Received: 6 August 2020 | Revised: 24 September 2020 | Accepted: 5 October 2020

DOI: 10.1111/ijlh.13380

ORIGINAL ARTICLE

ISLH International Journal of Laboratory Hematology WILEY

# Evaluation of machine learning–driven automated Kleihauer-Betke counting: A method comparison study

Zhuoran Zhang[1] | Ji Ge[1] | Zheng Gong[1] | Jun Chen[1] | Chen Wang[2,3] | Yu Sun[1,4,5,6]

[1]Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, ON, Canada

[2]Lab Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

[3]Division of Hematology, Mount Sinai Hospital, Toronto, ON, Canada

[4]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada

[5]Department of Electrical and Computer Engineering, University of Toronto, Toronto, ON, Canada

[6]Department of Computer Science, University of Toronto, Toronto, ON, Canada

**Correspondence**
Chen Wang, Rm 602 - 600 University Ave, Toronto, Ontario M5G 1X5, Canada.
Email: chen.wang@sinaihealthsystem.ca

Yu Sun, Rm 419 – 5 King's College Road, Toronto Ontario M5S 3G8, Canada.
Email: sun@mie.utoronto.ca

**Funding information**
University of Toronto; Natural Sciences and Engineering Research Council of Canada

## Abstract

**Introduction:** The Kleihauer-Betke (KB) test is the diagnostic standard for the quantification of fetomaternal hemorrhage (FMH). Manual analysis of KB slides suffers from inter-observer and inter-laboratory variability and low efficiency. Flow cytometry provides accurate quantification of FMH with high efficiency but is not available in all hospitals or at all times. We have developed an automated KB counting system that uses machine learning to identify and distinguish fetal and maternal red blood cells (RBCs). In this study, we aimed to evaluate and compare the accuracy, precision, and efficiency of the automated KB counting system with manual KB counting and flow cytometry.

**Methods:** The ratio of fetal RBCs of the same blood sample was quantified by manual KB counting, automated KB counting, and flow cytometry, respectively. Forty patients were enrolled in this comparison study.

**Results:** Comparing the automated KB counting system with flow cytometry, the mean bias in measuring the ratio of fetal RBCs was 0.0048%, with limits of agreement ranging from −0.22% to 0.23%. Using flow cytometry results as a benchmark, results of automated KB counting were more accurate than those from manual counting, with a lower mean bias and narrower limits of agreement. The precision of automated KB counting was higher than that of manual KB counting (intraclass correlation coefficient 0.996 vs 0.79). The efficiency of automated KB counting was 200 times that of manual counting by the certified technologists.

**Conclusion:** Automated KB counting provides accurate and precise FMH quantification results with high efficiency.

KEYWORDS

automation, fetomaternal hemorrhage, Kleihauer-Betke test, machine learning

## 1 | INTRODUCTION

In North America, there are over 6 million pregnancies annually. Approximately 10% are of a Rhesus D (RhD)-negative woman with an RhD-positive baby. During pregnancy or childbirth, small amounts of fetal blood can enter the maternal circulation (fetomaternal hemorrhage or FMH) due to obstetric complications, trauma, fall, accident, domestic abuse, placental abruption, or sometimes no identified causes and finally grow into alloimmunization with a high risk.[1,2] In subsequent pregnancies, red blood cell (RBC) alloimmunization will lead to fetal hydrops, severe fetal anemia, heart failure, or even fetal death.[3,4] Therefore, reliable detection and quantification of FMH are necessary

Zhuoran Zhang and Ji Ge contributed equally to this work.

for physicians to make treatment decisions, for instance, the administration of an appropriate therapeutic dose of RhD immune globulin (RhIG) to prevent immunization of the patient.[5,6]

Fetomaternal hemorrhage quantification requires the determination of the ratio of fetal to total RBCs in a blood sample. The task involves the accurate identification of the low number of fetal RBCs and the counting of the total number of RBCs. The most reliable way to identify and count fetal RBCs is by flow cytometry.[7-9] Sample preparation and the operation of flow cytometry have strict skill requirements, and due to the high demand of flow cytometry in hospitals, its use for FMH quantification is restricted or limited (in less than 5% laboratories in the US[10]). Additionally, flow cytometry laboratories are not always operational at nights or on weekends. For these reasons, most hospitals still use the Kleihauer-Betke (KB) test for FMH quantification, which is based on differential resistance of fetal and adult hemoglobin (ie, HbF and HbA) to acid (HbF is significantly more resistant). Because of the resistance to acid, fetal RBCs show a slightly brighter red color than maternal RBCs. A certified lab technologist places the prepared KB slide on a microscope stage and looks through the eyepieces to count the RBCs. Typically, the technologist counts approximately 2000 RBCs, in 15 ~ 20 minutes, and calculates the percentage of fetal RBCs.

