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Automated Anti-D Titration with Calculated Titre Score using the Bio-Rad IH-500®

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Introduction

During pregnancy it is of vital importance to categorise the nature of the anti-D detected (whether immune or passive)[1] to avoid the risk of sensitisation.

The UK Antenatal Testing Guidelines were revised in 2016[2]; introducing a recommendation to measure anti-D concentration by continuous flow analysis (CFA). This method is considered the gold-standard for quantifying the concentration of anti-D present in a patient's sample, and therefore the likelihood of the antibody being immune in nature. Currently NHS Blood and Transplant (NHSBT) laboratories have access to this method which can be costly for hospital laboratories.

In a multi-centre comparative study Evans et. al.[3] demonstrated the automated titre score (TS) (figure 1, figure 2) method gave results that could be statistically linked to the results obtained by CFA. The study recommended the titre score can be used as an in-house screening tool to differentiate between passive and immune anti-D when using the Ortho Vision® immunohaematology platform.

Column Reaction Strength	Titre Score
4+	12
3+	10
2+	8
1+	5
0.5+	3
0	0

Figure 1. Individual column reaction strengths are converted to a score

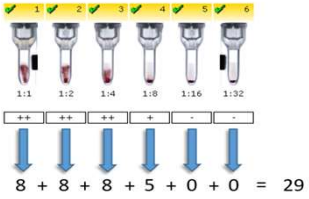

Figure 2. Worked example. Each column reaction is converted to a score. The sum of the scores is reported as the Titre Score.

Aims

Due to the recent change in immunohaematology analyser, we wanted to demonstrate correlation between our new system, the Bio-Rad IH-500® vs the Ortho Vision® platform by performing a direct method comparison.

A secondary aim was to establish the measurement of uncertainty for the technique in order to inform a safe TS cut-off value.

Methods

122 samples were tested on both the IH-500® and Ortho Vision® platforms using the on-board serial dilution methods native to each system. Titration was performed against NHSBT OR1r reagent red cell (ref: PR045). Titre score values were calculated using the method described by Evans et. al.[3]

A comparison of the obtained titre scores was performed and statistical significance calculated using Pearson's correlation coefficient.

50 replicates of NIBSC Anti-D standard (ref: 73/517) were used to calculate uncertainty of measurement.

Results

Strong correlation was observed between the Ortho Vision® and Bio-Rad IH-500® platforms (r(120):0.99) (figure3) with all data points falling within 95% confidence limits (figure 4).

The IH-500 ® demonstrated good reproducibility with an uncertainty of measurement of 1.82 when measured at a titre score of 34 (the clinical decision limit). This equates to around 6% uncertainty.

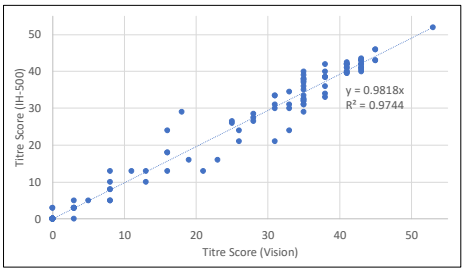

Figure 3. Linear regression

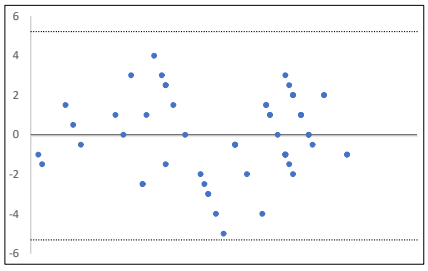

Figure 4. Bland-Altman plot. All data points within 95% confidence limit

Conclusions

Anti-D Titre score on the Ortho Vision® has been established as an alternative to CFA for differentiation between passive and immune anti-D in a previous study[3]. The method comparison performed here has demonstrated strong linear correlation between the Bio-Rad IH-500® and the previously validated method on the Ortho Vision® (r(120):0.99) therefore establishing the method as suitable to use on the Bio-Rad IH-500® platform. Due to the minor variations in the two technologies, an amended titre score cut off value of 34 was adopted using the Bio-Rad platform. This is due to the statistical correlation observed: $R^2 = 0.9744$.

It is a requirement of ISO15189 that measurement of uncertainty must be established for all methods. The measured uncertainty for the Bio-Rad IH-500® method was 1.82. As a result, we amended the original titre score cut-off to 32 allowing for a cautious approach to the risk associated with uncertainty. Organisations must appraise uncertainty of measurement within their own setting and apply this to the original recommended cut off (34).

A cautious approach should be adopted to design a decision making algorithm to aid interpretation of TS results. This should incorporate the following factors: whether any additional antibody specificities are present, whether anti-D was detected prior to injection of anti-D immunoglobulin, and whether the patient has received anti-D immunoglobulin within the last 12 weeks.

Taking into account all these factors allows laboratories to deploy the automated titre score in place of the CFA test, enabling faster turn-around times and a cost saving to the organisation.

Future Aims

We continue to collect data for patients with immune anti-D with the ultimate aim of establishing whether the titre score method could be extended to predict risk (low / medium / high) to the pregnancy in the same way CFA is currently used. Clinical input for this stage of the project will be sought.

References

1. SHOT Serious Hazards of Transfusion (SHOT UK2012); 2011 Annual SHOT report. www.shot-uk.org. Accessed May 10, 2023.
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3. Evans ML, Holmes B, Dowling K, et al. Evaluating automated titre score as an alternative to continuous flow analysis for the prediction of passive anti-D in pregnancy. Transfusion Medicine. 2021;31:36–42.
4. Bruce DG, Tinegate HN, Williams M, Babb R, Wells AG. Antenatal monitoring of anti-D and anti-c: could titre scores determined by column agglutination technology replace continuous flow analyser quantitifcation? Transfusion Medicine 2013;23:36-41