The Analysis of Blood Morphology on Peripheral Blood Smears using Manual Microscopy and an Automated Analyser, The CellaVision DM96

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Introduction

Full blood count (FBC) samples requiring morphological examinations are one of the most valuable investigations in the haematology laboratory due to their significant contribution towards the diagnosis and monitoring of disease processes. It is imperative that these procedures can be completed as quickly as possible especially in cases where immediate clinical action is required (Merino *et al*, 2018).

At the Christie Trust, FBC samples are processed on the ADVIA 2120 using optical scatter methods. The results are presented on cytograms where the populations of cells are displayed in distinct clusters demonstrated in Figure 1a to Figure 1e. Abnormal flags will indicate that the differential may not be correct and this alerts the operator to delete the differential and refer the sample for a peripheral blood smear to be examined.
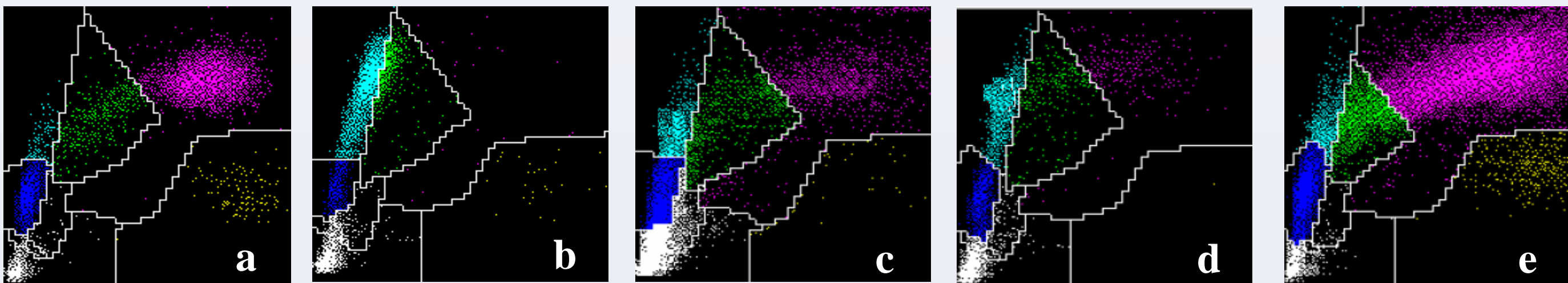
Figure 1: a) Perox Cytogram demonstrating WBC differential within normal reference ranges. b) Weak peroxidase staining with neutrophils shifting to the left c) Atypical lymphocytes in a sample obtained from a patient with lymphoma. d) Blasts in the large unstained cells region. e) Immature granulocytes appearing in the neutrophil region of the cytogram. (ADVIA® 2120/2120i Hematology Systems Operator's Guide, 2010).