In practice, counting is a difficult task because the technologist, while counting, must make quick judgment to differentiate a fetal RBC from maternal RBCs based on differences in color, size, and texture. As shown in Figure 1, a typical KB slide may also contain neutrophils, contaminants, and overlapping cells, making manual KB counting challenging and subjective. Thus, the results of manual counting heavily depend on the experience of technologists. High inter-observer and inter-laboratory variability has been shown,[11,12] calling for the reduction of human involvement and automated counting of KB slides.

Attempts have been made to automate KB counting,[13,14] in which automation referred to the use of a motorized microscope stage to scan the KB slide and capture cell images. Fetal RBCs were still manually identified and distinguished from maternal RBCs by technologists based on intensity and distribution patterns of staining. The total number of cells was also manually estimated (vs counted). To date, no system exists that is capable of automated counting of fetal and maternal RBCs.

We have developed a fully automated system for reading KB slides and quantifying the fetal RBC ratio in a blood sample.[15] The system was built on a standard upright microscope with a motorized microscope stage and a digital camera to automatically capture images of different fields of view of a KB slide. The system uses computer vision algorithms to extract cell features, including the pixel value, color, gradient, area, and roundness of the cell, and then uses a machine learning model to distinguish fetal RBCs from maternal RBCs. The system was trained to separate RBCs that overlap, identify cases of neutrophils and contaminants, and reject these cases from the determination of fetal RBC ratio.

Here, we report the results of a comparison study using randomly selected clinical blood samples from patients at risk of FMH. The aim was to assess the accuracy, precision, and efficiency of the automated KB counting system. Flow cytometry was used as a benchmark for evaluating the accuracy of automated and manual KB counting. The precision and efficiency of the automated system were compared with those of manual KB counting.

## 2 | MATERIALS AND METHODS

### 2.1 | Blood sample preparation

Blood samples were collected from the patients who were being investigated for FMH during pregnancy or postpartum at Mount Sinai Hospital, Toronto. Collection and analysis of blood samples were approved by the Research Ethics Board of Mount Sinai Hospital, and informed consent was obtained from all subjects. All samples were processed within 4 hours of collection. Forty patients were enrolled in the study. The sample size achieved a power of 0.9 for method comparison (see "Sample Size" in Supplementary Information). No blood samples were voted out or excluded from the study.

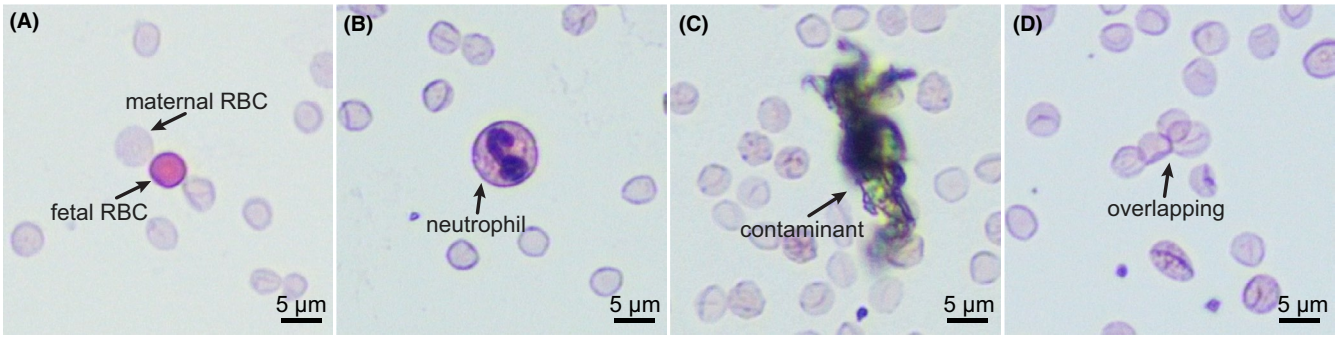


**FIGURE 1** (A) An ideal clinical Kleihauer-Betke (KB) slide image of fetal and maternal red blood cells (RBCs). Fetal RBCs show brighter red color than maternal RBCs. A typical clinical KB slide may also contain (B) neutrophils, (C) contaminant, and (D) overlapping cells, making it a challenging task to read/count KB slides [Colour figure can be viewed at wileyonlinelibrary.com]

