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Inter-Hematology Laboratory Quality Assessment with Reference to Hemoglobin Estimation, Packed Cell Volume and Total White Cell Counts

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Abstract

Majority of clinical decisions are said to be based on laboratory test results. Therefore, discrepant and unreliable laboratory results may cause serious consequences for the health of individuals and the society. This study is designed to evaluate the degree of accuracy and precision of laboratory test results from different laboratories. Fifty blood samples were collected from apparently healthy subjects and sent to 3 different participating hospital laboratories designated as A, B and C within northern Nigeria over a ten-week period. The laboratories, all using standard hematology techniques, undertook Hb estimation, total WBC counts and PCV measurements in an inter-laboratory quality control assessment. The study revealed that laboratories B and C obtained significantly lower mean values of 13.20 g/dl and 13.80 g/dl for hemoglobin respectively compared to 14.60 g/dl from the originating laboratory ($p<0.01$). Meanwhile, the mean WBC values for laboratories B and C appeared significantly higher than the accurate mean. Two laboratories (B and C) also obtained mean values of PCV slightly different from the accurate one while laboratory A had similar mean PCV value to the accurate one. Generally, higher variance ratios between laboratories than between samples ($P<0.01$) was observed in hemoglobin estimation and WBC count, while PCV showed a high variance ratio between samples than laboratories. However, the reproducibility of test results of participating laboratories was good. With the increasing reliance on laboratory test results for the diagnosis of diseases, clinical laboratories must embark on regular intra- and inter-laboratory quality evaluations to minimize misdiagnosis.

Keywords: Quality control; Accuracy; Precision; Inter-hematology; Packed cell volume; Hemoglobin

Introduction

Quality control in health laboratory results commonly refers to the set of checking procedures intended to ensure a reasonable degree of consistency in observations made and results reported [1,2]. Clinical laboratories, including the hematological, are faced with an increasing workload as well an increasing complexity and an over boarding range of tests [3]. At the same time, there is an increased demand for greater validity of the test results. It has been recognized that this demand can only be met adequately when every factor in the analytical process is appropriately controlled [4]. These factors include the continuous training and retraining of personnel, quality control of equipment, quality of regents and references preparations and finally the way in which tests are actually performed and quality control usually refers only to the control of the last-mentioned factor [5,6]. This involves assessing by control samples, the accuracy and the precision of the methods being applied in the daily practice.

Two facets of quality control protocols have been identified: the internal or intra-laboratory control, which involves the analysis of control samples selected daily by the laboratory supervisor, in order to check precision and accuracy within the laboratory. The second facet is external or inter-laboratory control. This involves the analysis of control samples periodically from the outside sources in order to compare accuracy levels of different laboratories [7]. A procedure is said to be under control when both the accuracy and the precision of the results are within generally accepted, though arbitrary, limits [8].

The practice of internal quality control originated spontaneously, its need been self-evident [9]. The demand for external quality control did not arise, however, until it became apparent that the differences, sometimes significant, existed between results in different laboratories. The wish to obtain comparable results is motivated not only from dissatisfaction from the analytical point of view but also by such reasons as the frequent movement of patients from one health service facility to another [10].

External quality assessment (EQA) can be organized at international, national and regional or local levels [11-13]. At the international level, the two main bodies involved in the organization of quality control trial are the International Committee for Standardization in Hematology (ICSH) which certifies standards and produces recommendations on methods and WHO involves in training and sponsoring the international external quality assessment scheme (IEQAS) [14,15]. At the national level, government and professional bodies are involved in education and EQA schemes; and such schemes can also function on a regional or local level were the turn round time can be much reduced [16]. We aimed to carry out an inter-hematology laboratory quality assessment at the local level in Zaria, Northern Nigeria. The study was designed to provide an independent check on the accuracy of results of the participating laboratories. It particularly assesses the quality of Hematology laboratory results including Hemoglobin (Hb) estimation, packed cell volume (PCV) determination and total white blood cell (WBC) counts from the participating laboratories to ensure reliability of laboratory test results as they relate to test results from the originating laboratory.

Materials and Methods

Study location

The three participating laboratories were all situated in Zaria, northern Nigeria and were within 5 KM of each other. The originating laboratory analyzed samples for accurate values at the Out-Patient Laboratory Unit, Ahmadu Bello University Teaching Hospitals, Zaria.

