ACTA SCIENTIFIC MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 4 Issue 1 January 2020

Research Article

Study on the Performance of SigTuple AI100 in the Analysis of RBC, WBC, Platelet Morphology and WBC Differential Count in a Large Hospital Setup

Renu Ethirajan[1]* and Rojaramani P[2]

[1]*Director Pathology - SigTuple Technologies Pvt Ltd., Bengaluru, Karnataka, India*

[2]*Manager Pathology - SigTuple Technologies Pvt Ltd., Bengaluru, Karnataka, India*

***Corresponding Author:** Renu Ethirajan, Director Pathology - SigTuple Technologies Pvt Ltd., Bengaluru, Karnataka, India.

Received: December 05, 2019; **Published:** December 12, 2019

DOI: 10.31080/ASMS.2020.04.0504

Abstract

In this study, we evaluate the performance of SigTuple AI100, powered by SHONIT, a cloud based artificial intelligence (AI) system for the analysis of peripheral blood smears against the haematology automated analyser and results of manual microscopy in a large hospital setup. A blinded, randomized comparative study between results of AI100 and 7-part haematology analyser values for WBC differential counts, WBC morphological classification, RBC morphology and platelet morphology was conducted. The results of RBC and platelet morphology classification given by AI100 were compared with the results of the manual microscopy. The mean-absolute-difference between 5 Part differentials reported by AI100 and the Sysmex 7-Part haematology analyser for Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil were 4.38%, 4.74%, 5.82%, 0.97%, and 0.53% respectively. The r^2 coefficient mean of results of haematology analyser vs Shonit (AI100) for neutrophil, lymphocyte and eosinophil were 0.97, 0.97 and 0.92 respectively. AI100 can increase the throughput and decrease TAT; thereby increasing the productivity and efficiency of the pathologist.

Keywords: SigTuple AI100; RBC; WBC; Platelet

Introduction

SigTuple AI100 with PBS Analyzer is an automated peripheral blood smear slide analyzer intended for in-vitro diagnostic use in pathology laboratories. It provides both morphological analysis and quantifies hematological parameters of various cells observed in peripheral blood smear slides. The peripheral blood smear slide can be digitized on SigTuple AI100 by any trained laboratory personnel but and the report generated after automatic analysis is meant for review only by a pathologist. Irrespective of the analyzer, approximately 15% of the blood samples require manual microscopic observation either because of biological rules or analyzer flags [1].

AI100 specifically provides

- Morphological analysis of WBCs, RBCs, and platelets.
- Analysis of anisopoikilocytosis and differential counts of WBCs.
- Total counts for WBCs, RBCs, and platelets.
- Key volumetric and non-volumetric indices for RBCs and platelets.

Shonit uses an ensemble of deep learning techniques for the localization and classification tasks. This is our attempt to use a deep learning network towards object localization in peripheral blood smear images.

The captured images are transferred to a compute cloud hosting the software component – an artificial intelligence (AI) based platform which analyses these images. All WBCs visible in the captured images are classified. Thousands of RBCs (approximately 30,000) and platelets (approximately 5,000) are also extracted and classified into different categories.

In this study we aim to highlight the classification accuracy of SigTuple AI100 in categorizing WBCs, RBCs and platelets including immature cells on peripheral blood smear. We further validate SigTuple AI100's results in providing WBC Differentials vis-à-vis state-of-the-art Hematology Analyzer (Beckman Coulter Unicell DXH800) and manual microscopy. In contrast to state of the art devices like the Cellavision DM9600, which creates digital scan of pre-defined area of any interesting specimen, the AI100 scans, extracts classifies and computes with the help of SHONIT powered by AI to give a 7 - part differential count [2].

Detailed case studies are discussed in all cases where there is discordance between the results provided by SigTuple AI100 with manual microscopic examination as an arbitration process. We quantified the correlation of WBC Differential Counts provided by AI100 against Beckman Coulter Unicel DXH800 using various statistical measures. The time taken by the AI100 for scan and release of report was found to be approximately 7 min whereas manual screening required appx. 8 min per slide on an average [3].

