Original article:

Cost effectiveness & accuracy analysis of mannual versus automated methods of estimation of basic haematological parameters in a resource poor setting

Richa Ranjan[1], R K Singh[2], Rigvardhan[3]

1. Richa Ranjan , Assistant Professor, Dept of Pathology, Command Hospital (CC) Lucknow-226002
2. R K Singh, Assistant Professor, Dept of Paediatrics, Command Hospital (CC) Lucknow-226002
3. Rigvardhan, Associate Professor, Dept of Pathology, Command Hospital (CC) Lucknow-226002

Corresponding author : Rigvardhan

Abstract

Background: A competent medical care starts with an accurate estimation of basic hematological parameter which primarily includes hemoglobin, total leukocyte count and differential leukocyte count. The primary concern in our country, being a resource challenged nation, is to cut down the cost of health care without compromising the quality.

Aim: Our study was done with the aim of comparing the cost incurred and accuracy of results while estimating hemoglobin, total leukocyte count and differential leukocyte count by both manual and automated methods.

Material and Methods: We ran 750 consecutive blood samples sent for Hb, TLC and DLC in the automated hematology counter (a three part differential counter) along with simultaneous manual estimation of the above mentioned parameters. Accuracy, precision and cost of both the methods were compared on SPSS computer software.

Results: The study found that the cost when adopting manual methods is less than half as compared to the automated method. The accuracy by both the methods gave a satisfactory result except for the mixed cell counts, which give significantly incoherent results with automated method.

Conclusion: When the volume of the laboratory investigations is not overwhelming and resources area constraint, performing basic hematological parameters by manual method is a viable option

Keywords: Accuracy, hemogram, Cost effectiveness, Manual versus Automated Methods, Resource poor setting.

Introduction:

Hemoglobin estimation along with total and differential leukocyte count is of utmost importance while investigating a patient and serves both as a diagnostic parameter as well as a baseline record for further management.[1] It is advised in almost every admitted case, most of the out-patient and in all the females undergoing antenatal checkup, accounting for sizeable part of laboratory work load.

Hemoglobin (Hb) is a porphyrin iron protein compound that transports oxygen from lungs to the body tissues where it is utilized for energy metabolism. .[2] Surveys of hemoglobin concentrations are also used as tools to provide public health data, such as nutritional status, and to monitor malaria interventions.[3] While Hb is done by cyanmethemoglobin method ,TLC is done by diluting the sample with WBC diluting fluid and charging the Neubaur chamber. For DLC, Leishman stained slide is used (1). Different methods are in use like acid hematin method, cyanmethemoglobin method by

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photometer and automated methods by cell counters. .[4]

Automated methods for Hb, TLC and DLC counts are based on impedance or light scattering technology based on the coulter principle was given by Wallace and Coulter in 1956. .[5] Automated method is based on impedance principle for cell counting while lysing and calorimetry for Hb estimation. A lytic reagent is added to the blood solution to selectively burst the red cells, leaving only white cells and platelets intact, and then the solution is passed through a second detector. This allows the differential count of rbcs against wbcs and platelets to be obtained. The platelet count is easily separated from the WBC count by the smaller impedance spikes they produce in the detector due to their small volume. (5)Cost for any procedure consists of material, labor and expenses. Direct costs are those costs that can be identified entirely to a particular department or a product or a service. Indirect cost cannot be entirely identified to a particular dept, product or a service. While the direct costs are controllable costs the indirect costs are not controllable.[6]

Material and methods:

