

Evaluation of a prototype software for automated Kleihauer-Betke fetomaternal haemorrhage estimation

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Introduction

- Kleihauer-Betke (KB) method: used in Blood Transfusion laboratories for the estimation of fetomaternal hemorrhage (FMH) during pregnancy/post-delivery:
 - Labor-intensive and time consuming manual method, associated with high intra- and inter-observer variability
 - Used worldwide, as a screening method and/or as a low-cost alternative to flow cytometry
 - KB method principle: fetal hemoglobin (HbF) is resistant to citric acid treatment, while adult hemoglobin (HbA) is denatured by it. Many different commercial kits are available for KB film preparation - fetal cells appear pink when counter-stained, whilst adult cells appear as ghost cells.
- Flow cytometry: gold standard for FMH quantification but only available in specialist laboratories due to high costs and required expertise

Objectives

- Develop a prototype software for the automated analysis of KB films, on the CellaVision® DC-1 digital morphology analyzer (Figure 1)
- Evaluate prototype software performance against manual FMH estimation and flow cytometry.

Methods

- Prototype software development:** by CellaVision®; neural network trained using routine KB films and adult blood samples spiked with different concentrations of fetal cells
- Evaluation:** KB films made from adult blood samples spiked with known concentrations of fetal RBCs. Stain: Guest Medical Fetal Red Cell Detection Kit (Guest Medical Limited, UK)
 - Precision: FMH estimation (mL) from the prototype software, before and after user review, was compared to flow cytometry (Level 1, 2 and 3 at 0.2, 0.5 and 1.6% of fetal RBCs, respectively).
 - Effect of the analyzed number of RBCs on precision: average %CV (N=10) from Level 1, 2 and 3, was analyzed at 10 000, 25 000, 75 000 and 100 000 RBCs.
 - Method comparison study: performed on 30 samples (0 - 6% of fetal RBCs)

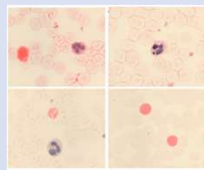


Figure 2: High resolution images captured by the prototype software (representative images only)

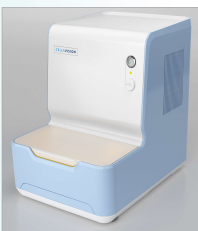


Figure 1: CellaVision® DC-1 analyzer

Results

	PROTOTYPE PRE-CLASSIFIED	USER REVIEWED	MANUAL
Bias	4.38	0.84	-0.91
SD	4.93	3.38	7.49
Lower LOA	-5.29	-5.77	-16.47
Upper LOA	14.05	7.46	14.65

Figure 3 – Bias, SD and limits of agreement prototype and manual methods (vs flow cytometry)

Manual method was associated with higher SD and wider limits of agreement



Figure 4 - Effect of analysed number of RBCs on precision (user reviewed)

CV (%) reached a minimum value at 50000 cells and 25000 cells for Level 1 and Level 2-3 respectively.

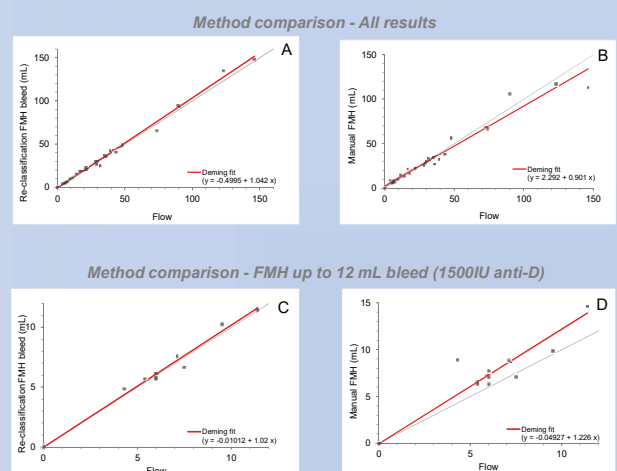


Figure 5 – Comparison of user-reviewed prototype results and manual method against gold standard, flow cytometry

User-reviewed prototype results are a closer fit to the best fit line (grey line) compared to manual method (Fig. 5A and B); such association is more pronounced when lower end of results is analysed (Fig. 5C and D)

Conclusion

- The new prototype software is associated with acceptable within-run precision and good correlation with the gold standard flow cytometry and manual method. However, further development of this new method is needed.
- Automated digital image analysis of KB films represents a viable and promising alternative to manual count by increasing precision and accuracy of KB method