In the process we have evaluated the usability of Shonit as a telepathology enabling solution to provide remote access to blood smear analysis results which is at par with other state of the art devices available in the market like Vision Hema Ultimate [4].

Materials and Methods

A blinded, randomized comparative study between AI100 and 7-Part hematology analyzer values of Peripheral Blood Smear (PBS) for WBC differential values and WBC morphological classification was done.

A total of 250 peripheral blood smears were selected from a normal workload of the laboratory. Sample selection for PBS preparation was as per the following criteria: 1. Normal blood samples (Approximately 25% of total samples) 2. Abnormal samples or flagged by the automated analyzer.

Anonymized peripheral blood samples were run in the 7-Part hematology analyzer at the laboratory in Apollo Hospital, Bannerghatta. Anonymized peripheral blood smears were prepared from the samples using Hemaprep auto-smearer and staining was done manually using Leishman stain. The stained slides were shared with SigTuple. The stained slides were scanned in AI100. WBC differentials, along with the morphological classification of WBCs, were produced as a result by AI100. The 7-Part hematology analyzer reports were provided to SigTuple and a comparative analysis between WBC differential values provided by AI100 and the 7-Part hematology analyzer at the laboratory in Apollo Hospital, Bannerghatta were performed for evaluation of correlation and mean difference of WBC differential counts provided by AI100 and the 7 -part hematology analyzer. WBC morphological classification results produced by AI100 for each slide was verified by SigTuple's in-house haemato-pathologist.

Results

Statistical analysis: Correlation analysis between AI100 and 7part DC from hematology analyzer: The r2 coefficient mean of observations between ShonitTM and 5-Part hematology analyzer for neutrophil, lymphocyte and eosinophil were 0.967, 0.964, 0.921 respectively. The correlation plots for the same are shown below.

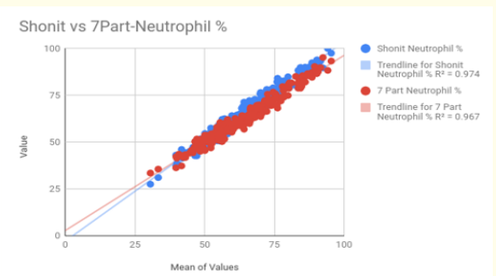

Figure 1: Graph of Neutrophil % comparison (7-part HA and Shonit- within acceptable limits).

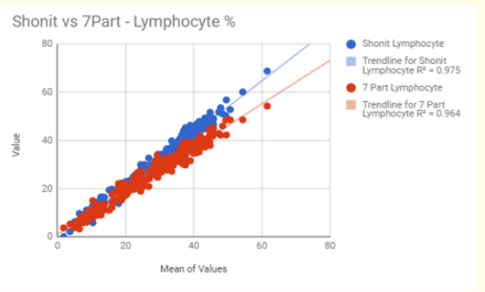

Figure 2: Graph of Lymphocyte % comparison (7-part HA and Shonit-results are within acceptable limits).

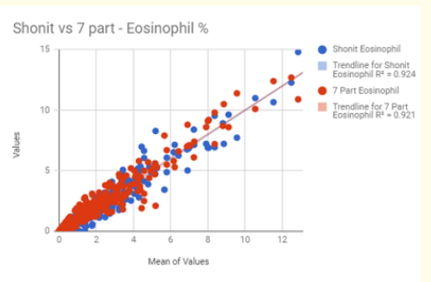

Figure 3: Graph of Eosinophil % comparison (7-part HA and Shonit- results are within acceptable limits).

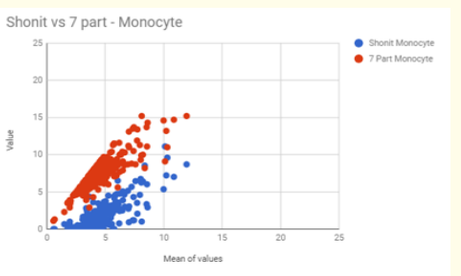

Figure 4: Graph of Monocyte % comparison (7-part HA and Shonit) The hematology analyzer overpredicted the monocytes by wrongly classifying the IGs as monocytes.