A manual differential is extremely crucial in distinguishing the cells of the myeloid and lymphoid lineages particularly in the case of acute promyelocytic leukaemia where an urgent diagnosis is required in order to begin treatment immediately (Merino *et al*, 2018).
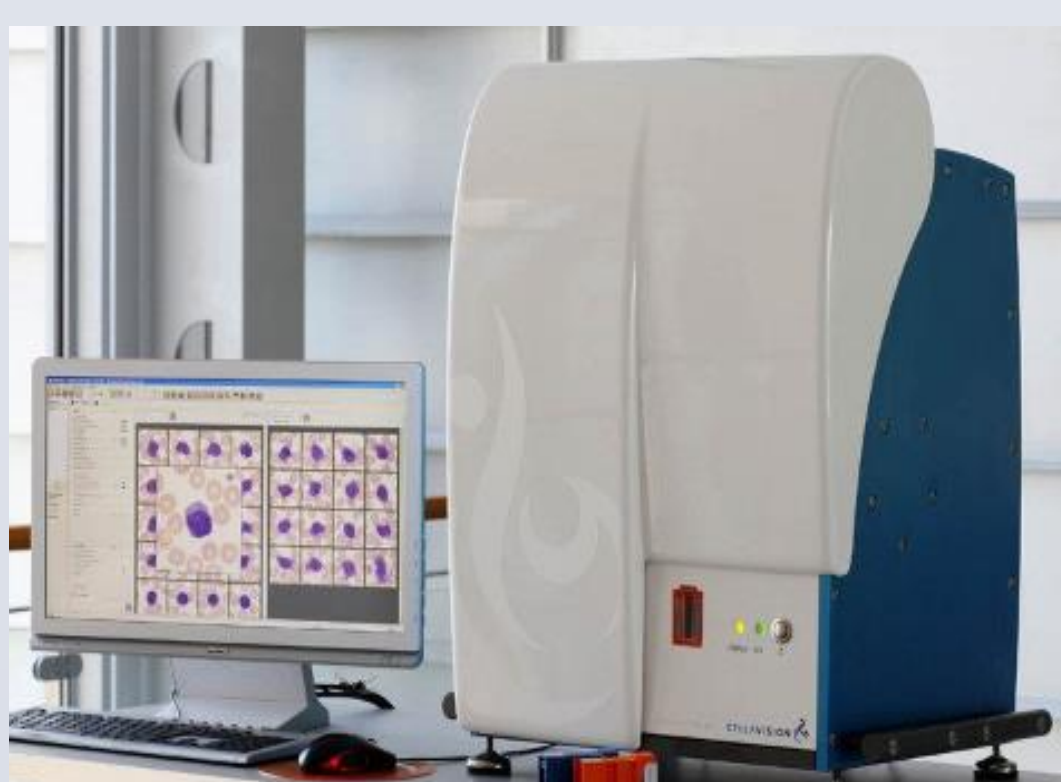
Limitations of manual differentials:

- The requirement of trained biomedical scientists with experience in morphology and expert knowledge in the classification of immature granulocytes.

- Subjective interpretation and classification of immature granulocytes are made by various individuals.

