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External Quality Assessment of Haemoglobin Measurement: A Comparison of Different Analysers

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Abstract
The utilization of clinical laboratory test results in the diagnostic decision making process forms an essential aspect of clinical medicine. Inconsistent and unreliable laboratory test results may have severe effects on the health of the patient and society. The aim of this descriptive cross-sectional study was to evaluate the accuracy of most commonly used haemoglobin analysers in determining a standardised reference for low, normal and high haemoglobin values in blood and to compare the manual and automated methods of haemoglobin estimation. A total of 292 laboratories received three samples with low, normal and high haemoglobin concentrations for analysis, after which their results were evaluated for accuracy by comparing with the reference values. Accuracy of the analysers was evaluated using one-way analysis of variance while Coefficient of Variation was calculated as a measure of inter- method variability. Mean deviation from the expected mean of the references reflected the bias of each analyser. Overall 58.4 % (n=7/12), 17.6% (n=2/12) and 25% (4/12) gave accurate, underestimated and overestimated haemoglobin values respectively. Celltac, Humalyzer, Medonic, Mindray, Colourimeter, Hemocontrol and Sysmex produced results that were not significantly different from the reference values (P>0.05). Diaspect and Sahli underestimated while Hemocue, Urit and Mission overestimated Hb values (P<0.05). Automated methods were more precise than the manual methods with Coefficient of Variation for automated, semi-automated and manual methods being 7.08%, 7.04% and 34.26% respectively. With increasing reliance on the utilization of laboratory test outcomes for clinical decision- making, laboratories mustfrequently participate in External Quality Assessment in order to provide reliable results. Laboratories should embrace automation which gives more accurate and precise results.
Keywords: *Haemoglobin, External Quality Assessment, Accuracy, Bias.*

Introduction

There are two main elements of a quality assurance programme, namely, IQC and EQA[1]. EQA is described by the World Health Organization (WHO), as a method used by an external agency or facility for objectively verifying the performance of a laboratory [2]. EQA's main objective is to establish inter-laboratories, inter-methods, including inter-instrument comparability and agreement with a reference standard as well as detecting systematic errors (bias)[3].

Clinical laboratories of developed countries highly regard quality assurance programmes as one of the key players in quality of laboratory results and thus there is widespread enrolment and participation into these programmes. However, this is not the case in clinical laboratories in resource limited settings. In addition, the general perception that EQA is expensive has hampered extensive laboratory enrolment into these programmes[4].

Medical laboratories have the objective of providing accurate results to facilitate clinical decisions regarding the health of patients. To achieve this objective, laboratories must consider the quality of measurement procedures (MPs) results, engage in routine internal quality control (IQC) assessment, participate in external quality assessment schemes (EQAs) and comparison of their own results with those of other laboratories [5]. Quality assurance in haematology is designed to ensure the reliability of laboratory results.

Laboratory test outcomes may influence 45% of medical decisions in primary healthcare in East Africa [6]. It is therefore important that laboratories generate reliable and accurate results that will guide clinical decision making in order to improve the health of the patient. However, in the resource-limited settings most of the laboratory services provide results of unknown quality [7].

Haemoglobin (Hb) concentration is the most frequently used indicator of anaemia at the individual and population level[8] and is used to screen for anaemia as well as evaluation of responses to intervention programs [9].The Hb test is precise, easily standardised and may be performed either manually or by using automated hematology analysers[10]. The International Committee for Standardisation in Haematology (ICSH) recommends the Drabkin's method as the standard method for determining the Hb concentration of whole blood [11]

In this study, the accuracy and reliability of most commonly used Hb analysers in Kenyan clinical laboratories was compared to evaluate the results of known samples against reference values. Further the study aimedat comparing the manual and the automated methods of Hb measurement.

Materials and Methods

A descriptive cross-sectional study involving a total of 292 laboratories sampled from 21 out of 47 Counties in Kenya and which voluntarily consented to participate in the study was conducted from January to July 2014.Cluster random sampling of the laboratories from the 21 Counties was used so that all categories of laboratories in both public and private hospitals were sampled. The study was based on determination of Hb concentration in three EQA samples (blood haemolysate) with low (sample A), normal (sample B) and high (sample C) Hb concentrations**,** which were prepared in the Central Laboratory at the African Medical and Research Foundation (AMREF), Nairobi, Kenya. The Hb concentration (reference values) for the three samples was sample A (6.2g/dl), sample B (13.6g/dl), sample C (18.1g/dl), whose values were assigned using the reference system (Sysmex XS-1000i laboratory)

The samples were placed in leak proof plastic vials which were properly labelled with the unique code numbers. The samples were transported to the laboratories at 4^0 C in icepacks by carrier. Laboratories were instructed to process the samples within two days after delivery using their current methods of analysis by performing duplicate assays and to analyse the samples in the same manner as routine samples. Alongside the

study, each laboratory also received a questionnaire so as to collect data on Hb-related analytic information. Laboratories recorded their results in the worksheet provided and the results collected from the laboratories within one week. The results of each laboratory were evaluated for accuracy by comparing with the reference method (Sysmex XS-1000i laboratory).

