Analysis of potential interference in antibody quantification assay utilised in antenatal testing

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Background

Haemolytic Disease of the Fetus and Newborn (HDFN) is the destruction of fetal and newborn red cells by maternal red cell antibodies, specific for paternally inherited red cell antigens.

HDFN can lead to severe anaemia requiring intrauterine transfusions (IUTs), hydrops fetalis and in some circumstances fetal death.

Approximately 1% of pregnant women are found to have clinically significant red cell antibodies, with more than 50 red cell allo-antibodies known to cause HDFN (Smith et al., 2013).

Anti-D identification and strength in antenatal patients is determined by performing an indirect antiglobulin test (IAT) and an antibody quantification assay.

Antibody quantification aids in predicting clinical outcome of the fetus. It indicates the risk level of developing HDFN and indicates if fetal assessment by middle cerebral artery (MCA) Doppler ultrasonography is warranted.

Antibody quantification is performed in antenatal patients with passive and immune anti-D. Result thresholds are available to inform patient management with regards to specialist fetal medicine referral and the requirement of receiving prophylaxis.

The significance of levels of anti-D

Anti-D concentration	Predicted clinical outcome
Less than 4 IU mL^{-1}	HDFN unlikely, continue to monitor
4-15 IU mL^{-1}	Moderate risk of HDFN, requiring referral to a fetal medicine specialist
More than 15 IU mL^{-1}	High risk of HDFN requiring referral to a fetal medicine specialist

Table 1: Anti-D threshold levels. BSH guidelines (White et al., 2016

Interference from other antibodies could impact on the antibody quantification levels reported. Such antibodies include the presence of other red cell allo-antibodies, auto-antibodies, cold reactive antibodies and enzyme only antibodies.

It is critical to distinguish the accurate anti-D concentration in order for the patient to receive the appropriate level of care.

This study determines if red cell antibodies reactive with enzyme treated cells and cold reactive antibodies interfere with antibody quantification methods and results, therefore potentially impacting on patient management.

Methods

Serological testing was performed by the Red Cell Immunohaematology (RCI) Laboratory at the Irish Blood Transfusion Service (IBTS).

•A total of 14 patient samples containing anti-D and either enzyme only antibodies (n=4) or cold reactive antibodies (n=10) were analysed by the Continuous Flow Analyser (CFA) and Flow Cytometry (FC) methods.
•The CFA analyses haemolysed cells optically which is inversely proportional to the concentration of the anti-D.
•The FC method allows for indirect binding of bound anti-D and was performed on a BD FACSCanto™ II.
•Antibody levels are determined by comparison to a standard curve.
•Results were evaluated to investigate if methods are subject to potential interference.

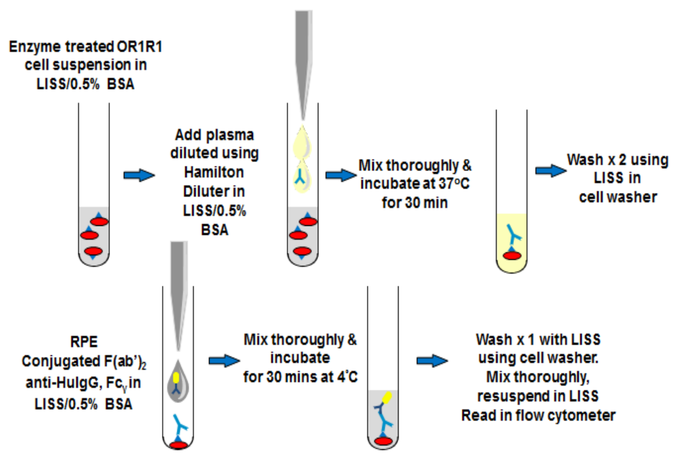

Figure 1: FC method-Indirect antibody binding using a fluorescent labelled anti-human IgG quantitating bound anti-D (Green., 2014). [Personal communication]

Results

Samples 1,2 and 3 obtained from the same individual showed potential interference due to the presence of a strong enzyme only antibody with CFA but not with FC (9.29 vs. 0.81 IU/ml, 9.60 vs. 0.81 IU/ml and 7.87 vs. 1.15 IU/ml).