- Time consuming method especially in leukopenic samples.
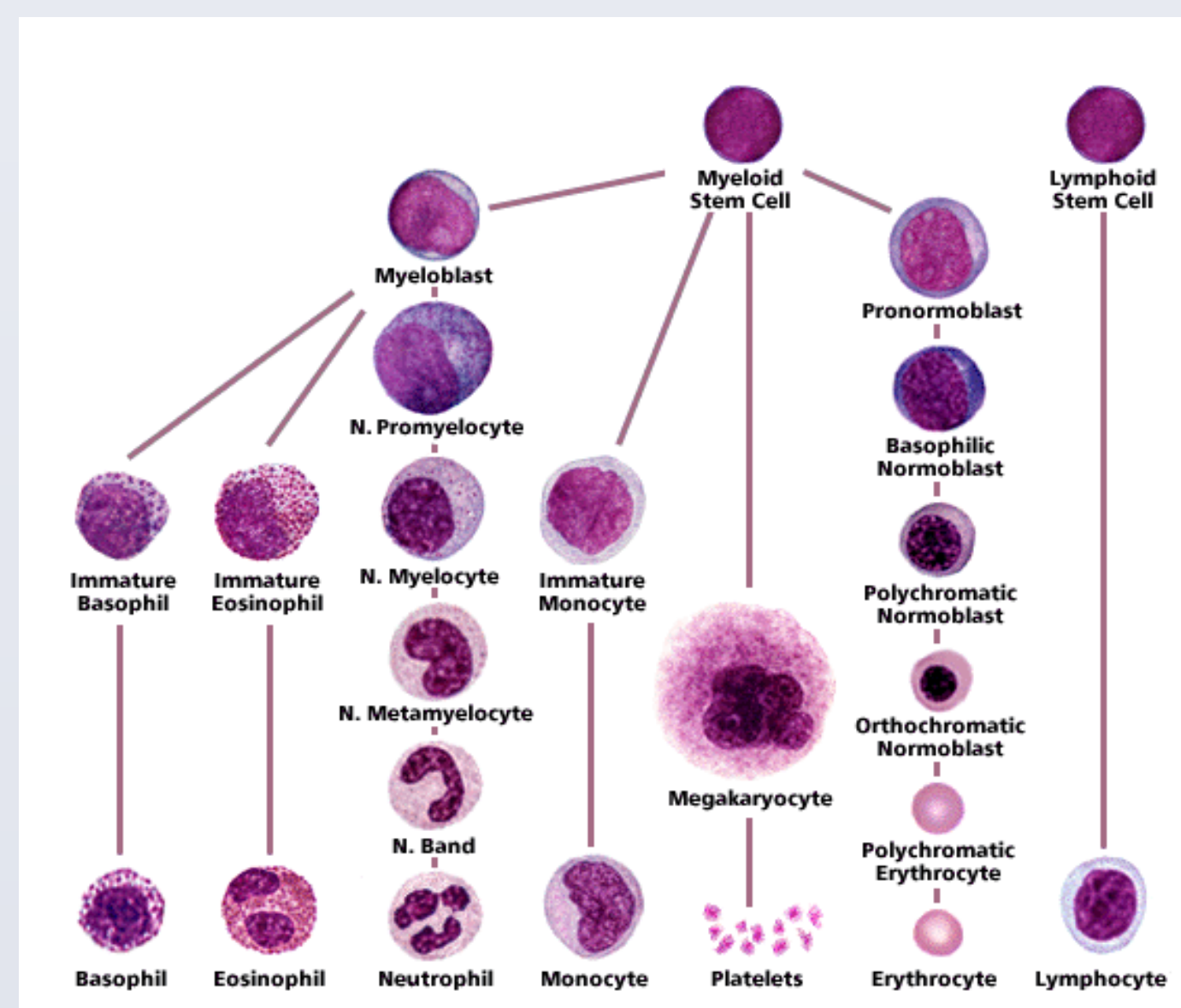
Figure 2: The stages of white blood cell maturation in the lymphoid and myeloid lineage (University of Minnesota, 2008).

The CellaVision DM96 System (Figure 3) is a digital morphology analyser that allows automated analysis of peripheral blood smears.

- Identifies WBCs and classifies them into the different categories of immature granulocytes.

- In leukopenic samples, a higher number of WBCs can be counted compared to manual microscopy.

- WBCs that are pre-classified by the system are neutrophils, lymphocytes, monocytes, eosinophils, basophils, blasts, promyelocytes, myelocytes and metamyelocyte. These are then presented to the operator for verification or reclassification of the cells.

**Figure 3:** CellaVision DM96 Digital Morphology Analyser (CellaVision DM96 Operator's Guide, 2014)

Aim

The aim of this study was to determine whether manual microscopy can be replaced by an automated digital morphology analyser at The Christie in order to reduce the turnaround times for the reporting of blood films.

Materials and Method

- FBC samples from the routine workload were processed on the ADVIA 2120 analysers

- A total of 20 normal FBC samples and 80 samples that were referred for manual differentials due to abnormalities were selected for the study for the examination of a peripheral blood smear using manual microscopy and the CellaVision DM96 analyser.

- A 100 cell manual differential on each slide was performed by two biomedical scientists. The final result was reported as the average of the manual differential counts obtained for each cell type and the average of the time taken to perform the manual differentials were also calculated for the abnormal slides.

- The same smears were then processed on the CellaVision DM96 by loading them on to the system where they were scanned and the blood cells were located and preclassified.

- Each cell type that was classified by the system was then either approved or reclassified to another cell type by the biomedical scientists. The time taken to complete this process was also recorded in order to compare to the time taken to perform the manual differentials.

