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COMPARISON BETWEEN HAEMATOLOGICAL PARAMETERS BY THE MERILYZER CELQUANT 360 AUTOMATED HAEMATOLOGY ANALYSER AND SYSMEX XP-100 HAEMATOLOGY ANALYSER WITH THE MANUAL METHODS

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ABSTRACT
This study was a comparative cross-sectional study to determine the correlation between haematology parameters by Merilyzer CelQuant 360 Automated Haematology Analyser with Sysmex XP-100 Cell counter and manual methods. Study included random selected subjects, those who have different blood disorders. The 3 ml of venous blood sample was collected aseptically from each subject into K_2EDTA for the analysis of haematological parameters using the automated and the manual methods during August through October 2017. The present correlation studies of haematological test parameters like Haemoglobin, WBC Count and Platelets counts shows minimal differentiation between analyzer to analyzers (Merilyzer CelQuant 360 versus Sysmex XP-100) and Merilyzer CelQuant 360 versus standard manual methods. From the results of our study, it can be concluded that the automated haematology analyser readings correlated well with readings by the standard manual methods and having added advantages are non-toxic and biodegradable reagents, reliable and uniform results and network facility with the help of LIS.

KEYWORDS: Validation, Haemoglobin, WBC Count, Platelets Count, Microscopy.

INTRODUCTION
The automated haematology analyser for the estimation of complete blood count (CBC) (White blood cells, WBC; Haematocrit, Hct; Haemoglobin, Hb; and Platelets, PLT) is commonly used in routine practice in laboratory but many other laboratories still work on manual procedure. Now-a-days, the availability of automated cell counting instruments (Cell Counter) has great advantages over manual methods in analysis of a huge number of routine samples for haematological test parameters with better precision and shorter turn around time in a short time for laboratories processing large-scale analysis, in addition to the benefits of reducing variability associated to human-error.[1] No automated cell counter can equal the performance of manual differentiation for the presence of old results for WBC. The WBC are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders.[2] The traditional review of all automated haematology analysers results by preparation, staining and microscopic examination of blood film examination has disappeared in most laboratories, because more accurate detection of specimens with distributional or morphological abnormalities by the traditional method.[3]

Hct is a measure of the percentage of blood red blood cell (RBC). This is referred to as packed cell volume (PCV). It is considered as an integral part of a person's CBC, along with Hb concentration, WBC count and PLT counts.[4]

The actual PLT counting is required particularly in severely thrombocytopenic patients, post chemotherapy bleeding and prophylactic PLT transfusion. Also, there is need for accurate count for increasing cases of thrombocytopenia associated with various conditions such as leukaemia, chemotherapy for various malignancies and viral disease (mainly, Dengue fever).

The electronic PLT counts were not accepted by clinicians especially for haematology and oncology patients. The manual counting of PLT (by the use of haemocytometer chamber), confirmed with PLT estimate by the stained thin blood film, and comparing the result with the automated PLT count for the same sample at the same time has been used to give conclusive advice.

Also, false low WBC counts may be observed because of agglutination in the presence of ethylene di amine tetra acetic acid (EDTA).[5]

We evaluated cell counter performance towards accuracy, precession, linearity and range, through a validation. For this procedure, the sample preparation was standardised and adjusted, in order to obtain viable cell counters, using CHO-K1 cells (CRL-CCL 61) and U 937 (CRL-1593.2) that were acquired from ATCC (Manassas, VA). These cells are employed for the biosynthesis and bio assays of recombinant proteins respectively. The stability of these methodologies was evaluated through a validation according to the ICH Q2 R1 guideline.[6]

OBJECTIVE

To evaluate the performance of Merilyzer CelQuant 360 automated haematology analyser vis-a-vis Sysmex XP-100 haematology analyser, and to compare automated methods with the manual methods using randomly selected human subject's blood samples.

MATERIALS AND METHODS

A comparative cross sectional study was conducted during August through October 2017 to assess the analytical performance between manual procedure and automated methods for the CBC determination for EDTA blood sample by Merilyzer CelQuant 360 automated haematology analyser and Sysmex XP-100 haematology analyser for a medical testing laboratory. It included 17 reportable parameters and three parts differential, which included the results in histogram for WBC, RBC and PLT.