Study population

The study covered a total of 292 haematology benches from 292 hospital laboratories sampled from the 21 counties in Kenya. The main study site was the Central Laboratory at African Medical and Research Foundation, Kenya Located in Nairobi, from where the samples were prepared, packaged and distributed to all the participating laboratories.

Inclusion Criteria

Both public and privately-owned laboratories that perform Hb measurements and gave free informed consent for participation and are registered by Kenya Medical Laboratory Technicians and Technologists Board (KMLTTB) were included in the study. All methods of Hb determination being used in the laboratories were applicable.

Exclusion criteria

Laboratories which are not registered by KMLTTB and which did not give informed consent were excluded from the study.

Ethical Approval

The study was approved by Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee (Ref: KNH – ERC/A/1).

Data Analysis

Data were analysed using XLSTAT statistical software (XLSTAT Version 2013.3.03). Using ANOVA, Significant differences ($P<0.05$) between means were assessed. Accuracy of analysers was analysed by calculating the difference (denoted as the bias) between the Hb concentration from the participating laboratory and the reference values. Differences that had p-values ≤ 0.05 were considered to be statistically significant.

Results

A sum of 292 laboratories participated in the study and 27 different analysers were used across all the laboratories (Table 1). Analysers that were used by less than five laboratories were excluded from the analysis, leaving a total of 12 analysers.

Table 1: The Different Analyzers and the Number of Laboratories Using Each Analyser Type

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
Hemocontrol	264	44
Hemocue	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
UritHb Meter	30	5
Erma	12	2
	1752	292

Applying the ±10% Allowable Deviation from the Reference Values, most of the analysers gave results that were accurate (comparable with the reference values) for all the three samples A, B and C, with only a few either underestimating or overestimating the Hb values, as shown in Table 2.

Table 2: Summary of the Performance of the Analyzers as per the ±10% Allowable Deviation from the Reference Values

Performance Of The Analyser	No of analyzers And Percentages		
	Sample A (Allowable Error, ±0.62g/dl)	Sample B (Allowable Error, ±1.3g/dl)	Sample C (Allowable Error, ±1.8g/dl)
Underestimated Hb values	1(8.3%)	2(16.7%)	2(16.7%)
Comparable with reference values	8(66.7 %)	7(58.3%)	6(66.6%)
Overestimated Hb values	3(25%)	3(25%)	2(16.7%)
TOTAL	12(100%)	12(100%)	12(100%)

Accuracy of the various analysers

The accuracy of Hb analysers in estimating low, normal and high Hb concentration is shown in Tables 3, 4 and 5 respectively. The mean Hb measurements from 22% (n=6/27) of the analysers (Sahli, Mission, Hemocue, Diaspect, Urit and Mission) were significantly different from the mean of the reference values (F (27) = 17.382, P<0.001,) while 78% (n=21/27) (Celltac, Humalyzer Junior, Medonic, Mindray, Colourimeter, Hemocontrol and Sysmex) gave values that were not significantly different from the mean of the reference values (P>0.05). The most accurate analysers, which gave results within the allowable bias for the three samples A, B and C were Celltac (bias 0.234, 0.231, 0.600) Colourimeter (bias 0.438, -0.031, -0.708) Hemocontrol (bias 0.048, 0.357, 0.759) Humalyzer Junior (bias -0.100,-0.600, -0.460) Medonic (bias 0.620, 0.620, 0.440) Mindray (bias -0.210, -0.270, 0.250) and Sysmex (bias 0.250, -0.175 and 0.150) for sample A, Band C respectively as depicted in tables 3, 4 and 5.

Table 1: The accuracy of the most commonly used analysers in Kenya in estimating low Hb value (6.2 g/dl)

Analyzer	Value	Standard. error	T statistic	P value
Intercept	6.200	0.284	21.823	< 0.001
Celltac	0.234	0.395	0.594	0.553
Colourimeter	0.438	0.498	0.880	0.380
Diaspect	-2.137	0.351	-6.083	< 0.001
Hemocontrol	0.048	0.361	0.132	0.895
Hemocue	2.167	0.339	6.397	< 0.001
Humalyzer Junior	-0.100	0.719	-0.139	0.889
Medonic	0.620	0.719	0.890	0.374
Mindray	-0.210	0.546	-0.384	0.701
Mission	5.050	0.666	7.579	< 0.001
Sahli	-0.396	0.414	-0.956	0.340
Sysmex	0.025	0.594	0.042	0.966
UritHb Meter	2.240	0.719	3.117	0.002

Table 2: The accuracy of the most commonly used analysers in Kenya in estimating normal Hb value (13.6 g/dl)

Analyzer	Value	Standard error	T statistic	P value
Intercept	13.600	0.447	30.418	< 0.001
Celltac	0.231	0.621	0.372	0.710
Colourimeter	-0.031	0.784	-0.039	0.969
Diaspect	-3.988	0.553	-7.213	< 0.001
Hemocontrol	0.357	0.568	0.628	0.530
Hemocue	2.556	0.533	4.795	< 0.001
Humalyzer Junior	-0.600	1.131	-0.530	0.596
Medonic	0.620	1.131	0.548	0.584
Mindray	-0.270	0.860	-0.314	0.754
Mission	2.917	1.049	2.782	0.006
Sahli	-3.158	0.652	-4.846	< 0.001
Sysmex	-0.175	0.935	-0.187	0.852
UritHb Meter	3.760	1.131	3.324	0.001