- Statistical analysis was performed using correlation coefficient values to establish the relationship between the values obtained for each white blood cell type using both methods of microscopy. Bland Altman plots were used in order to investigate the agreement between the two methods and Passing Bablok regression analysis was performed to determine any systematic or proportional differences between both methods.

Results

Figure 4 indicates that there was a good agreement for the values obtained for neutrophils and lymphocyte counts and less agreement for monocytes, eosinophils and basophils. A negative bias was observed for basophils seen in Figure 4. Immature granulocytes demonstrated less agreement than the mature cell types.
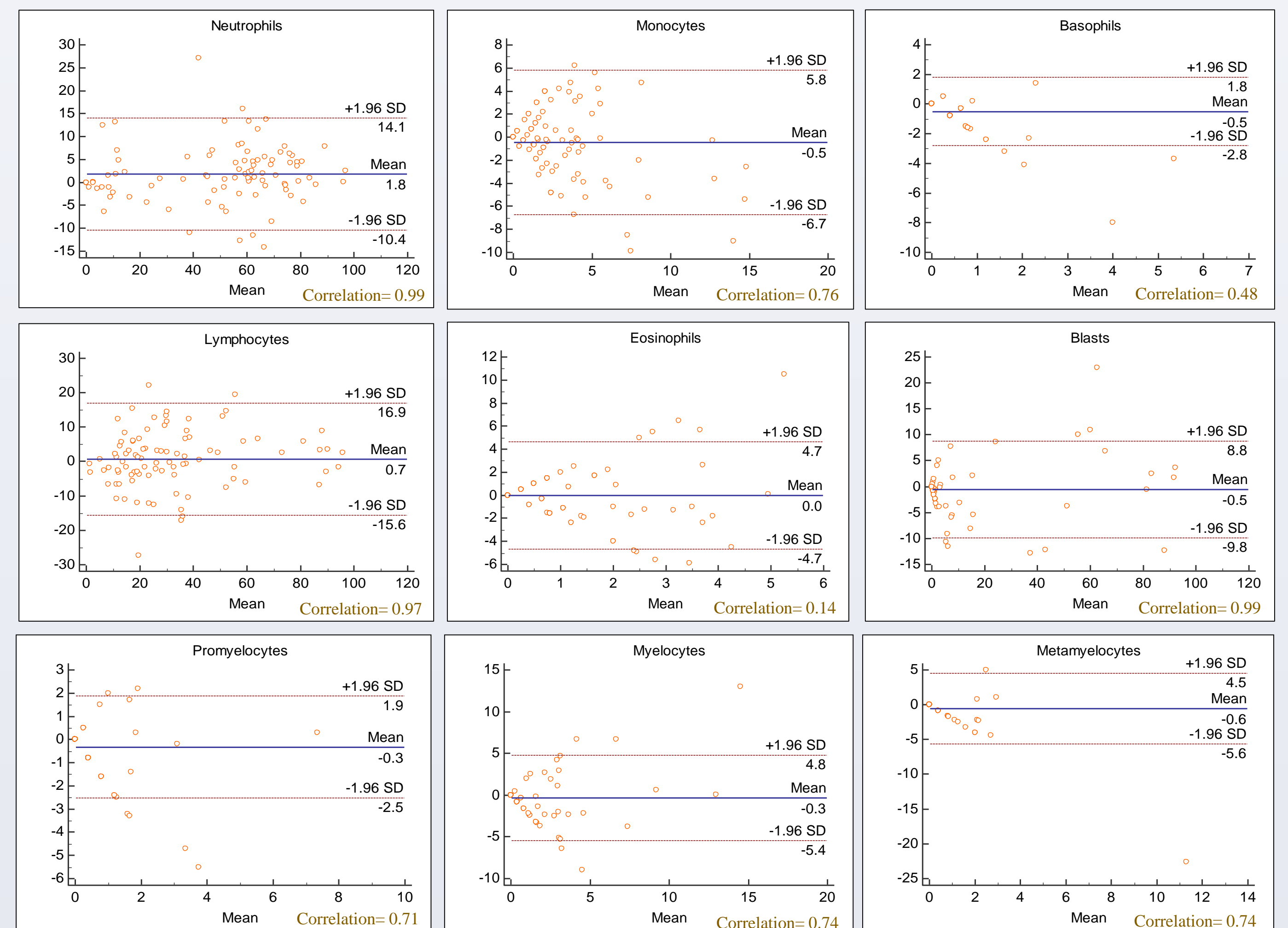
Figure 4: Agreement of each white blood cell count obtained via manual microscopy and CellaVision DM96.