### 2.2 | Flow cytometry analysis

Flow cytometry analysis used mouse monoclonal antibody specific to human fetal hemoglobin (Phycoerythrin-conjugated anti-HbF clone HbF-1; Invitrogen). Blood specimens were diluted with PBS according to RBC counts as determined on an automated hematology analyzer (Sysmex XE 2100; Sysmex Lincolnshire). An aliquot of 20 μL of whole blood (approximately $2.5 \times 10^7$ RBCs) was fixed in 1 mL cold 0.05% glutaraldehyde in PBS, mixed by vortex, and incubated at room temperature for 10 minutes. 100 μL of the mixture was then treated in 0.4 mL of cold 0.1% Triton X-100 (Sigma-Aldrich) in 0.1% BSA-PBS at room temperature for 5 minutes. 10 μL of the permeabilized sample was incubated with anti-HbF antibody in a total volume of 100 μL in the dark at room temperature for 15 minutes. The mixture was added to 0.5 mL of 1% formaldehyde in 0.1% BSA-PBS, and the suspension was used for flow cytometry analysis. A total of 50 000 cell events were acquired from each sample on a flow cytometer (FC500; Beckman Coulter). The RBC area was gated by the measurements of forward scatter (FSC), side scatter (SSC), log orange fluorescence, and log green fluorescence for autofluorescence. The regions of fetal RBCs and adult RBCs were set based on three levels of control cells (FETALtrol; Trillium Diagnostics) that were used in each test of patient samples. Data analysis was performed with the CXP Software (Beckman Coulter).

### 2.3 | Preparation of KB slides

Each KB slide was made with 2 ~ 3 μL of the diluted whole blood with equal volume of saline. The slides were air-dried, fixed in 80% ethanol for 5 minutes, and rinsed twice with deionized water. Acid elution was performed by immersing slides in citric acid/phosphate buffer, pH 3.2, at 37°C for 5 minutes. The citric buffer was freshly prepared by mixing stock solutions of 147 mL 0.1 M citric acid ($C_6H_8O_7{\cdot}H_2O$, MW 210.14) and 53 mL 0.2 M disodium phosphate ($Na_2HPO_4$, MW 141.96). The slides were rinsed with deionized water, first stained with hematoxylin at room temperature for 5 minutes, washed again, and then placed in 0.5% eosin Y solution at room temperature for 5 minutes. The slide preparation was complete following final wash and air-dry, and ready for microscopic analysis. Control samples of normal adult blood spiked with cord blood were included in each test of patient samples to validate the KB procedure.

### 2.4 | Manual KB counting

Each KB slide was counted independently by three certified technologists. Fetal cells and adult ghost cells were counted and recorded on the entire field until 2000 cells were counted in total. The total time for counting 2000 cells in each KB slide was recorded. The percentage of fetal cells was calculated by dividing the number of fetal cells by the total number of cells.

### 2.5 | Automated KB counting

The same KB slides were put on the automated system. For each KB slide, the system automatically controlled the motorized stage to capture 60 fields of view of the KB slide, which typically contained more than 20 000 cells. The 60 images were then automatically analyzed to calculate the ratio of fetal RBCs. The total time for counting each KB slide, including image capture and image analysis, was recorded. A supervised machine learning model was trained to use the extracted features as input to classify each cell into fetal RBC or maternal RBC. The training dataset contained images of 10 000 fetal RBCs (as identified and agreed upon by the three certified technologists) and another 10 000 as non-fetal RBCs (see Supplementary Information).

### 2.6 | Statistical analysis

Passing-Bablok analysis and Bland-Altman analysis were performed to calculate the agreement between two methods, that is, automated KB counting versus flow cytometry, and manual KB counting versus flow cytometry. The intraclass correlation coefficient was calculated to evaluate the precision of automated KB counting and manual KB counting, respectively. For all statistical analyses, $P < .05$ was considered significant difference. All statistical analyses were performed using MedCalc 18.11.3.