Ethical considerations and study consent

Approval for this study was granted by the Ethics Committee of the Institute of Health, Ahmadu Bello University Teaching Hospital, Zaria after an application was made with copies of detailed study proposal. After obtaining individual consent from the study participants, venous blood samples were collected and labelled to aid identification, classification and differentiation of data.

Study cohort

Fifty apparently healthy individuals, comprising medical laboratory science students and staff of Ahmadu Bello University teaching hospital Zaria between the ages of 20-30 years were recruited for this study regardless of gender, ethnicity, socio-economic status, and religion.

Sample collection and processing

From each study participant, 10 ml of venous blood was collected into universal bottles containing 10 mg of Ethylenediaminetetraacetic acid (EDTA tripotassium salt); mixed and 2.5 ml were dispensed into each of the labelled Z/5 universal plain plastic sterile tubes. One batch of sample aliquots was kept and processed by the originating laboratory for hemoglobin value, packed cell volume and total white cells count to obtain the “accurate value” while the remaining three tubes were delivered to the participating laboratories within 30 minutes of the collection to analyses for same parameters. The 4 sample aliquots were given the same identity. Reproducibility test was also carried out at the last part of the study using 40 ml of blood from a donor blood bag. Each participating laboratory was given 10 ml; 2 ml was dispensed into each of five Z/5 universal plain plastic sterile tubes given different identities to check the reproducibility of the participating laboratories.

Study sample size estimation

This study was aimed at evaluating for discrepancies in hematology laboratory results from the different participating laboratories in Zaria. The interest lies in the variance ratios between laboratories and samples. Sample size calculations are based on a Bonferroni adjusted α level of 0.00625 to control the overall type I error rate at 0.05. Therefore, the parameters used for the sample size calculation for comparison between groups with an equal number of subjects include the statistical power at 80%, α level at 0.00625, the standard deviation of 1 and clinically relevant difference of 1. This gives an overall sample size of 50 study participants.

Laboratory protocol for determination of accurate values

Packed cell volume estimation: Packed Cell Volume or hematocrit measures the ratio of the volume occupied by red blood cells to the volume of whole blood in a sample of capillary, venous or arterial blood. The ratio is measured after appropriate centrifugation and is expressed as a decimal fraction. Method: Each sample was treated in duplicate. The plain capillary tube was filled to about ¾th of its length with the well-mixed EDTA venous blood sample using a tube roller mixer. The dry end of the tube was sealed with plasticine clay to about 2 mm deep, and tubes were placed in the groove of the microhematocrit centrifuge. Hawksley Microhematocrit Reader was used to determine the value of hematocrit after centrifuging the tubes at 15,000 rpm for 5 and 10 minutes respectively. The average of the duplicate values was reported as PCV in L/L. The maximum difference between the duplicate tests was 0.4% and the readings at 5 and 10 minutes were usually the same. The mean of the duplicate values was reported as the accurate value.

Total white cells count: The anticoagulated blood sample was diluted with Turk’s solution. The cells were then counted in a calibrated chamber, and their number in undiluted blood reported as WBC/cubic millimeter or in × 10^9/L. Method: 20 µL of a well-mixed anticoagulated blood sample was washed into 380 µL of diluting fluid in a clean khan tube. The improved Neubauer Counting chamber was cleaned with glass cloth and the cover glass applied to obtain Newton’s rings. The chamber was then charged with 1-2 drops of the preparation of diluted blood when a cover glass is placed firmly on the chamber (Newton's ring). After allowing cells to settle for 2 minutes, cells were counted from the four corner squares under low

power (10×), and the number of cells counted was multiplied by 50, and the average value was reported as the number of WBC per cubic millimeter or in × 10^9/L.

Hemoglobin estimation: The hemoglobin concentration of each blood sample was determined in duplicate by diluting 20 µL of well-mixed EDTA blood sample in 5.0 ml of Drabkin's solution [17]. After allowing 10 minutes for complete conversion of the hemoglobin to cyanmethemoglobin, each dilution was read in a Corning colorimeter at the yellow-green filter (540 nm wavelength) calibrated with the cyanmethemoglobin standard. The readings were repeated at least once within 3 hours. The mean of the different estimations per sample was taken as the accurate value for the Hb. In all the 50 samples, the maximum difference between the different values of each estimation was 0.3 g/dl.