Venous blood sample was obtained from randomly selected patients attending hospital during the study period. Data of age and sex of patients were collected from the laboratory test request forms without meeting patients. The 3 ml of blood from vein of selected subjects was collected in a tube containing di-potassium ethylene di amine tetra acetic acid (K_2EDTA) anticoagulant. This was well mixed by gentle inversion for CBC analysis.

All samples were processed by automated cell counter using the Merilyzer CelQuant 360 and Sysmex XP-100, following the manufacturers operational guidelines. Also, all manual samples were analysed using standard haematological method.[7] All samples were analysed within 30 minutes of collection.

Hb was estimated by the cyan-methemoglobin method. The percentage of PCV was measured manually by filling plain capillary tube and sealing with modelling clay and centrifuging at 3000 g for 5 minutes, tabulating the result using Hct reader. Also, WBC counting was done manually using counter chamber. After adding 20 µl of blood to tube containing 0.4 ml glacial acetic acid, WBC count was measured by equation summation of two parts of chamber and multiplied by 100. Mean Cell Haemoglobin Concentration (MCHC) was calculated from Hb and PCVcount. PLT was estimated by the thin blood film, stained with Lishman's stains.

The quality control was maintained by running three levels controls daily and Levey-Jennings Control Chart. This study utilised both internal and external quality control procedure and obtained consistently satisfactory results, using quality control sera of BioRad, USA.

Data processing was performed by the SPSS statistical programme.

The study was in accordance with Declaration of Helsinki[8] and guidelines on good clinical practice locally available. It was also approved by institutional review board and ethics committee. This study was dealing with blood samples from the laboratory. All the obtained data were handled confidentiality, instead of reporting the name of patients, serial numbers were used.

RESULTS

The comparative studies of Hb, WBC and PLT counts were carried out between Merilyzer CelQuant 360 automated haematology analyser and Sysmex XP-100 haematology analyser and Merilyzer CelQuant 360 automated haematology analyser versus manual methods by regression analysis.

The test results reveals that Hb, WBC and PLT counts are positively correlated along the regression line between Merilyzer CelQuant 360 automated haematology analyser and Sysmex XP-100 haematology analyser, and Merilyzer CelQuant 360 automated haematology analyser versus manual methods (Figure 1 to Figure 6). All the data points are homogeniously distributed along the regression line in all three test parameters (Hb, WBC and PLT) excepting in one occasion in PLT studies (Figure 5 and Figure 6). The slop and intercepts of regression lines are variable depending upon nature of test parameters, but regression patterns remains identical in all cases.

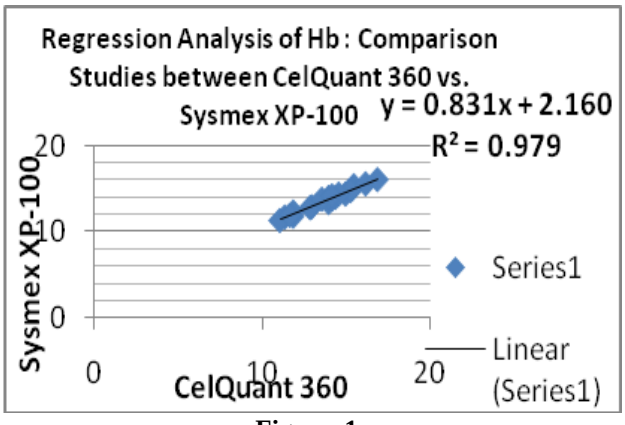
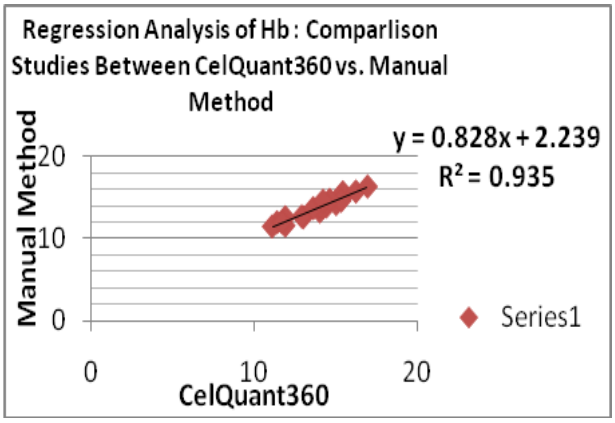


Figure. 1.