## 3 | RESULTS

### 3.1 | Automated KB counting provides as accurate FMH results as flow cytometry

To evaluate the accuracy of the automated KB counting system in measuring the ratio of fetal RBCs, we first compared the counting results by the automated KB counting system to that by the gold-standard flow cytometry. Passing-Bablok regression analysis (n = 40) showed a slope value of 1.03 with a 95% confidence interval (CI) of 0.98 to 1.08, and an intercept value of −0.010% with a CI of −0.011% to −0.0032% (Figure 2A). The Cusum test for linearity showed no significant deviation from linearity with $P = .38$. Bland-Altman analysis (n = 40) comparing the automated KB counting results and flow cytometry results showed a mean bias of 0.0048% with a CI of −0.032% to 0.042%. The limits of agreement ranged from −0.22% to 0.23% (Figure 2B). Further regression analysis of the difference in Bland-Altman analysis showed an insignificant trend of increasing/decreasing (slope value: −0.022 with a CI of −0.055 to 0.011, $P = .18$), indicating that the differences between the two methods did not increase or decrease with the measured ratio of fetal RBCs. Collectively, these results showed that there were no fixed differences between the automated KB counting and flow cytometry in measuring the ratio of fetal RBCs.

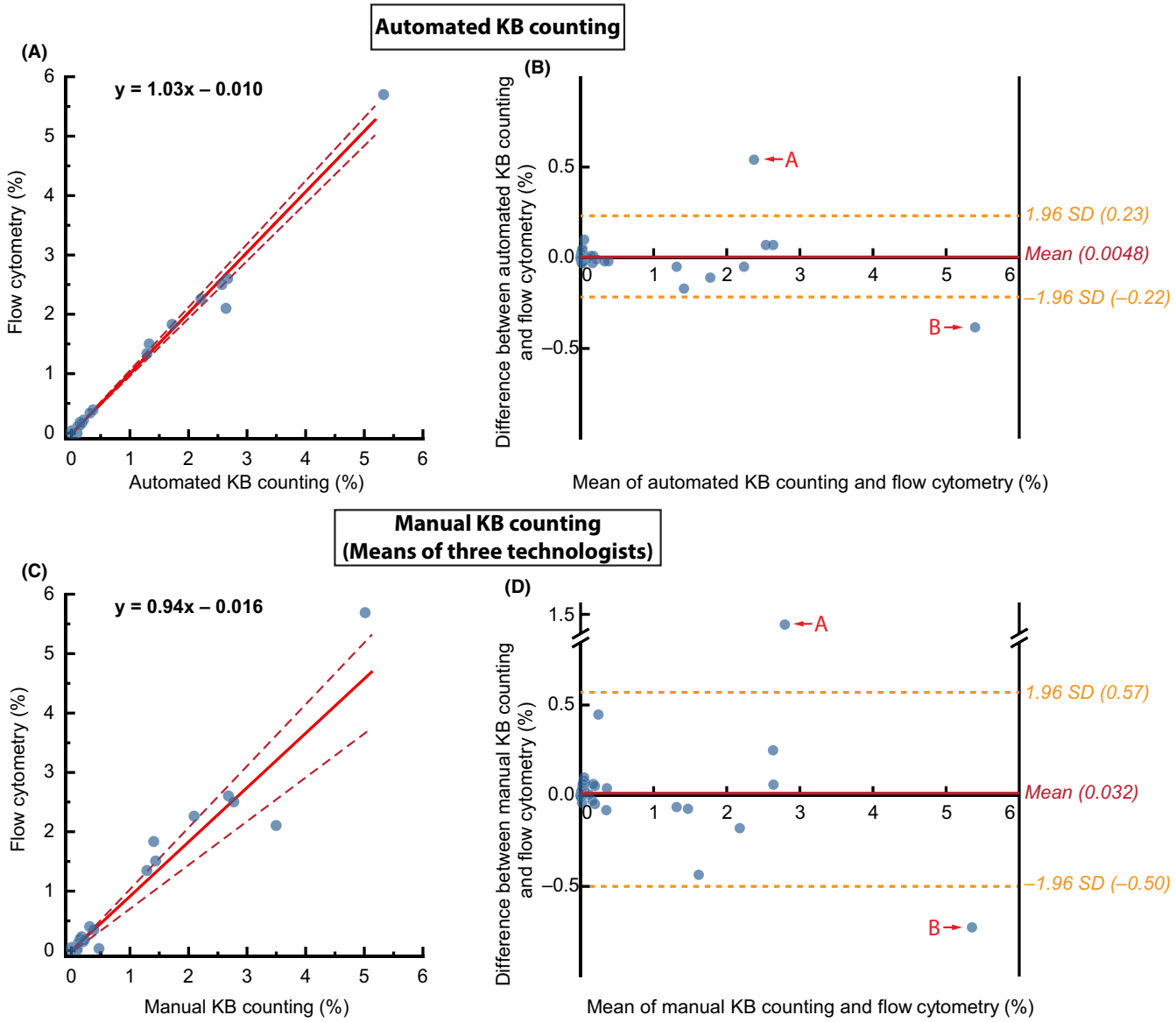


**FIGURE 2** Comparison of the ratio of fetal red blood cells (RBCs) from 40 patients measured by (A-B) automated Kleihauer-Betke (KB) counting vs flow cytometry, and (C-D) manual KB counting vs flow cytometry. The means of counting results from three technologists were calculated to represent manual KB counting. (A,C) Passing-Bablok regression analyses. The solid red line represents the regression line, and the dashed lines represent the 95% confidence bands. (B,D) Bland-Altman analyses. The solid red line represents the mean bias between the two methods, and the dashed yellow lines represent the limit of agreements. The arrows A and B label points that fall outside of the limits of agreements [Colour figure can be viewed at wileyonlinelibrary.com]

To further evaluate the diagnostic accuracy, the 40 samples were divided into FMH positive (fetal RBCs ≥ 0.1%[14]) and FHM negative (fetal RBCs < 0.1%[14]) according to the results from flow cytometry. Among the 40 samples, the automated KB counting gave 15 true positive and 25 true negatives (Table 1). No false positive or false negative results were given by the automated KB counting system.