Data management and statistical analysis: The means and standard error of means (SEM) of Hemoglobin, Packed Cell Volume and total White Blood Cell counts of the 50 samples from the participating laboratories and the originating laboratory were analyzed to determine differences between participating laboratories and the accurate value. A 2-way analysis of variance to evaluate for differences between results from the laboratories and between samples was also carried out. This was to evaluate for any significant difference in laboratory results of hematology laboratories in the region.

Results

Data were analyzed using statistical package for social sciences (SPP) version 23.0 software to compare mean values of PCV, Hb and total WBC counts between participating laboratories A, B and C, and the originating laboratory (accurate values). Differences that had p-values ≤ 0.05 were considered statistically significant, while those with p-values>0.05 were considered not statistically significant.

Table 1 presents the means and standard errors of mean for PCV values of 50 samples from the originating, and participating laboratories (A, B and C) were found to be 0.45 (45%), 0.44 (44%) and 0.45 (45%) respectively.

Table 1 The Mean and Standard Error of Mean (SEM) of Packed Cell Volume of 50 samples from the originating and participating Laboratories.

Laboratory	Mean PCV in L/L (%)	S.E. M.	Difference between laboratory value and accurate value
A	0.45 (45)	0.19	0
B	0.44 (44)	0.19	-0.01 (-1%)
C	0.44 (44)	0.19	-0.01 (-1%)
Accurate Value	0.45 (45)	0.19	

*Estimation from the residual mean square line in the analysis of variance table.

Meanwhile, **Table 2** displays the 2-way analysis of variance of determination of PVC in fifty (50) samples in four laboratories. Differences in PCV values between laboratories and between samples were statistically significant (p ≤ 0.01).

Table 2 Two-way analysis of variance of determinations of Packed Cell Volume of 50 samples in 4 Laboratories.

Source of variation	D. F.	Sum of square	Mean squares	Variance ratio	Probability
Between Labs	3	29.7	9.9	5.2	P<0.01
Between Samples	49	1626.7	33.2	17.5	P<0.01
Residual	147	383.6	1.9		
Total	199	1940			

Note: The PVC values were treated in percentage for the 2-way analysis of various determinations of P.V.C. in 50 samples in 4 Laboratories.

Table 3 shows the means and standard errors of mean (SEM) determination of Hb estimation from laboratories A, B, C and originating laboratory. The Hb mean values in g/100 ml are; 14.46, 13.20, 13.80 and 14.60 respectively.

Table 3 The Mean and Standard Error of Mean (SEM) of Hemoglobin values of 50 samples from the originating and participating Laboratories.

Laboratory	Mean value in g/100 ml	S.E.M.	Difference between Laboratory value and correct value
A	14.46	0.15	-0.14
B	13.20	0.15	-1.40
C	13.80	0.15	-0.80
Accurate Value	14.60	0.15	

*Estimated from the residual mean square line in the analysis of variance table.

Table 4 Two-way analysis of variance of determination of Hb estimation of 50 samples in 4 Laboratory.

Source of variation	D. F.	Sum of square	Mean squares	Variance ratio	Probability
Between Labs	3	62.7	20.9	19.0	P<0.01
Between Samples	49	237	4.8	4.4	P<0.01
Residual	147	162	1.1		
Total	199	461.7			

The percentage errors in the mean determinations for laboratories A, B and C as compared with the accurate ones are 0.9, 9.6 and 5.5 respectively.

In **Table 4**, the 2-way analysis of variance of determination of Hb estimation of fifty (50) samples from the laboratories was displayed. The differences in Hb values between laboratories and between samples were reported to be significant ($p \leq 0.01$).

Table 5 presents the mean and standard error of mean determination of WBC count from the laboratories A, B, C and the originating laboratory. The WBC mean values are reported as 4.47, 5.02, 4.96 and 4.4 respectively.

Table 5 The Mean and Standard Error of Mean (SEM) of total White Cells Count of 50 samples from the originating and participating Laboratories.

Laboratory	Mean total WBC count ($\times 10^9$/L)	S.E.M.	Difference between Laboratory value and correct value
A	4.47	0.19	0.07
B	5.02	0.19	0.62
C	4.96	0.19	0.56
Accurate Value	4.40	0.19	

*Estimation from the Residual mean Square Line in the Analysis of Variance table.

