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INTER-LABORATORY VARIABILITY OF HAEMOGLOBIN MEASUREMENTS OBTAINED FROM SELECTED CLINICAL LABORATORIES IN KENYA
E. W. Mandania, BSc, MSc, J. R. Ongus, BSc, MSc, PhD, Senior Lecturer, S. N. Kaggia, MBChB, MMed, Lecturer, Department of Medical Laboratory Sciences, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi and F. Abdallah, MBChB, MMed Lecturer, Department of Human Pathology, College of Health Sciences, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya

Request for reprints to: E. W. Mandania, P.O. Box 1206-01000, Thika, Kenya, Email address: emandania@yahoo.com

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E. W. MANDANIA, J. R. ONGUS, S. N. KAGGIA and F. ABDALLAH

ABSTRACT

***Background*: Accurate and precise tests results generated by clinical laboratories would assist correct decision making in the diagnosis and management of patients. Quality assurance, which comprises of internal quality control (IQC) and external quality assessment (EQA), is intended to ensure the reliability of the laboratory results.**
***Objectives*: To evaluate the inter-laboratory variation of haemoglobin (Hb) measurements.**
***Design*: A descriptive cross-sectional study.**
***Subjects*: EQA samples with low (A), normal (B) and high (C) Hb concentrations**
***Setting*: A total of 292 clinical laboratories selected from 21 out of 47 Kenyan Counties.**
***Main outcome measures*: Mean deviation from the expected mean of the references and coefficient of variation (CV).**
***Results*: A total of 68%, 64% and 51% of laboratories gave accurate results for the sample A, B and C respectively. Based on the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88) criteria, 61% of laboratories had acceptable performance. Inter-laboratory variation of 33.3%, 25.1% and 29.4% for samples A, B and C was recorded irrespective of the analyser a laboratory used. When grouped according to the type of analyser, CV reduced to 5.1% (Haemocontrol) to 41% (Urit) for sample A, 2.2% (Celltac) to 35% (Diaspect) for sample B and 3.4% (Medonic) to 42.6% (Diaspect) for sample C. These differences were statistically significant (p <0.001) across all Hb concentrations.**
***Conclusions*: The inter-laboratory variation in Hb measurements resulted from variation in methodologies and types of analysers. Regular participation in External Quality Assessment Schemes (EQAS) is essential in order to achieve inter-laboratory comparability of Hb results.**

INTRODUCTION

The measurement of blood haemoglobin (Hb) is one of the most common routine clinical laboratory tests (1). Accurate determination of Hb concentration is a common component in assessing the extent of anaemia and a significant variable in guiding transfusion therapy in patients (2).

A quality assurance programme has two main aspects, namely, internal quality control (IQC) and external quality assessment (EQA) (3). IQC and EQA play very crucial roles in ensuring reliability of analytical results (4). EQAs of clinical laboratories monitor inter-laboratory comparability of results as well as detecting bias (systematic errors) and overall review on the IQC programme (5, 6).

Although quality assurance programmes play an integral part in clinical laboratories of developed countries, these programmes have not been accorded the same degree of importance in the laboratories of developing countries. In Kenya, several organisations namely United Kingdom National External Quality Assessment Scheme (UKNEQAS), Kenya Accreditation Service (KENAS), Randox International Quality Assessment Scheme (RIQAS), Human Quality Assessment Scheme (HUQAS), East African Regional Quality Assessments scheme (EAREQAS), among others, regularly organise EQAS in haematology including Hb estimation. Despite the existence of the EQAS programmes in Kenya, however, laboratory participation is still very low and optional. Therefore, it is important that studies such as this present one are frequently carried out to help in the improvement of inter-laboratory comparison. This study sought to evaluate the extent of interlaboratory variation in Hb measurement across clinical laboratories of Kenya and to determine the ability of the participating laboratories to accurately differentiate normal, low and high Hb samples.

MATERIALS AND METHODS

This was a descriptive cross-sectional study of 292 laboratories sampled from 21 out of 47 counties in Kenya and which voluntarily consented to participate in the study. The study was based on determination of Hb in three blood haemolysate samples with low (sample A), normal (sample B) and high (sample C) Hb concentrations. The main study site was the Central Laboratory at the African Medical and Research Foundation (AMREF), Nairobi, Kenya from where all samples were prepared.

