Correlation of Serological Anti-D Reaction Strength by Indirect AntiglobulinTechnique to Anti-D Quantitation Level

Keogh M, Doyle B, Ni Loingsigh S.

## Introduction/Background

Despite the introduction of Anti-D Immunoglobulin (ADIg) prophylaxis, Anti-D remains the leading cause of Haemolytic Disease of the Newborn (HDN). Recent British Committee for Standards in Haematology (BCSH) guidelines recommend that all maternal samples with a serologically detectable anti-D at 28 weeks' gestation have antibody quantitation (AQ) with repeat referral every 2 weeks if anti-D remains detectable by indirect antiglobulin technique (IAT) (BCSH, 2016) regardless of prior administration of ADIg. It is not possible to distinguish between prophylactic anti-D or immune anti-D using serological techniques or using continuous flow antibody quantitation analyser.

Passive ADIg post-prophylaxis rarely exceeds 0.4 IU/mL - unless more than 1500IU ADIG has been administered (BCSH, 2016). In Ireland, the standard prophylactic anti-D dose is 1500IU - significantly higher than the standard UK dose (250IU or 500IU). This higher dosage regime results in passive anti-D being detected for a longer period in patients' plasma. Implementation of these guidelines in Ireland could result in up to 5-6 extra AQ assays if a patient has been given ADIg following a potentially sensitising event prior to 28 weeks' gestation.

The BCSH guidelines state that the serological strength of anti-D cannot be used to predict if the anti-D present is immune or prophylactic in nature. The aim of this study is to assess if there is a reliable correlation between serological reaction strength of anti-D and anti-D quantitation levels.

## Materials and Methods

Retrospective data analysis was performed on samples referred to the IBTS for anti-D quantitation using eTraceline LIMS and R8 GMP programme. Reaction strength by IAT of patient plasma versus an R2R2 cell tested using BioRad microtyping card was graded from 0 to 4+. Patients with an additional anti-E were excluded. One hospital (Hospital A) had already implemented the policy of referral of all samples containing Anti-D at 28 weeks' gestation, regardless of prior anti-D administration. Two data sets were analysed (1) samples referred from all hospitals for anti-D quantitation in 2015 (n=624) and (2) samples referred from Hospital A from Jan 2014- Feb 2016 where anti-D had been given prior to sampling (n=144).

## Results

For all samples tested in 2015, no quantitation level exceeded 0.4IU/ml where the reaction strength was 1.5 or less (Table 1). There was little correlation between reaction strength and quantitation level. When the reaction strength reached 2+, the quantitation levels ranged from <0.1 to 5.99IU/ml. In the subset where prophylaxis was given prior to sampling, no quantitation level exceeded 0.4IU/ml where the reaction strength was 2+ or less. Of note, one sample with a weak reaction strength of 0.5+ by IAT, had a quantitation level of 0.4 IU/ml. Again there was little correlation between reaction strength and quantitation level, however, the quantitation levels associated with a 3+ reaction strength (max 0.25IU/ml) were considerably less than in the general data subset (max 350.44IU/ml).

## Conclusion

A reaction strength of <2+ on our assay predicted an AQ of ≤0.4 IU/ml in our dataset. Reaction strengths of 2+ or greater correlate poorly with AQ levels.

**Reference** British Committee for Standards in Haematology (2016): White J, Qureshi H, Massey E, Needs M, Byrnes G, Daniels G, Allard S. Guidelines for blood grouping and antibody testing in

pregnancy. Available at: http://www.bcshguidelines.com/documents/2016-02-23_BCSH_Grouping_Ab_testing_in_pregnancy.pdf accessed 30/08/2016