The 2-way analysis of variance of determination of WBC count of fifty (50) samples in four the laboratories are shown in **Table 6**. A statistically significant difference in the values of total WBC count between the laboratories and between samples was observed.

Table 6 Two-way analysis of variance of determinations of WBC count of 50 samples in Laboratories.

Sources of variation	D. F.	Sum of squares	Mean square	Variance ratio	Probability
Between Labs	3	15.6	5.2	2.9	P<0.01
Between Samples	49	207	4.2	2.3	P<0.01
Residual	147	263.4	1.6		
Total	199	486			

Table 7 represents the reproducibility of results in the form of the coefficient of variation measurement of PCV, WBC and Hb between the participating laboratories A, B, C and the originating laboratory. The coefficient of variation of PCV between laboratory A, B, C and the originating laboratory are; 2.0, 2.4, 1.4 and 0 respectively. The coefficient of variation of WBC between laboratories A, B, C and originating laboratory are 1.8, 4.1, 4.2 and 3.8 respectively. Also, the coefficient of variation of Hb between laboratories A, B, C and originating laboratory are 0.95, 3.15, 3.45 and 1.08 respectively.

Table 7 Reproducibility of results: Coefficient of variation measurements.

Laboratory	CV (%)		
	P.C.V.	W.B.C.	Hb
A	2.0	1.8	0.95
B	2.4	4.1	3.15
C	1.4	4.2	3.45
Accurate Value	0	3.8	1.08

Discussion

There is no doubt, the purpose of a laboratory quality control is to detect laboratory errors; and the objective is to assure the highest standard of performance in accuracy and precision that is both practical and useful in the conditions of medical practice [18,19]. Unfortunately, there is no absolute standard of laboratory accuracy, and the results of the analysis fall within a range determined by the method, skill and circumstances under which it was performed. The setting of the range of acceptability must, therefore, be to a large extent, arbitrary. However, this can be related to what can be achieved by some laboratories operating under similar conditions and using similar methods and equipment [20]. The analysis of PVC, WBC and Hb depend on many variables, and that is why there is an acceptable range of variation for each of the estimations. Unlike other estimations like prothrombin time, folic acid, vitamin B assay, electrolytes where different laboratories are allowed to fix in-house normal ranges, there is no such allowance for PCV, Hb and WBC.

In essence, the results for the hematological parameters should be near to the actual values as possible, irrespective of the laboratory determining them when operating under similar conditions and using similar methods and equipment [20]. That is to say, a patient whose PCV is determined to be 0.45 L/L (45%) by one laboratory cannot be estimated as 0.35 L/L (35%) by another when the patients under the same conditions of health. It is pertinent to mention that; these differences may not be significant at the "normal range" but highly significant at the critical range. For example, an error of ± 0.10 L/L (10%) at a PCV of 0.45 L/L (45%) little or no significance as a single determination, but an error of this order at a critical level of 0.30 L/ L (30%) may determine whether a patient is correctly diagnosed as anemic. A similar situation holds for Hb and WBC.

As earlier mentioned, clinical laboratories including the hematological, are faced with an increasing workload as well as an increasing complexity and an ever broadening range of tests [21]. At the same time, there is an increased demand for a greater validity of the test results: the need for accuracy in hematology laboratories has been a concern to most hematologists in the world [22] and the Nigerian society for Hematology and Blood Transfusion.

The results of PCV were not too bad, though **Table 2** shows that differences between the samples and the laboratories are significant (p=0.01). In trying to find the source of the difference from the table of means, I found that slight differences exist between the mean PCV values of laboratories

'B' and 'C' and the accurate value (-1%) while laboratory A recorded exactly the same mean value as the accurate mean. Compared with the accurate mean, the proportionate errors in laboratories B and C is 2.2%. Now considering differences between samples, it was found that the result was not so good. A PCV of 0.39 L/L (39%) was recorded as 0.31 L/L (31%) by one laboratory and as 0.35 L/L (35%) by another (4-8%) and the highest variation was recorded when one laboratory estimated PCV to be 0.49 L/L (49%) as against 0.39 L/L (39%), of the accurate value (10% difference). It was shown that 29 results differed by 1-3% from each other and 16 results differed from each other by 1% only. As earlier mentioned 1-3%, 4-8% and even 10% may be of little significance at the normal but very significant at the decision- making or critical level especially that of 10%.