Bland altman analysis

We plotted Bland Altman charts for neutrophil, lymphocyte, eosinophil, monocyte and basophil by comparing the WBC differential values reported by the mean results of 7- part analyzer and AI100 Vs AI100

- Around 95% of the points plotted on Bland Altman charts for neutrophil, lymphocyte, eosinophil, monocyte and basophil lie within the acceptable range between the upper and lower level of agreement.
- A mild positive positive bias was noted for the results of lymphocytes obtained by AI00. This was since these cases had immature granulocytes which were not included in the 5 -part differential counts. This resulted in a mild increase

in the lymphocyte differential count. However, the increase was within 3% and not found to be medically significant.

- Monocyte results showed a negative bias. As mentioned earlier this was due to the overprediction of the hematology analyzer in cases of increased immature granulocytes. The hematology analyzer had wrongly classified the immature granulocytes as monocytes. The results of monocytes differentials count correlated with the results of manual microscopy.

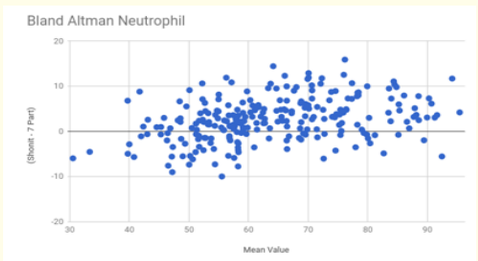

Figure 5: Bland Altman Chart (Neutrophil) – within acceptable limits.

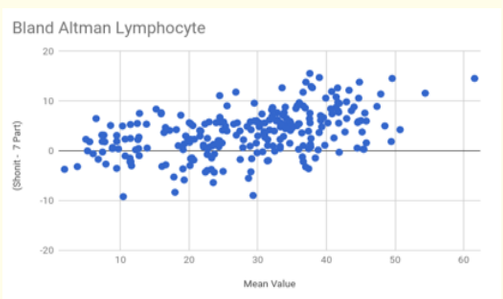

Figure 6: Bland Altman Chart (Lymphocyte)- mild positive bias by AI100. This is due to the exclusion of immature granulocytes in the 5 -part differential count resulting in mild increase in lymphocyte%. However this was medically insignificant.

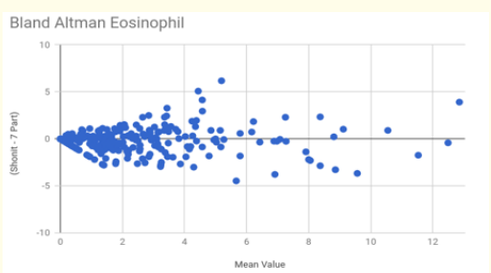

Figure 7: Bland Altman Chart (Eosinophil) – within acceptable limits.

For cases where there was a discrepancy between monocyte differentials reported by AI100 and the 7-Part hematology analyzer values reported by AI100 correlated better with the manual differential counts, proving that monocyte identification and enumeration was better than the 7-Part Hematology Analyzer.

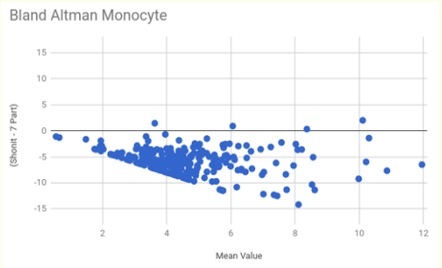

Figure 8: Bland Altman Chart (Monocyte) - discrepant due to the misclassification of immature granulocytes by the 7-part hematology analyzer as monocytes. Results of AI100 correlated with manual microscopy.

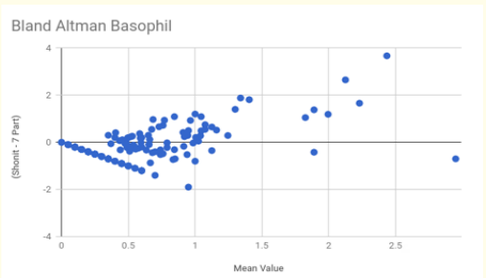

Figure 9: Bland Altman Chart (Basophil)- within acceptable limits.

