

TITRATION OF RED CELL ANTIBODIES IN PREGNANCY UK PERFORMANCE AND POLICY

J White, R Haggas, M Rowley

UK National External Quality Assessment Service (NEQAS) for Blood Transfusion Laboratory Practice (BTLT), Watford, UK

Background

UK NEQAS ABO titration EQA exercises have demonstrated wide variation in titration results within and between technologies. In the UK, titration (or quantitation) of non-ABO clinically significant red cell antibodies is used to inform clinical management in pregnancy. The British Society for Haematology (BSH) guidelines for blood grouping and antibody testing in pregnancy (2016) provide algorithms for testing and referral. Pregnant women with Kell system antibodies (unless their partner is confirmed to be antigen negative) should be referred to a fetal medicine unit (FMU) when the antibody is first identified; titration should be undertaken at four-weekly intervals up to 20 weeks, two-weekly thereafter and referral made for non-invasive testing using cell free fetal DNA (cffDNA) at a gestation recommended by the reference laboratory.

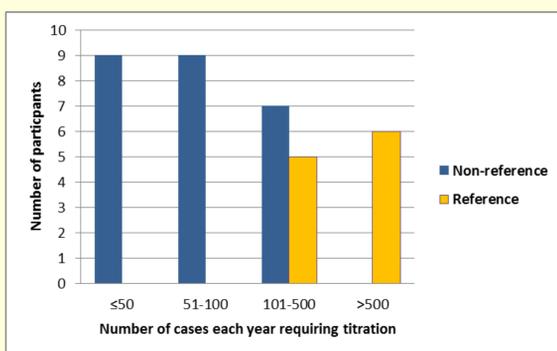
UK NEQAS exercise 17E7

In 2017, UK NEQAS BTLT offered optional assessment of titration on an EQA sample containing anti-K presented as an antenatal 'booking' sample; in the UK, the initial 'booking' appointment would be at approximately 12 weeks gestation. Laboratories were requested to use their in-house titration method and locally selected reagent red cells, and to answer a short questionnaire. The aim was to investigate UK practice in antenatal titration and referral, and whether there is requirement for and interest in an EQA Scheme for antenatal titration.

Titration results obtained by IAT were returned by 45 laboratories from the UK and Republic of Ireland. Five reported titration reaction grades but no titration value; in these cases an interpretation was made based on the reaction grades recorded and the endpoint (weak, 1+ or 2+) stated to be used in answer to the questionnaire. 40/45 (89%) answered the questionnaire.

Profile of participating laboratories

Figure 1 – shows the number of reference and non-reference laboratories participating, and their annual titration workload



Titration policy

The questionnaire explored the extent to which BSH guidance was followed with respect to selection of red cells for titration, parallel titration vs. the previous sample where possible, and use of the National Institute for Biological Standards (NIBSC) anti-D reference plasma. There was also a question on the titration end point used when testing clinical antenatal samples:

Endpoint of titration

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

Selection of heterozygous red cells

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

Comparison with previous sample

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

Use of NIBSC reference material

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

Results

Titration results

Figure 2 shows the number of participants using each IAT technology and the results obtained. The overall median result was 32.

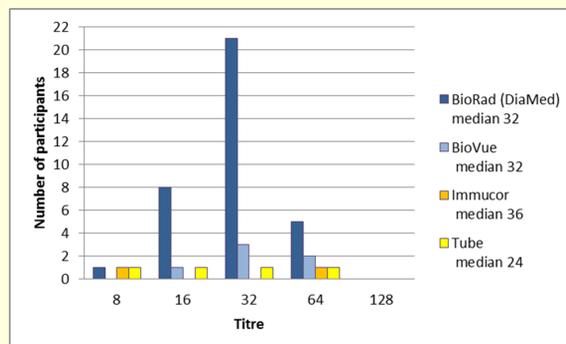
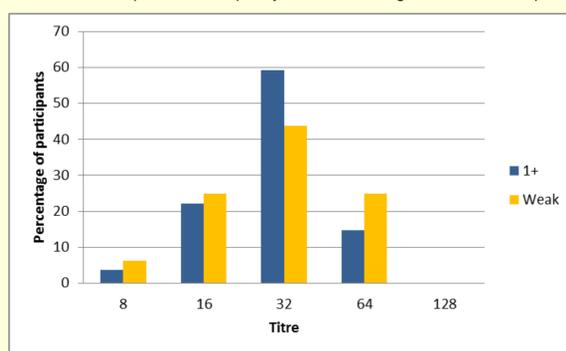


Figure 3 shows a comparison of the reported titre vs. the reaction grade used as an end point, based policy for determining the titration endpoint.



Actions triggered by titration result

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

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) ¹
Request a paternal sample	35 (80)
Request cffDNA	7 (16)
Request a repeat sample	29 (66)
Other ²	8 (18)

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

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

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

Figure 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 (12 weeks gestation).

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

Titration results

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.

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.

Clinical use of results

In this exercise, where anti-K was present in an antenatal booking sample, 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 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 Haemolytic Disease of the Fetus and Newborn (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 time².

It is important that clinical monitoring continues until it is confirmed conclusively that the fetus is K negative.

Conclusions

Patterns of variation in IAT titration results by technology, as seen in ABOT data, were not observed in this exercise.

For anti-K, titration results are used to make decisions on testing strategies and referral to a fetal medicine specialist; however, the trigger points used to make these decisions are not consistent.

For antibodies other than anti-K (or anti-D and anti-c which are quantified in IU in the UK), a titre of 32 is widely used as a trigger for further action, but this result does not necessarily represent the same concentration of antibody in all laboratories.

Following a positive response from participating laboratories, UK NEQAS is introducing a pilot scheme for antenatal antibody titration (ANT) to monitor antenatal titration results and raise awareness of guidelines for testing and referral.

References

¹Guideline for blood grouping and red cell antibody testing in pregnancy <http://www.b-s-h.org.uk/guidelines> (accessed 18/5/18)

²International 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://ibgri.blood.co.uk> (accessed 18/5/18)