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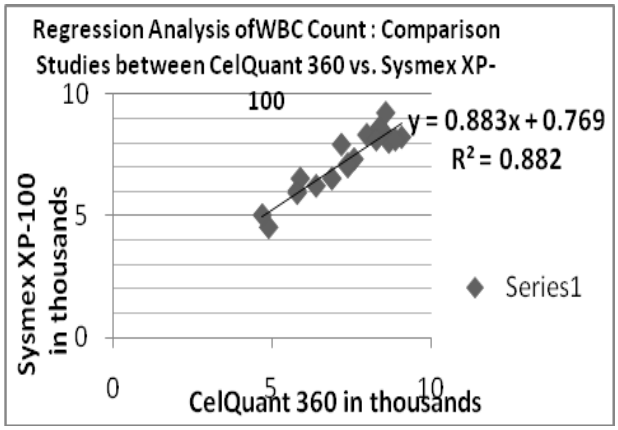

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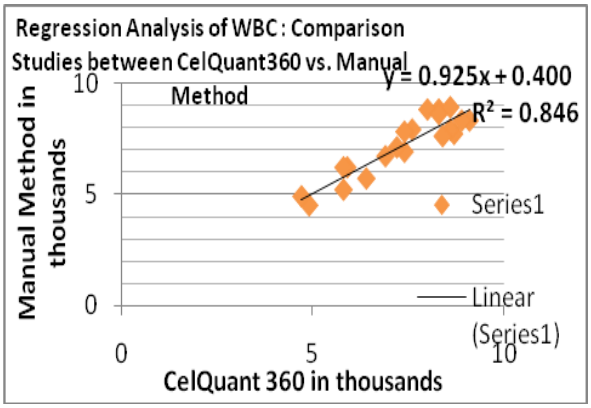

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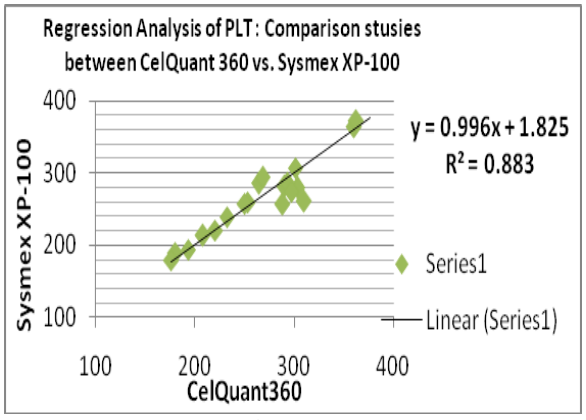


Figure. 5.

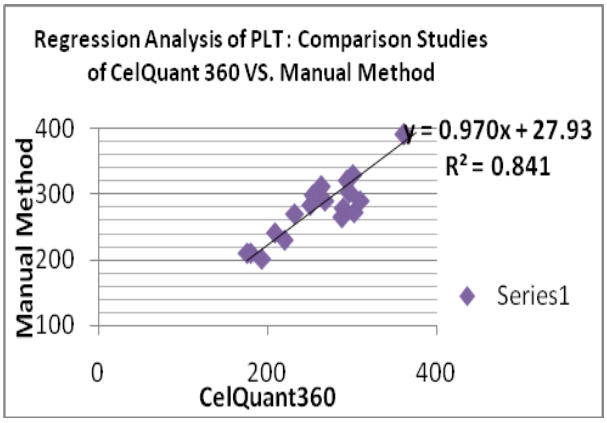


Figure. 6.

Harmonisation studies of HB shows that majority of data points are homogeniously distributed along the regression line towards positive directions with a maximal difference of +0.8 both in case of Merilyzer CelQuant 360 automated haematology analyser versus Sysmex XP-100 haematology analyser and Merilyzer CelQuant 360 automated haematology analyser versus manual methods (Figure 7 and Figure 8). This implied that for both tests WBC and Hct correlated positively when the two methods were compared.