Preparation of the Haemolysate: Three EQA samples with low, normal and high Hb values (samples A, B and C) respectively were prepared as follows:

One pint (450 ml) of blood in a blood bag which had tested negative for Human immunodeficiency virus, hepatitis B virus surface antigen, syphilis and hepatitis C virus antibodies was obtained from the Kenya National Blood Transfusion Service (KNBTS). Using a centrifuge tube, 40 ml of the blood was centrifuged to separate plasma and Buffy coat aseptically. To the red blood cells deposit, 2-3 fold volume of physiological saline (9 g/L Sodium Chloride)(NaCl) was added, mixed well and centrifuged at 2000g for five minutes. The supernatant and any remaining buffy coat was discarded completely. This saline wash was repeated two times to ensure complete removal of plasma, white cells and platelets. To the washed cells, half its volume of carbon tetrachloride (neat), 99%, was added and the mixture shaken vigorously in a mechanical shaker (Vibrofix VF1, Janke and Kunkel Model) at 2500g/minute for one hour. The mixture was then stored at 4°C overnight thus forming a semi-solid interface of lipid/cell debris between carbon tetrachloride and lysate. The mixture was centrifuged at 2500g for 20 minutes and the upper lysate layer was carefully pipetted out into a clean Winchester bottle. Sterility and stability of the haemolysate was maintained by the addition of preservatives and broad-spectrum antibiotics. To each 70 ml of lysate, 30 ml of glycerol was added followed by addition of 25-50 mg of penicillin and 25-50 mg of gentamicin per 500 mL of material. To make haemolysate with lower Hb concentration, an appropriate volume of 30 % (v/v) glycerol in 9 g/L NaCl was added to the stock and mixed well using a roller for one hour. The Hb target or reference values for the three samples were assigned using the reference system. While stirring continuously, 1 mL aliquots were dispensed aseptically into 2 mL sterile vials, capped, sealed and labelled appropriately. The samples were assigned unique codes A, B and C for sample with low, normal and high Hb concentration respectively. The samples were preserved at 2-80 C in the refrigerator awaiting dispatch.

Sample packaging and transportation: Each participating laboratory received three samples labelled sample A, sample B and sample C. The samples were placed in leak proof plastic vials which were properly labelled with the unique code numbers. After preparation of aliquots, samples were checked for any leakage, spillage and if they were correctly labelled with well sticking labels. The quality of the samples was maintained during transport by use of icepacks so as to maintain temperatures of 2-8^0 C. Samples were also secured during transport so that there is no leakage, spillage or contamination. In order to ensure that all the necessary requirements were followed by the participating laboratories, the samples were accompanied by a complete set of instructions with respect to storage, handling and deadline for analysis.

Sample processing: The laboratories were instructed to process the samples within two days after delivery using their current analytical procedures, to process the samples in the same way as routine samples and record the results in the worksheet provided. Samples were analysed in duplicate by performing two assays with a difference of not more than six hours between the assays.

Alongside the study, each laboratory also received a questionnaire so as to collect data on analytical aspects related to Hb determination. This included methods of analysis used, reference ranges used by each laboratory, control materials and reagents used for analysis, use of Standard Operating Procedures and previous participation in EQAS. The laboratories were also required to state clearly, by filling the questionnaire, the date the samples were received, date of analysis and the type of equipment/method used. The results were collected from the participating laboratories within one week after dispatch of the samples.

Ethical Considerations: Approval to carry out the study was obtained from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (Ref: KNH – ERC/A/1).

Confidentiality was maintained by assigning unique codes to the participating laboratories rather than using their names and the coeds were only known by the principal investigator.

Data analysis: Data were entered in Ms excel worksheet, coded and edited using consistency checks, checks for duplicate entries and range checks. Data were analysed using XLSTAT statistical software (XLSTAT Version 2013.3.03). For inter-laboratory variability, coefficient of variation (CV) was used. The variation was also assessed using analysis of variance (ANOVA). Significant differences ($P<0.001$) between means were assessed by ANOVA. For evaluation of

performance of participating laboratories, acceptable performance criteria given by the CLIA' 88 was used (7). Accuracy of results was analysed by calculating the difference (expressed as the bias) between the Hb concentration provided by the participating laboratories and target values. We tested for the effect of the Hb concentration on the proportion of the laboratories that performed well based on CLIA'88 criterion using Chi-Square analysis. Data presentation was done using tables and graphs.

RESULTS

Two hundred and ninety two laboratories sampled from both public hospital laboratories and privately-owned laboratories participated in the study. All levels of laboratories, that is, national reference laboratories, accredited-District, Sub District and Health centres were considered. A total of twenty seven different analysers were used across all the laboratories (Table 1).