## 3.2 | Automated KB counting provides more accurate and precise results than manual KB counting

The accuracies in measuring the ratio of fetal RBCs by automated KB counting and by manual KB counting were both benchmarked to flow cytometry. The results from the three technologists were averaged as mean of manual KB counting. Passing-Bablok regression analysis and Bland-Altman analysis were performed on both mean of manual KB counting and on results from each technologist (Figure 2C-D). Note that averaging the results from the three technologists significantly reduced the bias and narrowed the limits of agreement in manual measurement (Figure S4 and Table S1 in Supplementary Information). Even compared to the mean of manual KB counting, automated KB counting showed a smaller bias and narrower limits of agreement in measuring the ratio of fetal RBC (Figure 2). Collectively, the data showed that the automated system provided more accurate counting results than manual counting.

When analyzing the same KB slide, each of the certified technologist showed a different mean bias and different

**TABLE 1** Contingency table comparing automated Kleihauer-Betke (KB) counting, manual KB counting, and flow cytometry

| | | Flow cytometry | | |
|---|---|---|---|---|
| | | Negative | Positive | Accuracy |
| Automated KB counting | Negative | 25 | 0 | 100% |
| | Positive | 0 | 15 | |
| Mean of manual KB counting | Negative | 24 | 0 | 97.5% |
| | Positive | 1 | 15 | |
| Technologist 1 | Negative | 23 | 1 | 92.5% |
| | Positive | 2 | 14 | |
| Technologist 2 | Negative | 24 | 0 | 97.5% |
| | Positive | 1 | 15 | |
| Technologist 3 | Negative | 22 | 3 | 85% |
| | Positive | 3 | 12 | |

**TABLE 2** Comparison of precision and efficiency of automated Kleihauer-Betke (KB) counting and manual KB counting

| | Automated KB counting | Manual KB counting |
|---|---|---|
| Precision (Intraclass correlation coefficient) | 0.996 (CI: 0.994-0.998) | 0.79 (CI: 0.68-0.87) |
| Number of cells counted | ~20 000 | ~2000 |
| Time to count a KB slide | 1 min | ~20 min |
| Efficiency/Speed | ~20 000 cells/min | ~100 cells/min |

limits of agreement to flow cytometry (Figure S4 and Table S1 in Supplementary Information). Each technologist also achieved a different diagnostic accuracy (Table 1), indicating the subjectivity in manual KB counting. To evaluate and compare counting precision, we calculated the intraclass correlation coefficient (ICC) for both manual KB counting and automated KB counting. As shown in Table 2, the ICC for manual KB counting was 0.79 with a CI of 0.68 to 0.87. The automated system also counted each of the KB slide for three times and showed a higher ICC of 0.996 (CI: 0.994 to 0.998).