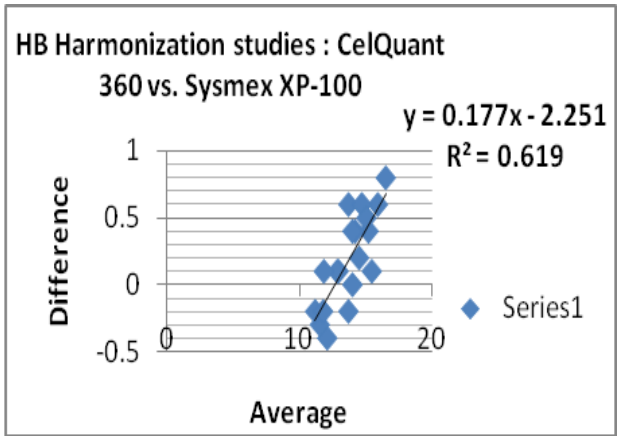


Figure. 7.

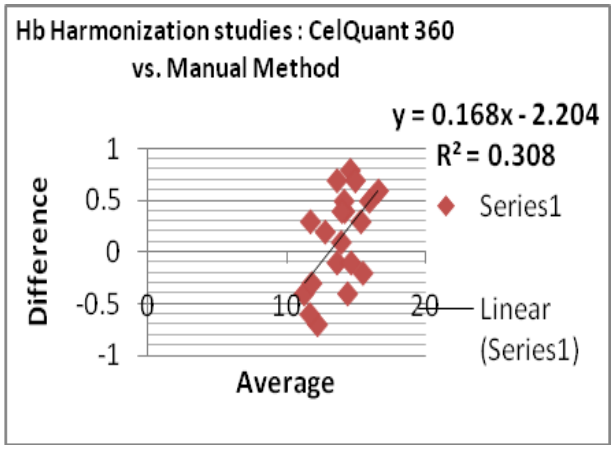
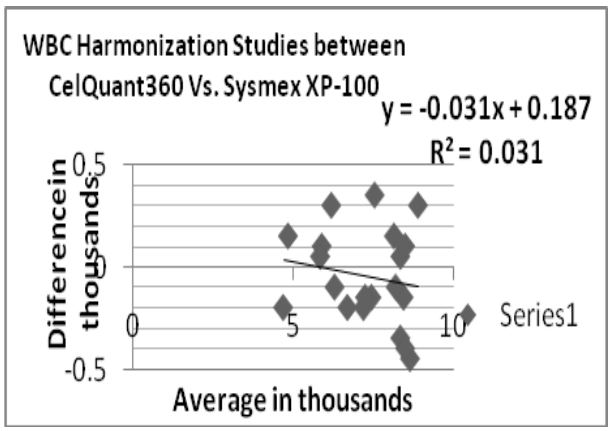


Figure. 8.

In contrast, heterogenous distribution of data points are noted in both WBC and PLT counts studies irrespective of Merilyzer CelQuant 360 versus Sysmex XP-100 and Merilyzer CelQuant 360 versus manual methods with a maximal differences of + 1000 in case of WBC counts (Figure 9 and Figure 10) and + 50 in case of PLT Figure 11 and Figure 12.



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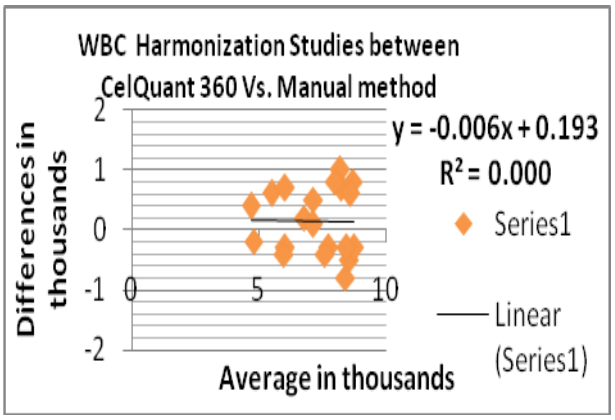

Figure. 10.

