Validation of the Automated Antibody Titration with the IH-500 System at the Blood Transfusion Centre of Slovenia

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Background

During pregnancy, when a clinically significant antibody is detected and identified, it must be titrated to support the prediction and management of hemolytic disease of the fetus and newborn (HDFN). The standard for this semi-quantitative method is still the conventional tube test (CTT). It is known that other methods such as the gel column agglutination technology test (CAT), which is becoming a suitable alternative to the standard, results in higher titers and therefore must be validated with clinical findings and laboratory data to ensure appropriate interpretation. In our laboratory for prenatal testing, we routinely perform the manual CTT and CAT methods. However, manual methods have several limitations, including poor reproducibility, measurement errors depending on the experience and accuracy of the operator, changing working conditions, and variation in result interpretation among different technicians. On the other hand, the CAT method, proven reliable for many immunohematology tests, is compatible with automation, which improves reproducibility and objectivity and allows storage of immunohematology test results.

Aims

This study evaluates the first step of validating the automated antibody titration and compares manual and automated antibody titration using the CAT method to assess whether automated titration is accurate and reliable enough to predict HDFN.

Methods

Thirty-six plasma samples from pregnant women, who had clinically significant antibodies, were evaluated. Antibody titration was performed by serial two-fold dilutions of plasma by manual and automated methods. The manual titration was performed with a selection of different commercial reagent red blood cells (RBCs) from Ortho Clinical Diagnostics (United Kingdom), used routinely, and from Bio-Rad (Switzerland), used for comparison on Bio-Rad's LISS/Coombs ID-Cards. The automated titration was performed on Bio-Rad's IH-500 System with the ID-Titration Solution. The dilution showing the last agglutination grade 1+ defined the final titer, while the dilution with the last reactive result defined the end-point titer. For more objectivity, we considered the automated interpretation of the analyzer. The selected RBCs expressed the corresponding antigen in a single dose. In the presence of several alloantibodies, the titers were determined individually. The final titers obtained automatically were compared with the final titers obtained manually using RBCs from different as well as the same manufacturers.
We also tested the reproducibility of the automated titration, using the same and different RBCs with a single dose of the corresponding antigen.

Results

Forty-one titrations including 2 anti-K, 11 anti-D, 3 anti-C, 1 anti-c, 16 anti-E, 3 anti-C^w, 3 anti-M, 1 anti-s, and 1 anti-A_1 were performed and evaluated. According to the set criteria, the final automatically determined titers, in comparison to the final automatically determined titers, were the same in 56% (23/41) of the antibodies tested (100% of anti-K, anti-C, anti-c, anti-s and anti-A_1, 67% of anti-C^w, 50% of anti-E, 45% of anti-D, and 0% of anti-M), lower in 3 cases (67% of anti-M and 10% of anti-D), and higher in 15 cases (50% of anti-E, 45% of anti-D, and 33% of anti-M). The titers ranged between 2 and 2,048 for both methods. A difference within two titers was found in 94% (17/18) and one result within four titers (one case of anti-M). The automated method's final titers and end-point titers were the same in 23 cases (56%) and differed for no more than two titers in the remaining 18 cases (44%). In 19 cases, the final titers determined manually were identical to the end-point titers determined automatically which increased the proportion of the same results from 56% to 73%. We confirmed the reproducibility of the automated titration results when using the same RBCs. When different RBCs with a single dose of the corresponding antigen were used, different final and end-point titers were seen (i.e., anti-M), highlighting the importance of the selected RBCs.

Summary / Conclusions

Although our results were the same in 56% of the compared titers between the routine manual method and the automated one, our results included the most clinically significant antibodies (e.g., anti-K, anti-c, anti-C, anti-D) in the antenatal context. Moreover, the difference overall was within two titers, which is widely acceptable. The differences in anti-D titers may have been because the manual method interpreter exercised more caution, resulting in higher variability than the automated analyzer interpretation. Expected higher deviations were found in the titers for anti-E and anti-M, a combination of IgGs and IgMs, which are more sensitive to working conditions such as temperature. In addition, we did not use dithiothreitol (DTT) treatment, as it is not part of the routine even in the manual method. We believe automated antibody titration using the CAT method, such as Bio-Rad's solution, is one of the next steps toward standardization because of its reliability, accuracy, and traceability. Finding a correlation with clinically relevant HDFN and defining a critical level of antibodies is the next step in our validation.

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