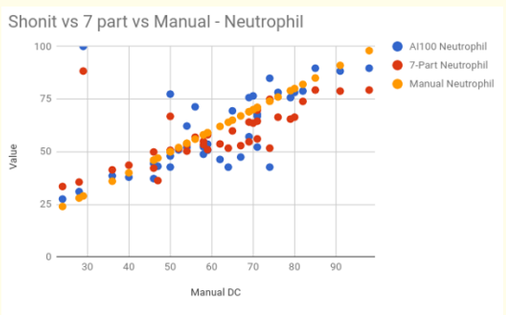

Figure 10: Results of AI100 correlated with 7-part hematology analyzer results and manual microscopy.

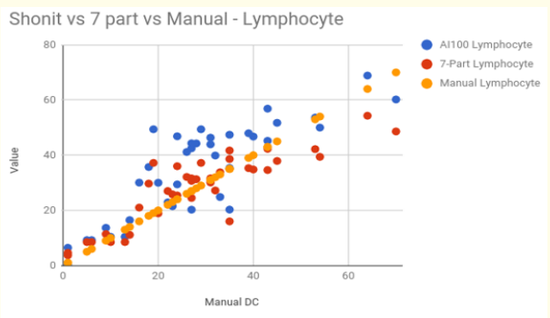

Figure 11: Results of AI100 correlated with 7-part hematology analyzer and manual microscopy in more than 95% of the cases. Mild positive bias in lymphocyte % was seen in a few cases were immature granulocytes were excluded from the 5 – part differential count.

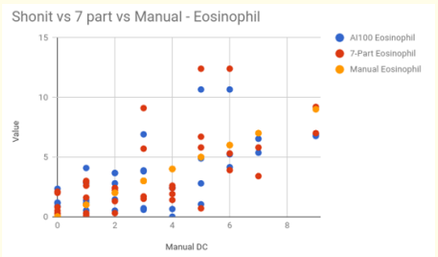

Figure 12: Results of AI100 correlated with results of 7-part hematology analyzer and manual microscopy.

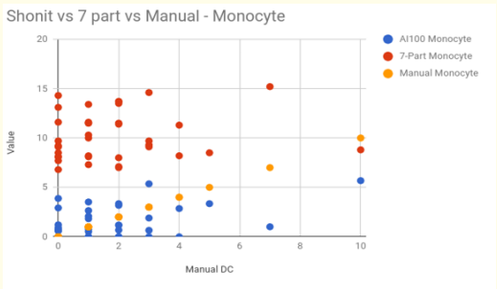

Figure 13

RBC - morphology analysis

RBC morphology in all 250 cases were compared with the blood cell indices.

Manual microscopy was performed in 75 cases and the grading was assigned according to the ICHS guidelines. The grading was compared with the results derived from AI100. AI100 showed an accuracy of more than 99% in the classification of anisocytosis and poikilocytosis. Anisocytosis was subclassified into Microcytes, normocytes, round macrocytes and oval macrocytes. Poikilocytosis were classified into echinocytes, ovalocytes, elliptocytes, target cells, tear drop cells and fragmented cells. Below is the confusion matrix and outlier analysis of the values obtained by manual microscopy and the hematology analyzer.

Poikilocytosis sensitivity and specificity on AI100.

Platelet morphology

AI100 classifies platelets into normal, large/giant categories. It can also give an accurate estimation of platelet clumps. AI100 demonstrated a 100% sensitivity, specificity and accuracy in normal and large platelets and platelet clumps.