Patient management implications vary significantly between methods. The remaining 11 samples were not subject to potential interference and minor differences were encountered between methods.

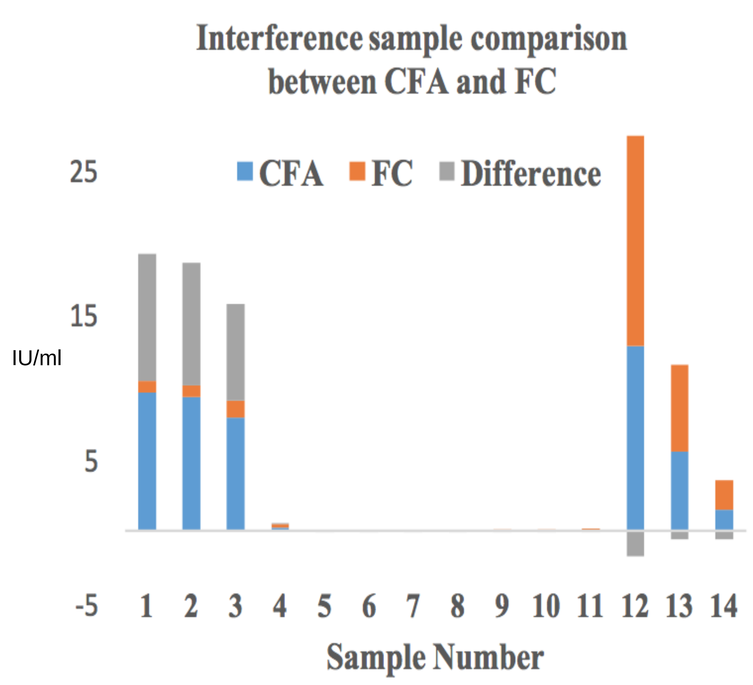

Figure 2: A stacked histogram comparing the CFA (blue), FC (orange) results (IU/ml) and the difference (grey) between the two datasets.

Conclusion

The RCI lab in the IBTS provides a national antibody quantification service in the Republic of Ireland. The objectives of anti-D quantification are to identify pregnancies potentially at risk of HDFN and aid in the distinction of immune and prophylactic anti-D.

A 92 sample cohort was previously analysed by FC and compared to CFA results (unpublished data). The study found a Pearson's correlation coefficient (r) of 0.94. between the two methods and a p-value > 0.05, indicating no clinical significance between datasets and overall good agreement between methods.

In this study, samples 1, 2 and 3 clearly show a marked difference in results between FC and CFA which would completely alter the management of the patient if the potential interference had been unknown. The CFA quantification technique uses pooled enzyme treated red cells, therefore the potential for these cells to cross react with enzyme reactive allo or auto antibodies with specificities other than anti-D is possibly resulting in a higher concentration threshold level.

In this case, it is postulated that the enzyme only antibody present is IgM in nature and cross-reactivity cannot occur with the IgG specific binding FC method, therefore this method is not subject to the interference. The weakly reacting enzyme antibody detected in sample 4 had no implication on the patient's management concluding that antibody strength is also a factor. It is suggested care should be taken when testing patient's with a strong (>3+ reaction) enzyme antibody as this could produce a falsely elevated result.

The potential impact with regard to patient management when interference is present include; referral to the fetal medicine assessment, increased follow up appointments and hospital attendance, increased sample volume for laboratories and increased stress to the mother. However, there is no risk to the fetus.

Where prophylaxis has been given, interference could over estimate the level of anti-D and lead to an erroneous conclusion whether the anti-D present is immune or passive in nature.

Current practice in the RCI laboratory is to quantitate the anti-D sample versus rr (dce/dce) phenotyped cells to ascertain the extent of the interference. It is possible that FC may have an advantage over CFA when testing patients with additional enzyme reactive antibodies in an antenatal setting.

Care must be taken to distinguish true quantification results from potential interfering antibodies ensuring appropriate patient management.

References

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