750 consecutive samples for Hb, TLC and DLC were run in the automated counter KX21 (Sysmex) along with manual estimation of the above mentioned parameters. These samples included those which were received both on routine basis (daytime) and emergency basis (off-working hours). Samples with hemolysis were excluded from the study. Blood samples were drawn in vaccutainers, having K_3 EDTA anticoagulants. The automated hematology cell counter KX 21 (Sysmex) was recalibrated. R1, R2 and R3 controls (EIGHT check) from Sysmex were used. After proper mixing, hemoglobin was estimated by hematology cell counter KX 21 (Sysmex). Parallel estimation for Hb count was done manually by spectrophotometer using cyanmethe-moglobin method. 20µl of well mixed blood sample was taken in 5 ml of Drabkin's reagent and incubated at 37˚C for 10 minutes. Absorbance was taken at 540 nm.[4] Commercial control was also run with each batch. Accuracy, precision and cost of both the methods were compared on SPSS computer software. TLC estimation manually was done on 20µl of well mixed blood sample and diluting it in 0.38 ml of lysing fluid which has Gentian violet and Glacial acetic acid. The function of Glacial acetic acid is lysis of RBC. The Neubaur chamber was charged after thorough mixing and TLC was done in the outer four squares of Neubaur chamber at 10 X magnification. DLC count was done on a well made peripheral smear. Entire smear under a low power was scanned for quality of smear. Relative proportion of the various types of wbcs was observed under 40X magnification. 100 wbcs are counted using exaggerated battlement method.

These tests were done by a technician employed permanently by this laboratory who works around 08 hours on an average basis. Cost of all the 750 test samples was worked out based on the cost of the consumable materialcost which was common to both the procedures was calculated and added to the individual cost of both the methods. Cost of the equipment like the microscope, automated analyzer, photometer were not taken into account as they are nor recurring expenses.Indirect cost, salary of the technician, electricity and rent was not done ,To keep the study simple. In other words, in this study we calculated primarily ,the cost of consumables

Results:

The results were accessed using both the parameters of cost , time taken and accuracy of the results. The

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cost of common variables for both the methods was Rs 6.15/test. The expendables for manual method were Rs 8.90/test while for automated method was Rs 25.7/test. The total cost thus was Rs 15 and Rs 32 per test for manual and automated method respectively. How the cost was worked out is given in (Table1, 2 and 3).As far as time is concer-ned total time taken by manual method and automated method for all the 750 tests was 500 and 40 hrs respectively. (Table 4). The accuracy of the results obtained by both the methods are given in Table 5.

Tab 1: Common expenses for 750 investigations were as such

Equipment	Cost (Rs.)
Test tubes	490
Syringe	3570
Swabs	375
Total:	4615
Cost per test	6.15

Tab 2: Reagents expended for manual methods were as such:

Variable	Cost (Rs)
Drabkin's solution (5ml/test)	700
Control solution	150
TLC fluid	910
Leishman stain	2622
Buffer	1690
Glass Slide	500
Hb Pipettes	45
Bulb	30
TLC Pipettes	50
Total	6697
Cost/ test	8.9
Total Cost/ test	15

Tab 3: Reagents expended for automated methods were as such:

Variable	Cost (Rs)
Annual maintenance contract	1000
Cell pack (20 lit):	6000
Stomatolyzer (500 ml)	8600
Controls	3400
Paper roll	300
Total	19300
Cost per test:	27.7
Total Cost of each test	32

Tab 4: Time taken for procedure

Procedure	Time
Sampling time	2 min
Each set of automated test	50 sec
Each set of manual test	40 min
Total time for each manual test: 40 mins	42 min
Total time for each automated test:	3 mins
Total time for manual tests: in a month	500 hours
Total time for automated tests	40 hrs

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Tab 5: Comparison of accuracy by both manual and automated methods

Variable	Sysmex counter	Manual
Mean Hb (gm %)	13.7	13.2
Mean TLC	7636	7780
Mean neutrophil (%)	64.18	64.4
Mean mixed cell (%)	7.5	4.6

Discussion:

Hemoglobin TLC and DLC are the most commonly advised test in any Laboratory across the world. Hemoglobin estimations are used in hospitals forIndividual patient management, to guide transfusion practice, and in the Management of therapy. Different methods are in used for Hb estimation like acid hematin method, cyanmethemoglobin method by photometer and automated methods by cell counters (4). Different laboratories need different methods depending upon number of patients, technical skill of staff and availability of funds. .[5]The total count is done by using counting chambers that hold a specified volume of diluted blood are used to calculate the number of red and white cells per liter of blood. The total wbcs is determined in whole blood in which red cells have been lysed. .[6] The drawbacks of this method are: field error Dilution errors, technical errors When TLC counts are too low or high appropriate dilutions are need to be done .[1]DLC count consists of the systematic examination of a well made stained peripheral smear and enumeration of the relative proportion of the various types of wbcs. Entire smear under a low power is scanned for quality of smear. Then using 40X magnification, 200 WBC are counted using either linear strip method or exaggerated battlement method. The disadvantages are that even when 200 cells are counted the errors are of the order of 7% .[7] And if only 100 cells are counted, a 10% error is expected.

Automated methods for Hb, TLC and DLC counts are based on impedance or light scattering technology.[4] The coulter principle was given by Wallace and Coulter in 1956.[8] Electrical analysis involves passing a dilute solution of the blood through an aperture across which an electrical current is flowing. Impedance counting is based on the principle that RBC is a poor conductor of electricity whereas diluents are good conductors. The passage of cells through the current changes the impedance between the terminals (Coulter principle). As direct current resistance changes the blood cell size is detected as electrical impulses. Blood cell count is calculated by counting the pulses and a histogram of blood sizes is plotted by determining the pulse sizes. As large numbers of cells are counted, precision is high .[8] The light scattering principle is also used in certain equipments. Counting of blood cells is based on the volumetric impedance method, directly measuring white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), platelets (PLT), mean corpuscular volume (MCV) and mean platelet volume (MPV) and automatically calculating hematocrit (HCT), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), RBC distribution width (RDW), plateletcrit (P-LCR) and platelet distribution width (PDW). A lytic solution is added to the blood solution to selectively burst the red cells, leaving only white cells and platelets intact and then the solution is passed through a second detector. This allows the differential count of rbcs

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against wbcs and platelets to be obtained. The platelet count is easily separated from the WBC count by the smaller impedance spikes the produce in the detector due to their small volume. [9]

The instrument differentiates the subpopulations of lymphocytes, granulocytes and the mid-cell fraction (eosinophils, basophils, monocytes and precursors of wbcs) by electronic sizing. Specially formulated reagents cause the WBC membrane to shrink around the nucleus while keeping the cell intact, allowing separation of white cells according to their volume. Lymphocytes fall within the small-cell region, neutrophils within the large-cell region and the remaining cells into the mid size cell region. The three part differential screen is provided with a region flagging criteria (R-flags) system based on computer check of the three different cell populations' peaks and valleys histogram. Suspect flags are generated after the instrument has evaluated the three-part differential indicating the possible distribution or morphological abnormalities. .[9]Manual counting is subjected to sampling error because so few cells are counted compared with automated analyzers. .[1]Comparison of automated and manual methodsIn general, the automated counter counts as compared reasonably well with routine manual counts if the instrument is assigned only two functions that is performing differential counts on normal samples an flagging the abnormal samples. If a differential count shows other than distributional abnormalities there is no substitute of the human observer for the recognition and enumeration of abnormal cells. .[1]Also, a percentage of normal patient's platelets will clump in EDTA anticoagulated blood. In these cases the automatic analyzers will give a falsely lower platelet count. On looking manually at the slide in these cases, clumps of platelets will be visible. .[1]

Cost effectiveness of both automated and manual methods.

In general, people understand costs in terms of monetary price or the amount of expenditure incurred on goods and services. .[6] But economist understands cost as the sacrifice made in order to obtain a goods or a service. Also one pertinent point is that resources do not have a monetary value and cannot be exactly measured.