Cell	Outliers	Reason	Remarks	Accuracy
Microcytes Manual: NIL AI100 <10%	6	In all 6 cases the detection of microcytes was missed on manual microscopy whereas AI100 proved to demonstrate better sensitivity.	Higher sensitivity by AI100	100%
Normocytes	0			100%
Round Macrocytes Manual: NIL AI100 <10%	9	In all 9 cases the detection of round macrocytes was missed on manual microscopy whereas AI100 proved to demonstrate better sensitivity.	Higher sensitivity by AI100	100%
Oval Macrocytes Manual NIL AI100 <2%	6	In all 6 cases, the detection of oval macrocytes was missed on manual microscopy whereas AI100 proved to demonstrate better sensitivity.	Higher sensitivity by AI100	100%
Round	0			100%
Elliptocyte 11cases: Manual NIL. AI100 <5% 4 cases: Manual >5 <10AI100 <5%	15	In 11 cases the elliptocytes were missed on manual microscopy and detected by AI100. In 4 cases there was a borderline discrepancy in the grading. However, this was medically insignificant.	Borderline discrepancy in 4 cases. Medically insignificant.	94%
Ovalocyte Manual: NIL AI100 <10%	16	In all 16 cases, the ovalocytes were missed on manual microscopy and detected by AI100.	Higher sensitivity by AI100	100%
Target 17cases: Manual NIL AI100 <5% 8 cases: Manual <5 AI100 NIL	25	In 17 cases, the detection of target cells were missed on manual microscopy but were picked up by AI100. In 8 cases manual microscopy had detected occasional target cells which were not detected by AI100. However, these were medically insignificant according to the ICSH guidelines.	Good sensitivity by AI100. Outlier-cases were-medically insignificant according ICSH	89%
Teardrop	0			100%
Echinocyte Manual NIL AI100 <5%	26	In all 26 cases the echinocytes were missed on manual microscopy and detected by AI100.	Higher sensitivity by AI100	100%
Fragmented cells 12cases: Manual: NIL AI100 <1% 11cases: Manual: NIL AI100 >1% 3 cases: Manual <1 AI100 >1%	36	The outliers in all 36 cases were due to artifacts. The artifacts resembled fragmented RBCs. Such cases were due to scratches created on the slides.	Good sensitivity by AI100	100%

Table 1: RBC morphology – outlier analysis (AI100 Vs Manual microscopy).

Class	Sensitivity	Specificity
fragmented	98%	100%
target	100%	100%
echinocyte	100%	99%
teardrop	92%	100%
round	100%	98%
ovalocytes	99%	100%
elliptocytes	97%	99%

Table 2

Figure 14

Cell	Outliers	Reason	Remarks	Accuracy
Normal Platelet	0			100%
Large platelet- Manual NIL AI100 <10%	4	In all 4 cases, large platelets were missed on manual microscopy but detected on AI100	AI100 demonstrated high sensitivity and specificity in identification of large platelets.	100%
Platelet Clump 18cases: Manual NIL AI100 < 5 16cases: Manual NIL AI100 > 5	34	In all 18 cases, the platelet clumps were missed by manual microscopy but detected by AI100.	AI100 demonstrated a high sensitivity for the identification of platelet clumps.	100%

Table 3: Platelet morphology outlier analysis.

Discussion

AI100 provides value add by identifying basophils, eosinophils, monocytes and immature granulocytes more accurately than the hematology analyzer especially in the case of monocytes and IGs. The hematology analyzer overpredicted the monocytes by wrongly classifying the IGs as monocytes. For cases where there was a discrepancy between monocyte differentials reported by AI100 and

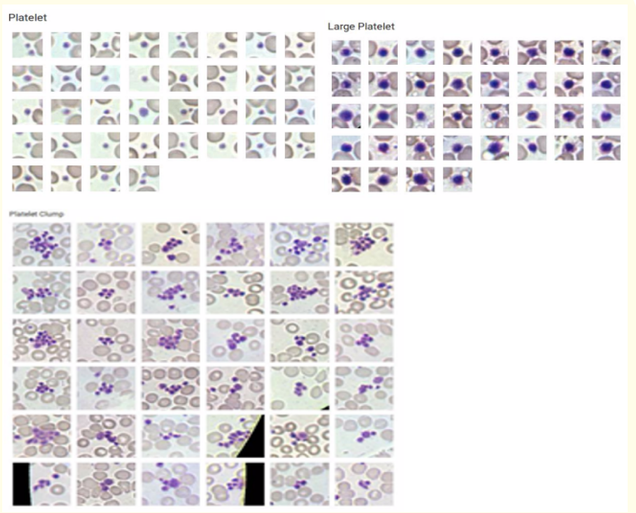

Figure 15

the 7-Part hematology analyzer values reported by AI100 correlated better with the manual differential counts, proving that monocyte identification and enumeration was better than the 7-Part Hematology Analyzer. Absolute difference of WBC differential values between reports generated by AI100 and 7-part hematology analyzer reports from Apollo Bangalore are within the acceptable range of 10% for neutrophils, lymphocytes and monocytes, 5% for eosinophils and 1.5% for basophils.