Figure 5 demonstrates a linear regression between both methods of microscopy for counts obtained for all the WBC types. An intercept value of 0.00 and slope of 1.0222 indicates that there were no systematic or proportional differences between the two methods.

$$y = 0.000 + 1.022\ x$$
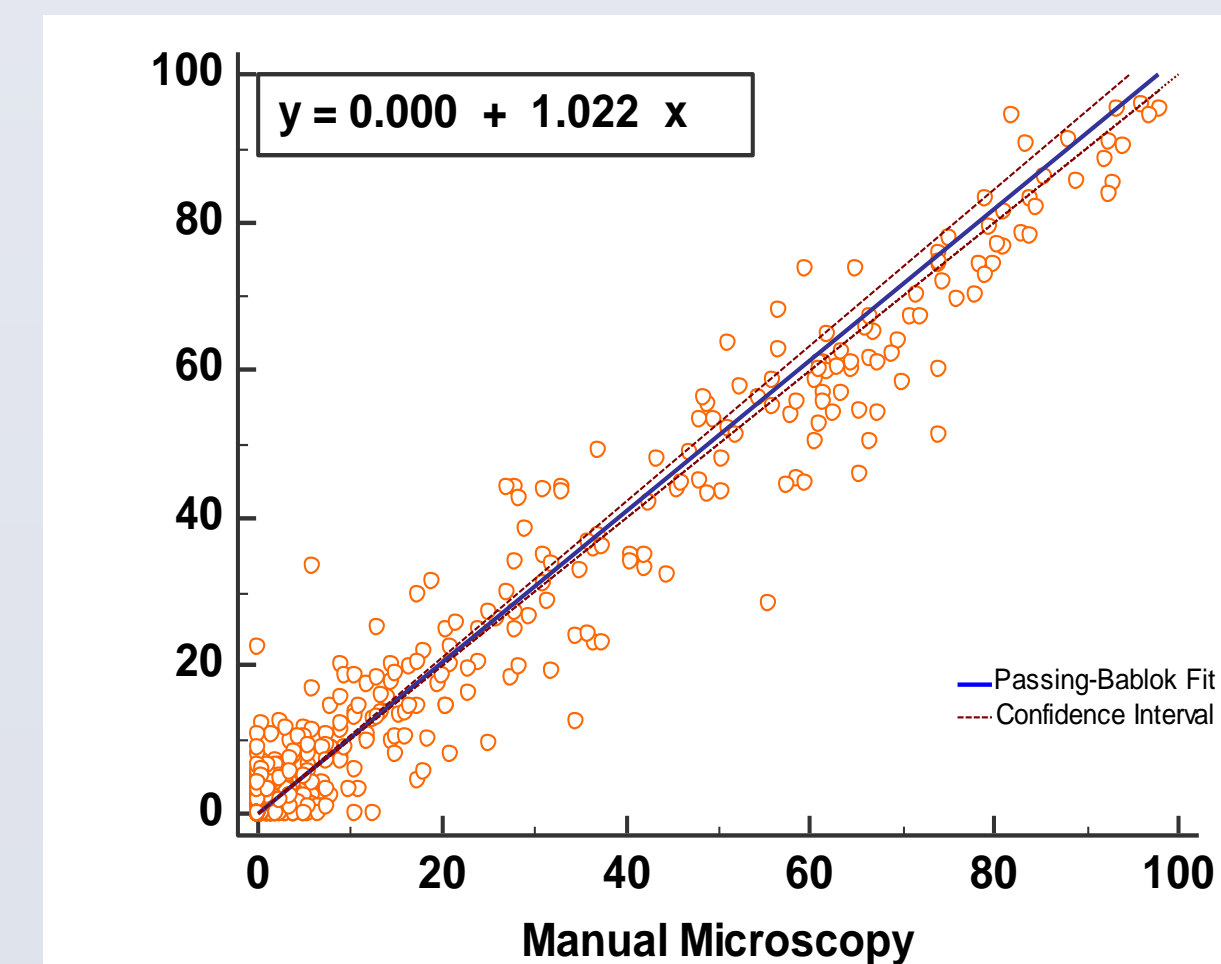
Figure 5: Passing Bablok regression analysis representing the comparison between the counts obtained for all white blood cell types using the CellaVision DM96 analyser and manual microscopy.