Cost is of material, labor and expenses. Direct costs are those costs that can be identified entirely to a particular department or a product or a service. Indirect cost cannot be entirely identified to a particular dept, product or a service. It is important to note that direct costs are controllable costs whereas indirect costs are not controllable. .[6] Material is the substance from which the product is made. It may be a raw product or a product of output. Material costs can be direct and indirect.Indirect material costs is what is used for purpose ancillary to the production of service and which cannot be easily assigned to a specific physical unit is termed as indirect material e.g. Consumable stores, oil and wastes printing, stationary material. .[6]

Direct labor cost is of the labor which takes an active and direct part in the production of a particular commodity or in provision of a service is called direct labor, e.g. Salary of a technician. The indirect labor cost is employed for the purpose of carrying out activities incidental to the goods reduced or services provided is called indirect labor.

Direct expenses are those expenses that are directly, easily and wholly allocated to specific costs centers or cost units, e.g. Hiring instruments for a diagnostic purpose. Indirect expenses: are expenses that cannot be directly, easily and wholly allocated to costs centers or cost units, e.g. Rent building,

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lighting.There have been few studies which have tried to work out the most cost effective and yet an accurate method of Hemoglobin estimation. One study was done in Multan (Pakistan) by Waqar Azin et al.[10] And other is a study from Malavi (Africa) by A Medina Lara et al.[3] Waqar Azin et al concluded that the both manual as well as automated methods are accurate and precise for hemoglobin estimation, with reference range 2.61% more in manual method. It is recommended that with small samples and with parameters like hemoglobin or hemoglobin with erythrocyte sedimentation rate, manual method is cost effective and feasible. However, with multiple parameters like absolute values and with very large batches, like in tertiary laboratory, automated method is time effective and feasible, provided the laboratory can bear the cost .[10] The African study also concluded that it is needed to take account not only of cost, but also simplicity, accuracy, speed, available manpower and technical skills of their laboratory work force and the health needs of the population before we choose a method. .[11, 12]

In this study, the automated cell counter costs Rs. 32/- per sample for hemoglobin, TLC and DLC estimation while method performed manually costs Rs. 15/- per sample only. This cost is comparable with the study by Waqar Azim *et. Al* while concluded that the cost of automated analysis was Rs. 50/- per test, while manual method costs Rs. 3.5/- per test . The cost estimation in this study does not take in to account many factors like cost of machine procurement, minor breakage of laboratory ware, emoluments given to the technician, electricity bills, rental for the laboratory, general maintenance of the lab area.

While working out tests for significance the p value was less than 0.005 for only the mixed cell count while the p value for other parameters of hemoglobin, TLC, neutrophil and lymphocyte counts were > 0.005. The gold standard or the reference standard for the hemoglobin, TLC and DLC is manual. The statistical analysis, points out that by using automated methods, the variation that occurs in the values of hemoglobin, TLC, neutrophil and lymphocyte counts is acceptable. On the other hand, the mixed cell count on the automated counter does not tally well with the manual method. The proposed reason for this could be that the three part differential count uses volume parameter to identify the cells. Increase in size of lymphocytes, say due to a viral infection (leads to activation) and thereby this lymphocyte gets counted as a mixed range cell. Time taken by manual method for a single sample is around 450 mins, compared that of automated method which is approximately 3 mins. The difference seems pretty large. Most of the manual method estimations are done in a batch that is collection of sample is done and when reasonable numbers of samples are collected they have to process together as a batch. This saves on the time and fatigue of the technical staff. So the manual method works well for routine samples but for an urgent case manual method takes far too much time compared to the automated method.

Conclusion:

The study was done on 750 samples for hemoglobin, TLC and DLC, using a three part hematology cell counter and manual methods. We conclude that the automated cell counter costs per sample is more than double compared to tests performed manually. Therefore cost wise manual method is an option in a small hospital with a moderate work load. As time taken automated method is much less compared to

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manual methods , manual method can be resorted to only when the samples received are not too many.As far as accuracy goes, both the methods give a satisfactory result except for the mixed cell counts, which give significantly incoherent results with automated method. Making manual method a superior choice compared to a three part differential counter.To use a manual method of estimation, a well trained technical staff is a definite prerequisite. In case, it is not so automated methods with the correct use of control samples are a better option.

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