### 3.3 | Automated KB counting is more efficient than manual KB counting

Besides accuracy and precision improvement to manual KB counting, the automated KB counting system also showed a higher efficiency than manual counting. For each KB slide, the automated system was able to analyze an order of magnitude higher number of cells (20 000 cells vs 2000 cells) in a shorter time (~1 minute vs ~20 minutes) than manual counting (Table 2). The efficiency of the automated system was two orders of magnitude (approximately 200 times) that of manual KB counting.

## 4 | DISCUSSION

In this study, we evaluated the accuracy, precision, and efficiency of the automated KB counting system for the quantification of FMH. The results of automated KB counting were in strong agreement with the benchmarking flow cytometry results. A wide range of the fetal RBC ratios was covered (from 0.00% to 5.70%), and the difference between the two methods did not significantly increase/decrease with the ratio of fetal RBCs. This suggests that the automated KB counting system could be used as an alternative to the state-of-the-art flow cytometry quantification of FMH.

Compared to manual KB counting, automated KB counting showed an improvement in accuracy. The improvement could, in part, be attributed to the higher number of cells analyzed by the automated system. For samples where the ratio of fetal RBCs was low (eg, <0.1%), manually counting a low number of cells (eg, 2000) resulted in a low incidence of fetal RBCs (eg, <2 cells); thus, the counting result was less tolerant to errors in classifying each cell. Another potential reason for the improved accuracy could be the high data quality in training the machine learning classification model of the automated system. Indeed, the performance of the machine learning–based classification depends on the quality of labeling in the training dataset. In the automated system, the training dataset was independently labeled by the three certified technologists. A cell was labeled as a fetal RBC only when all the three technologists agreed that the cell was a fetal RBC. The data were discarded when the three technologists had conflicting labeling results. This helped mitigate the effect of subjective manual judgment in training the classification model and avoided "garbage in, garbage out" in machine learning.[16]

The automated KB counting system uses manually stained and prepared KB slides, and the quality of staining could influence the results of KB test. For instance, for point A in Figure 2B, the automated KB counting system overestimated the ratio of fetal RBCs by ~0.5%. We re-evaluated the slide and observed that the stained maternal RBCs appeared darker than surrounding cells and had a high chance of being falsely identified as fetal RBCs. Analyzing the same slide, the majority of the three technologists also gave a higher ratio of fetal RBCs than flow cytometry. Poor staining quality could also cause underestimation of the ratio of fetal RBCs. For

instance, for point B in Figure 2B, the colors of some fetal RBCs appeared much lighter than typical fetal RBCs shown in Figure 1. These fetal RBCs were identified to be maternal RBCs by both manual and automated KB counting, resulting in a lower ratio of fetal RBCs. To ensure consistent staining quality, techniques could be developed to automate the staining and KB slide preparation process, which is beyond the scope of this study.

Automation enables higher efficiency and reproducibility/precision than manual KB counting. The automated system could free laboratory technologists from the labor-intense KB counting task while providing more accurate results than manual counting. Different from flow cytometry, the automated KB counting system only requires minor modifications (ie, installing a motorized stage) to the standard setup for counting/reading KB slides and reduces the infrastructure cost along with operating and reagent costs in flow cytometry. More importantly, automated KB counting provides easier access than flow cytometry which is only available for the quantification of FMH in less than 5% laboratories in the United States. The automated KB counting system has the potential to standardize how RBCs are counted in clinical KB test and the potential to provide better diagnostics and patient care to pregnant women.

## ACKNOWLEDGEMENTS

The authors would like to thank the Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, ON, Canada, for patient KB slides' preparation and flow cytometry tests. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) through an Idea to Innovation grant, and the University of Toronto via a Connaught Innovation Award.

## CONFLICT OF INTEREST

The authors declared no competing finical interests.

## AUTHOR CONTRIBUTIONS

CW and YS conceived and designed the study. ZZ, JG, ZG, and JC developed the automated KB counting system. JG collected data. ZZ and JG analyzed and interpreted the data. All authors contributed to the manuscript and approved the final version before submission. ZZ and JG contributed equally.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author YS (sun@mie.utoronto.ca) upon reasonable request.