Around 95% of the points plotted on Bland Altman charts for neutrophil, lymphocyte, eosinophil, monocyte and basophil lie within the acceptable range between the upper and lower level of agreement.

The mean-absolute-difference between 5 Part differentials reported by AI100 and the Sysmex 7-Part hematology analyzer for Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil were 4.38%, 4.74%, 5.82%, 0.97%, and 0.53%. The r2 coefficient mean of observations vs Shonit for neutrophil, lymphocyte and eosinophil were 0.97, 0.97 and 0.92 respectively. Leukocyte morphology analysis done by AI100 is accurate and within interobserver limits. Leukocyte differential values provided by AI100 has advantages over manual microscopy. AI100 could identify rarer cells in the smear especially in pancytopenic samples, as it scans >100 FOVs from each smear. Enumeration of nucleated red blood cells is performed as an independent parameter due to which a correction of the total WBC count is not required. AI100 can accurately classify RBC anisocytosis and poikilocytosis [5]. AI100 can correctly classify platelets into normal, large/giant platelets. It can provide an accurate estimation of platelet clumps in the smear. The existing state of the art hematology analyzers make our routine smoother, more efficient, free up time for other more important tasks and also makes a genuine difference in patients' lives [6]. AI100 has taken this journey a step ahead and demonstrated a superior TAT compared to existing methodologies. Angulo., *et al.* in their publication

presented a technique to automatically detect the working area of peripheral blood smears stained with May Grünwald Giemsa. The optimal area was defined as the well spread part of the smear by Angulo., *et al.* This zone starts when the erythrocytes stop overlapping (on the body film side) and finishes when the erythrocytes start losing their clear central zone (on the feather edge side).

This observation has been adopted while developing the scan algorithms for AI100 which scans the well discernible monolayers to capture images [7].

Ritter., *et al.* presented an unsupervised blood cell segmentation algorithm for images taken from peripheral blood smear slide which unlike prior algorithms was fast and fully automated [8]. Benigo., *et al.* in their research discussed the motivations and principles regarding learning algorithms for deep architectures [9]. AI100 also works on similar algorithms designed by inhouse data science enthusiasts.

Once the images are captured and displayed on the screen of computer, the actual time taken for the pathologist to review and authenticate a report is within 2 minutes.

Conclusion

In conclusion AI00 has the potential to address the diagnostic, logistic and operational challenges in the laboratory environment. AI100 can increase the throughput and decrease TAT; thereby increasing the productivity and efficiency of the pathologist. AI100 creates an environment where a pathologist can gain remote access of digital images for verification and approval of reports. It obviates the need for the physical availability of slides and microscope during reporting. AI100 reduces manual error and removes ambiguity associated with the human eye and allows standardization, reproducibility and scalability. AI100 enables telepathology and creates a vista of opportunities for educational and research activities.

Bibliography

1. Cornet E., *et al*. "Performance evaluation and relevance of the CellaVisionTM DM96 system in routine analysis and in patients with malignant hematological diseases". *International Journal of Laboratory Haematology* 30 (2008): 536-542.

2. Cellavision Sweden Cellavision. DM9600.

3. Medica Corporation. Easycell. USA: Medica Corporation. Medica Easycell.

4. West Medica. Vision Hema Ultimate. Austria.

5. SigTuple. ShonitTM: The complete peripheral bloodsmear analysis solution. India: SigTuple (2006).

6. Sysmex. USA (2017) Sysmex. XN-3000TM Hematology System (2017).

7. Angulo J and Flandrin G. "Automated detection of working area of peripheral blood smears using mathematical morphology". *Analytical cellular pathology* 25 (2003): 37-49.

8. Ritter N and Cooper J. "Segmentation and border identification of cells in images of peripheral blood smear slides". *Australian Computer Society* (2007): 161-69.

9. Bengio Y. "Learning deep architectures for AI". *Foundations and Trends in Machine Learning* 2 (2009): 1-127.

Volume 4 Issue 1 January 2020