Assessment of Flow Cytometry and Kleihauer-Betke Method When Calculating Fetomaternal Hemorrhage and Rh Immunoglobulin Dose

To the Editor—Alloimmunization from fetomaternal hemorrhage remains an ongoing cause of hemolytic disease of the fetus and newborn. Though failure rates of postnatal prophylaxis have decreased to less than 1% to 2%, it is critical to minimize laboratory errors when calculating Rh immunoglobulin (RhIg) dose to prevent maternal alloimmunization. Flow cytometry and the Kleihauer-Betke method are 2 means of calculating the necessary RhIg dose, with flow cytometry considered a more precise, but possibly more expensive, method, and not readily available 24 hours a day.

Using data collected by the College of American Pathologists (CAP) fetal red blood cells (RBCs) detection surveys from 2014–2019 (24 proficiency testing samples to approximately 50 laboratories using flow cytometry and 1000 laboratories using Kleihauer-Betke) where the recommended RhIg dose was calculated from mean reported values with the CAP RhIg Dose Calculator, we compared differences in dose calculation. Paired t tests were used to assess differences in mean reported percentage of fetal RBCs and recommended RhIg dose. Results showed that laboratories using Kleihauer-Betke overestimated the percentage of fetal RBCs on average by 0.221% more (5.292 total percentage points over 24 instances) (95% CI, 0.213%–0.227%), with a standard deviation of 0.190. In 6 of 24 CAP surveys (25%), this average increase in reported fetal RBCs resulted in an increase of RhIg dose by 1 vial. In no instances did the Kleihauer-Betke method lead to a dose recommendation lower than the flow cytometry results.

Inherent limitations to using Kleihauer-Betke include lack of standardization, associated labor to perform, and relative imprecision with a coefficient of variation of 30% to 80% as demonstrated in the past 5 years of CAP proficiency testing (2016–2020). Advantages include its ease of access, inexpensive cost, and lack of special equipment needed. Although flow cytometry can analyze a larger batch of samples—resulting in greater quantitative accuracy and reproducibility, it is more precise with a coefficient of variation less than 20%, and can distinguish between adult F and fetal cells, it is relatively more expensive, time-consuming, and inaccessible.

Ultimately, Kleihauer-Betke is a less precise measure of fetomaternal hemorrhage quantification than flow cytometry; however, on average it will result in a similar to increased recommended dose of RhIg. Given the low incidence of severe adverse reactions to RhIg for antenatal and postnatal prophylaxis, greater RhIg dosing by 1 vial one-fourth of the time is generally safe. The risks of RhIg do not exceed risks associated with any other pooled human product, few incidents of viral transmission have been reported in North America, and RhIg is currently manufactured with viral inactivation products. Flow cytometry is currently not feasible for many laboratories owing to the need for special equipment and longer duration of turn-around times, which can impact patient care. Therefore, Kleihauer-Betke remains an adequate method for assessing fetal-maternal hemorrhage and RhIg dose. Standardizing training and methods for using Kleihauer-Betke may improve precision. Regardless of method used to quantify fetomaternal hemorrhage, use of tools including the CAP RhIg Calculator can improve quality of patient care and reduce inadequate dosing.

Catherine Gereg, BS1; Mark K. Fung, MD, PhD2
1Larner College of Medicine, University of Vermont, Burlington; 2Department of Pathology and Laboratory Medicine, University of Vermont Medical Center, Burlington

The authors thank Mary Griffin, MD, for her work in the initial data collection and analysis for this study.


Accepted for publication November 8, 2022.

The authors have no relevant financial interest in the products or companies described in this article.

doi: 10.5858/arpa.2021-0432-LE