## ORCID

*Zhuoran Zhang* https://orcid.org/0000-0002-8737-4210

## REFERENCES

1. Troìa L, Al-Kouatly HB, McCurdy R, Konchak PS, Weiner S, Berghella V. The recurrence risk of fetomaternal hemorrhage. *Fetal Diagn Ther*. 2019;45(1):1-12. https://doi.org/10.1159/000491788
2. Maier JT, Schalinski E, Schneider W, Gottschalk U, Hellmeyer L. Fetomaternal hemorrhage (FMH), an update: review of literature and an illustrative case. *Arch Gynecol Obstet*. 2015;292(3):595-602. https://doi.org/10.1007/s00404-015-3686-1
3. Ahmed M, Abdullatif M. Fetomaternal transfusion as a cause of severe fetal anemia causing early neonatal death: a case report. *Oman Med J*. 2011;26(6):444-446. https://doi.org/10.5001/omj.2011.113
4. Christensen RD, Lambert DK, Baer VL, et al. Severe neonatal anemia from fetomaternal hemorrhage: report from a multihospital health-care system. *J Perinatol*. 2013;33(6):429-434. https://doi.org/10.1038/jp.2012.142
5. Kumar S, Regan F. Management of pregnancies with RhD alloimmunisation. *Br Med J*. 2005;330(7502):1255-1258. https://doi.org/10.1136/bmj.330.7502.1255
6. Sandler SG, Sathiyamoorthy S. Laboratory methods for Rh immunoprophylaxis: a review. *Immunohematology*. 2010;26(3):92-103.
7. Kim YA, Makar RS. Detection of fetomaternal hemorrhage. *Am J Hematol*. 2012;87(4):417-423. https://doi.org/10.1002/ajh.22255
8. Wong L, Hunsberger BC, Bruce Bagwell C, Davis BH. Automated quantitation of fetomaternal hemorrhage by flow cytometry for HbF-containing fetal red blood cells using probability state modeling. *Int J Lab Hematol*. 2013;35(5):548-554. https://doi.org/10.1111/ijlh.12060
9. Pastoret C, Le PJ, Fest T, Roussel M. Evaluation of FMH QuikQuant for the detection and quantification of fetomaternal hemorrhage. *Cytom Part B Clin Cytom*. 2013;84(1):37-43. https://doi.org/10.1002/cyto.b.21052
10. Wylie BJ, D'Alton ME. Fetomaternal hemorrhage. *Obstet Gynecol*. 2010;115(5):1039-1051. https://doi.org/10.1097/AOG.0b013e3181da7929
11. Duckett JRA, Constantine G. The Kleihauer Technique: an accurate method of quantifying fetomaternal haemorrhage? *BJOG An Int J Obstet Gynaecol*. 1997;104(7):845-846. https://doi.org/10.1111/j.1471-0528.1997.tb12032.x
12. Gielezynska A, Stachurska A, Fabijanska-Mitek J, Debska M, Muzyka K, Kraszewska E. Quantitative fetomaternal hemorrhage assessment with the use of five laboratory tests. *Int J Lab Hematol*. 2016;38(4):419-425. https://doi.org/10.1111/ijlh.12518
13. Pelikan DMV, Mesker WE, Scherjon SA, Kanhai HHH, Tanke HJ. Improvement of the Kleihauer-Betke test by automated detection of fetal erythrocytes in maternal blood. *Cytometry*. 2003;54B(1):1-9. https://doi.org/10.1002/cyto.b.10017
14. Pelikan DM, Scherjon SA, Mesker WE, et al. Quantification of fetomaternal hemorrhage: a comparative study of the manual and automated microscopic Kleihauer-Betke tests and flow cytometry in clinical samples. *Am J Obstet Gynecol*. 2004;191(2):551-557. https://doi.org/10.1016/j.ajog.2004.01.007
15. Ge J, Gong Z, Chen J, et al. A system for counting fetal and maternal. *IEEE Trans Biomed Eng*. 2014;61(12):2823-2829. https://doi.org/10.1109/TBME.2014.2327198
16. Radakovich N, Nagy M, Nazha A. Review Machine learning in haematological malignancies. *Lancet Haematol*. 2020;7(7):e541-e550. https://doi.org/10.1016/S2352-3026(20)30121-6

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Zhang Z, Ge J, Gong Z, Chen J, Wang C, Sun Y. Evaluation of machine learning–driven automated Kleihauer-Betke counting: A method comparison study. *Int J Lab Hematol*. 2021;43:372–377. https://doi.org/10.1111/ijlh.13380