Table 1
Types of analysers used by laboratories

Analyzer	Number of Hb Measurements	Number of Labs
ABX Micros	6	1
ACT Diff Beckman Coulter	18	3
BTS 305	12	2
Celltac	174	29
Cera Check	18	3
Colourimeter	78	13
Coulter Counter	24	4
Diaspect	306	51
Drew	6	1
Easy Mate	12	2
Sahli	144	24
Hb Meter	12	2
Haemocontrol	264	44
Haemocue	384	64
Hichroma	6	1
Humalyzer Junior	30	5
Hybrid	12	2
Kyrot	6	1
Medonic	30	5
Mindray	60	10
Mission	36	6
Pentra ES 60	6	1
RMS	6	1
Stat	12	2
Sysmex	48	8
Urit	30	5
Erma	12	2

The performance of participating laboratories in differentiating low, normal and high Hb measurements: Based on the CLIA'88 criteria, 61% of laboratories had acceptable performance across all measurements. The analyses shown in table 2 revealed that laboratory performance using CLIA'88 criteria declined with increase in the concentration of the target Hb value: 67.98%, 64.04% and 50.68% of laboratories passing for the sample with low (6.2g/dl), normal (13.6g/dl) and high (18.1g/dl) Hb values respectively. These differences were statistically significant (p <0.001) across all haemoglobin concentrations for both reading 1 and reading 2 (Table 2).

Table 2
Assessment of Laboratories as per to CLIA' 88 Test Performance Criteria

Measurement	Reading 1			Reading 2			Total		
	Passed	Failed	Percent success	Passed	Failed	Percent success	Passed	Failed	Percent success
Low (A)	198	94	67.81	199	93	68.15	397	187	67.98
Normal (B)	191	101	65.41	183	109	62.67	374	210	64.04
High (C)	148	144	50.68	148	144	50.68	296	288	50.68
Chi square			21.16			19.50			40.30
P value			P<0.001			P<0.001			P<0.001

The inter-laboratory variability of the Hb measurements: The overall inter-laboratory CV was 33.3% for sample A, 25.1% for sample B and 29.4% for sample C irrespective of the analyser a laboratory used. When inter-laboratory CV was calculated across laboratories using the same analyser, the CV reduced to 5.1 % (Haemocontrol) to 41 % (Urit) for sample A, 2.2% (Celltac) to 35% (Diaspect) for sample B and 3.4 % (Medonic) to 42.6% (Diaspect) for sample C (Table 3).

Table 3
The Inter-Laboratory Variability of Hb Measurements for Each Type of Hb Analyser

		Mean ± 1 Standard Deviation			CV (%)		
Hb Analyzer	n	A	B	C	A	B	C
Celltac	29	6.43±1.09	13.83±0.30	18.7±0.97	16.9	2.2	5.2
Colourimeter	13	6.64±1.18	13.57±1.79	17.39±1.72	17.8	13.2	9.9
Diaspect	51	4.06±0.68	9.61±3.36	9.37±3.99	16.8	35.0	42.6
Hemocontrol	44	6.25±0.32	13.96±0.51	18.86±0.84	5.1	3.6	4.4
Hemocue	64	8.37±2.37	16.16±3.09	21.14±4.10	28.3	19.1	19.4
Humalyzer Junior	5	6.10±0.43	13.00±1.07	17.64±0.84	7.1	8.3	4.8
Medonic	5	6.84±1.56	14.22±1.85	18.54±0.63	22.9	13.0	3.4
Mindray	10	5.99±0.6	13.33±0.48	18.35±0.78	10.1	3.6	4.3
Mission	6	11.25±2.62	16.52±1.49	19.67±1.14	23.3	9.0	5.8
Sahli	24	5.80±1.83	10.44±2.55	13.2±2.89	31.6	24.4	21.9
Sysmex	8	6.23±0.52	13.43±0.73	18.25±1.07	8.3	5.5	5.8
Urit	5	8.44±3.47	17.36±4.98	21.76±3.07	41.1	28.7	14.1

DISCUSSION

As demonstrated in this study, 61% of laboratories met the CLIA'88 criterion for samples A, B and C. The percentage of laboratories reporting unacceptable results was 32.02% for sample A, 35.96% for sample B, and 49.32% for sample C. The ability of a laboratory to accurately determine the Hb values of a sample depended on the type of analyser/ method used and the application and adherence to quality control practices by the laboratory. The results in this study have demonstrated considerable variation of Hb results when laboratories analyse the same sample. These differences in the results were statistically significant. Such results will have potential implications in the classification of patients. For instance, assuming sample A, B and C was representative of an anaemic subject, a healthy normal subject and a subject with higher Hb concentration respectively. A total of 1.7 % (5 out of 292), 19.8 % (58 out of 292) and 37.3 % (109 out of 292) laboratories respectively, would have misclassified these cases. This study results are slightly different from those of a study conducted by Paleari *et al* (2007)to evaluate the extent of interlaboratory variation and accuracy in hemoglobin A2 assays among 48 Italian laboratories who reported that the proportion of laboratories reporting unacceptable results was 31.9% (15 out of 47) for sample A, 17.0% (8 out of 47) for sample B, and 31.9% (15 out of 47) for sample C(8).They also reported that no laboratories would have misclassified the two cases represented by sample A (normal healthy subject) and sample B (the carrier) and only 11 out of 47 laboratories would have misclassified the case represented by sample C. In this study, the percentages of unacceptable results for all the samples are higher than those reported in their study and also some laboratories would have misclassified the three cases. This could be due to the fact that most clinical laboratories in Kenya are currently using the manual methods of haemoglobin measurement which are less accurate and reliable than the automated methods, which were used in their study.