The lowest PCV recorded in this series was 0.31 L/L (31%), which makes it very impossible to demonstrate the havoc that can arise from such low PCVs (critical range) in this work. The commonly incriminating factors for inaccurate PVC are improper packing of the cells (technical error). Generally, the results of this study show a scatter at both (+) and (-) sides of the accurate values. Centrifuging the capillaries for a further 5 minutes (10 minutes) did not change the hematocrit values at the originating laboratory. The discrepancies observed in PCV results from the participating laboratories could be associated with improper use of hematocrit reader, insufficient mixing of samples and inappropriate labeling of capillary tubes which results in mixing up of results.

The Hb results showed that there were differences of up to 1.4 gm between the laboratories. **Table 4** shows that differences between the laboratories and samples are also highly significant ($p \leq 0.01$). From table of means (**Table 3**), these differences are clearly shown when laboratories A, B and C have mean values significantly lower than the accurate value. These results varied from 1.0 gm in one laboratory to 4.8 gm in another; 8 results varied by more than 2 gm; and four others by exactly 2 gm. Nine results varied by more than 3 gm while 3 (three) others varied by more than 4 gm and the remaining others fall below unity. This error is very alarming mainly for values within the critical range. Applying the factor (coefficient of variation) of ± 3.5% for Hb [23] of the college of American Pathologists, most of the results lie well outside this range. The variation found attributed by a number of factors which include pipetting, sampling and proper use of colorimeters and standards. Results of estimation will depend on how deep the pipette is dipped into the sample that stands for hours on the bench and mixed manually. From this study, it was made clear that many laboratory staff are aware and can trace the causes of inaccuracies but only a few works against them in practice.

Table 6 shows that differences between laboratories are not as significant as encountered in the samples. However, the mean values in **Table 5** showed errors of different proportions exist between the laboratories. The laboratory 'B' mean value is significantly higher than the accurate one and all the other two results. Laboratories A and C mean value are slightly higher than the accurate value. The percentage errors in the mean determinations for laboratories A, B and C as compared with the accurate ones are respectively 1.6, 14.0 and 12.7.

The WBC results showed that there exists a maximum difference of 0.6 × 10^9/L between the laboratories. Various differences exist between the samples; one result varied from 3.0 × 10^9/L in one laboratory to 8.1 × 10^9/L in another. Differences of 4.0 × 10^9/L, 4.2 × 10^9/L and even 5.2 × 10^9/L were also observed in three results of this series. 15 results showed differences of the range 2-3.5 × 10^9/L and a maximum difference of 1.9 × 10^9/L (1-1.9 × 10^9/L) was observed in twenty-three results.

The variation between the laboratories as illustrated in the 2-way analysis of the variance of determinations table (F-table) is accepted as against variation between samples ($p<0.01$). Low counts were shown in 15 results and more errors were observed with these results. The tendency for counting debris when the counts are low is suggestive of the errors encountered; though this should not arise if solutions are filtered and counting chambers cleaned before use. Some laboratories have the habit of prolonged storage of the specimens on the bench which affects the reliability of such results, that is, the length of time between the collection of samples and actual performance of tests is prolonged unnecessarily. Dilution of each sample was made in duplicate and results of the duplicate showed the best agreement.

Reproducibility of individual laboratories was good as illustrated by a test of reproducibility included in this series (**Table 7**).

Conclusion

We have conceived the impression that most hospitals in our locality may be turning out grossly inaccurate results. Conclusion drawn from this study indicates that most hospitals in Zaria and probably from other parts of Nigeria may be turning out grossly inaccurate and inconsistent results. This is so because other surveys revealed that similar situation is reported in the Western State of Nigeria. Considering the significant role of hematological parameters in the diagnosis, treatment and management of diseases, inter-laboratory quality control programs should be encouraged by the Medical Laboratory Science Council of Nigeria to enhance quality of laboratory performance and to minimize discrepancies in Hematology laboratory results for effective health care service. Furthermore, that as part of its overall responsibility to strengthen health care services, governments at all levels should have the prime responsibility for establishing official external quality assessment of health laboratories in Nigeria. It is also suggested that government should approve and support Medical Laboratory Scientists to organize, operate and evaluation and undertake National EQA Schemes across the country.

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