Limitations

- Neutrophils with toxic granulation incorrectly identified as basophils and erythroblasts by the CellaVision DM96 analyser.

- The time taken for verification on the CellaVision DM96 may not be an accurate representation of the time as the two biomedical scientists were unfamiliar with the software during the start of the study

- The peripheral blood smears were stained using Modified-Wright's stain whereas the CellaVision DM96 supports Romanowsky stains. This may have had an effect on the pre-classification and quantifiable properties of the cells as the colour intensity generated by the malignant blood cells is measured by the CellaVision DM96 to preclassify the cells.

Conclusion

The CellaVision DM96 did not reduce the time taken to perform manual differentials and the immature white blood cells had a lower percentage of pre-classifying agreement. The use of the CellaVision DM96 or other automated digital morphology analysers alone may not be appropriate for completing manual differentials. However, with modifications to the pre-fixed algorithms on the system, there is still potential to revisit the possibility of introducing an automated digital morphology analyser. The analyser is an excellent system that can be used as a tool for teaching, quality assurance and also for completing staff competency assessments

Future Experimentation

- Introduce an automated digital morphology system within a FBC analyser that will process the FBC, prepare and examine the peripheral blood smear and provide results that will be sent directly to the laboratory information system without any physical involvement.

- Modification is required in the fixed algorithms particularly of the cells that are less commonly encountered.

- More emphasis is required on the investigation of red blood cells in morphology as the CellaVision DM96 is very useful in the identification and grading of red blood cell abnormalities.

References

1. ADVIA® 2120/2120i Hematology Systems Operator's Guide. (2010).
2. CellaVision DM96 Operator's Guide (2014)
3. Merino, A., Puigví, L., Boldú, L., Alférez, S. and Rodellar, J. (2018). Optimizing morphology through blood cell image analysis. International Journal of Laboratory Hematology, 40, pp.54-61. Merino, A., Puigví, L., Boldú, L., Alférez, S. and Rodellar, J. (2018). Optimizing morphology through blood cell image analysis. International Journal of Laboratory Hematology, 40, pp.54-61.
4. Park, S., Park, C., Choi, M., Kim, M., Cho, Y., Jang, S. and Chi, H. (2013). Automated digital cell morphology identification system (CellaVision DM96) is very useful for leukocyte differentials in specimens with qualitative or quantitative abnormalities. *International Journal of Laboratory Hematology*, 35(5), pp.517-527
5. S., Moioli, V., Seghezzi, M., Previtali, G., Alessio, M., Simon Lopez, R., Ortolani, C., Ottomano, C. and Lippi, G. (2018). Evaluation and comparison of automated hematology analyzer, flow cytometry, and digital morphology analyzer for monocyte counting. *International Journal of Laboratory Hematology*, 40(5), pp.577-585.
6. University of Minnesota (2008), 'Hematography, blood cell maturation chart', [online] Available on: <http://hema.umn.edu/pages/matchart.html> [Accessed 20.02.2019]