In this study the range of Hb results obtained for each analyser/method varied widely. In the present study there was a high overall inter-laboratory CV for samples A, B and C. However, when the analysers were grouped according to the type of analyser, the variation of Hb results reduced to a great extent. This suggests that both method/analyser bias and laboratory-specific bias are the cause of the overall variability of inter-laboratory Hb results. Unfortunately, the inter-laboratory variation may have consequences for clinical practice depending on the case at hand. The results obtained in the present study are slightly different from those of Paleari *et al* (2007) in a study conducted to evaluate the extent of inter-laboratory variation and accuracy in Hb A2 assays among 48 Italian laboratories (8). They reported that the overall inter-laboratory co-efficient of variation was 8.0%, 7.9% and 6.0% and for samples with low, intermediate and high HbA2 levels, respectively which are lower than those reported in this study. These observed variations again could be due to the differences in the methods of analysis used by the developed and the developing countries. When different analysers were compared, the inter-laboratory CV ranged from 5.1 % (Haemocontrol) to 41 % (Urit) for sample A, 2.2% (Celltac) to 35% (Diaspect) for sample B and 3.4 % (Medonic) to 42.6% (Diaspect) for sample C. These inter-laboratory CVs are higher than those reported in another study conducted by Blerk *et al* (2007)to assess the reliability of Hb measurements in Belgian hospitals, which reported that inter-laboratory variation ranged from 0.6 % (Roche) to 6.7 % (IL) for sample 1 and from 2.0 % (Radiometer) to 4.5 % (IL) for sample 2 (9).

The study results indicate that very few (15%) laboratories participated in External Quality Assessment Schemes/PT programmes. A possible explanation to this may be due to lack of funds since EQAS is expensive, lack of awareness by some of the laboratories and lack of commitment by the laboratory staff and management as well. Regular participation in EQAS assists individual laboratories to continuously monitor their performance and to compare it with that of other laboratories. Also, it is an effective means for identifying problems that cause interlaboratory variation of laboratory results and initiates a process towards solving these problems thus improving the quality of service at the level of each individual laboratory. Lack of participation in EQAS is one of the factors among others, which cause inter-laboratory variation of laboratory results. Different laboratories used different reagents and control materials depending on the method of analysis/analysers used in their laboratory. Most laboratories reported not using quality control/reference materials when performing Hb test. This could be due to the fact that quality control materials are expensive and are not readily available to most laboratories. A close observation of the results indicate that even those laboratories that use quality control materials do not use all the three levels but only the normal Hb concentration. A good number (31.5%) of the laboratories did not use SOPs when performing Hb tests, however, most laboratories reported not experiencing any challenges in the analysis of the samples. These poor quality control practices further increase the problem of inter-laboratory variation of Hb measurements. To ensure standardisation of Hb measurements throughout the world, The International Council for Standardisation in Haematology (ICSH) in conjunction with Eurotrol, B.V. released a new lot of the haaemiglobincyanide (HiCN (Fe), HiCN) standard in 2008 for use in the

standardisation and calibration of whole blood Hb measurements on most haemoglobinometers and automated blood cell counters (10). However this reference standard is not readily available in the developing countries laboratories.

In conclusion, the average laboratory performance based on the CLIA'88 criteria across all measurements was 61%. The percentage of laboratories that gave accurate results for the sample with low (6.2g/dl), normal (13.6g/dl) and high (18.1g/dl) Hb values are 68%, 64% and 51% respectively. These differences were statistically significant ($p < 0.001$) across all haemoglobin concentrations

The overall inter-laboratory variation of Hb results was 33.3% for sample A, 25.1% for sample B and 29.4% for sample C irrespective of the analyser a laboratory used. When grouped according to type of analyser, the CV was lower, and ranged from 5.1 % (Hemocontrol) to 41 % (Urit) for sample A, 2.2% (Celltac) to 35% (Diaspect) for sample B and 3.4 % (Medonic) to 42.6% (Diaspect) for sample C.

RECOMMENDATIONS

Regular participation in External Quality Assessment Schemes (EQAS) in order to assess and improve laboratory performance, harmonisation of analytical methods and analysers for haemoglobin measurement, adherence to quality control procedures and use of quality control materials are essential in order to achieve inter-laboratory comparability of Hb results. In addition manufacturers should consider coming up with a single common type of calibration material that can be used for all analytical methods and analysers.

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