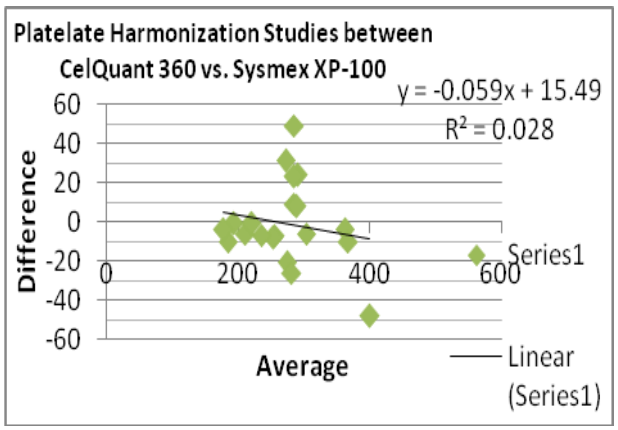

Figure. 11.

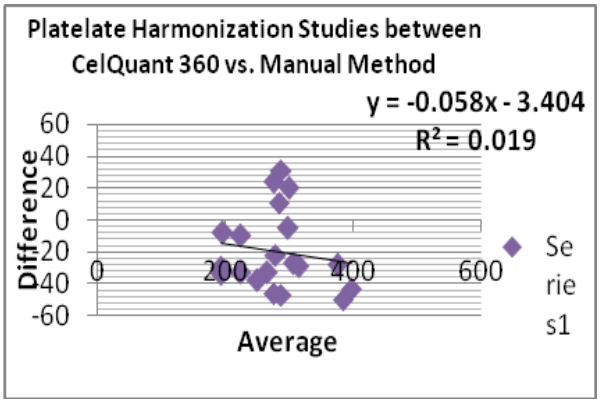

Figure. 12.

The mean PLT counts by both methods of the studied patients, it was found that the estimated mean PLT count did not show significant difference between the two methods.

DISCUSSION

It is to be noted that the Japanese Committee for Clinical Laboratory Standards has recently prepared minimum clinical allowance of blood cell count as follows: Hb: 3%, Hct: 4%, MCHC: 4% PLT: 10% and WBC: 7%.[9]

Automated peripheral blood WBC counts are widely accepted in routine practice. But many laboratories still reflexively perform manual CBC solely based on abnormal automated results or instruments "flags" before any manual triage step, to establish quality control.[9] In this study, WBC count by automated method demonstrated "un flagged" samples with incomplete WBC counts.[10] This is probably due to immature cells. However, this is in agreement with other study whose result indicated discrepancies in a quality control survey in a manual WBC count, which was attributed to poor differentiation.[11] Also, these analysers are able to provide more information about WBC count, like analytical interference of the cells like blasts, atypical lymphocytes, immature granulocytes and nucleated RBC.

However, Hb and PCV by the manual method may result in variations in the RBC indices. This is best seen with MCHC which may result in misclassification of values for diagnosis of anaemia. This indicated that, the manual method still has same advantages over the automated methods, although they are slow and at times cumbersome.[12] Other studies show that the Hct values determined by haematological analyser cannot replace the results obtained by manual microhaematocrit method.[13]

When all the PLT were analysed, we observed significant positive correlation between the result of both the methods. Till date, the only 'Gold Standard" in PLT counting available to assess the degree of accuracy of the automated cell count has been the manual phase-contrast microscopic method. The manual method itself has

significant limitations in terms of performance, particularly in the area of PLT. Recently, a Joint Task Force of the ISLH and ICSM has recommended a new immunological method for PLT count.[14] But this immunological method is not available in most of the laboratory.

CONCLUSION

In conclusion, it is plausible to note that homogenous and heterogenous distribution pattern of data points of Hb, WBC and PLT counts are related to inherent characteristic properties of the particular instrumental specificity like flow path, optical designing, electrical impedance properties and finally instrumental calibration properties.

The advantages of using automated system such as Merilyzer CelQuant 360 is that their hardware and software comply with the international regulations for working under a good manufacturing practice environment and the records or data obtained from the analysis are stored under a security – chronological records as per requirement of ISO 15189 standards.

Also, the manufacturers provide technical support, documentation and recommendations to the personnel at each laboratory to perform installation and operational qualification (IQ / OQ) procedures, which facilities the process of validation of instruments. In short, Merilyzer CelQuant 360 automated haematology analyser is an optimised space saving, cost effective instrument which can compete with any time tested cell counter available in the market.

CONFLICT OF INTEREST

The authors declare no conflict of interest in the present study conducted.

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