what actions would be triggered by this result, (assuming discussion locally with haematologist/obstetricians)?
- If requesting a repeat sample, and assuming no further information is available, when would you request the first repeat sample?

Titration policy

- What is the last positive reaction routinely taken into account to determine the endpoint of a titration?
- Please state the preferred zygosity of cells routinely selected for titration
- Is the previous sample (if available) routinely titrated in parallel with each new sample for titration?
- Is the NIBSC standard anti-D titrated in parallel each time a titration is performed?
- Where the antibody specificity identified in Patient 4 is found in a sample from an antenatal patient at booking, with no previous history of pregnancies affected by HDFN, would you refer to a fetal medicine specialist?
- If referring to a fetal medicine specialist at a specific titre, please specify titre

Pilot Scheme

- Would you be interested in taking part in a UK NEQAS pilot EQA scheme for IgG antibody titration?