Table 3: The accuracy of the most commonly used analysers in Kenya in estimating high Hb value (18.1 g/dl)

Analyser	Value	Standard error	T statistic	P value
Intercept	18.100	0.540	33.498	< 0.001
Celltac	0.600	0.751	0.799	0.425
Colourimeter	-0.708	0.948	-0.747	0.456
Diaspect	-8.733	0.668	-13.069	< 0.001
Hemocontrol	0.759	0.686	1.106	0.270
Hemocue	3.038	0.644	4.714	< 0.001
Humalyzer Junior	-0.460	1.367	-0.337	0.737
Medonic	0.440	1.367	0.322	0.748
Mindray	0.250	1.039	0.241	0.810
Mission	1.567	1.267	1.236	0.217
Sahli	-4.900	0.788	-6.221	< 0.001
Sysmex	0.150	1.130	0.133	0.895
UritHb Meter	4.525	1.504	3.008	0.003

Variation of Hb measurements due to methods

A total of 74.32%, (n=217) of the laboratories used manual methods, 24% used an automated methods (n=70) and 1.7%, (n=5) used semi-automated methods. ANOVA comparisons revealed that the mean Hb across the three methods were not significantly different from the mean of the reference values ($F_{(3)}$ =1.333, P=0.262, mean of reference value =12.633). However the CV for the automated and semi-automated methods were similar but was large for the manual method, with a Coefficient of Variation (CV) of 7.08%, 7.04% and 34.26% respectively (Fig 1).

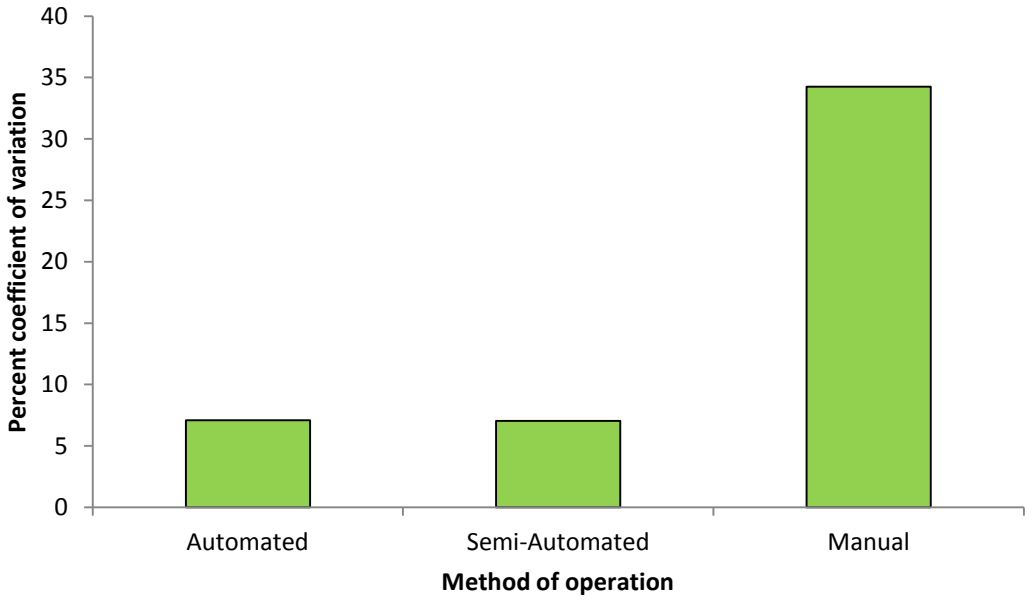

Figure 1: Coefficient of variation around the standard reference as a measure of reliability of the different methods of Hb analyser operation

Discussion

Considering that laboratory services play a major role in clinical decision making, then the reliability and quality of laboratory test outcomes is of utmost importance.

Laboratory quality control is intended to identify laboratory errors; and the objective is to guarantee the highest level of accuracy and precision [12, 13]. Both accuracy and precision of "standard" laboratory measurements are dependent on

multiple methodologic factors that affect them [14]. However, regardless of the method used, it is necessary that the different methods yield values that are as close as possible to the true value. The fact that the analysis of Hb is dependent on many variables, there is an acceptable range of variation for each of the Hb estimations.

Clinical Laboratories Improvement Amendment (CLIA) has proposed an acceptable range of deviation of Hb values of ±7% from the true value and a medically allowed error of 1g/dl. The CLIA specification variance is approximately1.0 g/dl for the normal Hb range of 13-15 g/dl, while asat the anaemic range of 10 g/dL the target variance is 0.7g/dL [15]. In other studies a 10% deviation from the reference method has been used because this variation constitutes only 1 g/dL Hb at the upper range of transfusion consideration (10 g/dL) which seems reasonable [16]. While all these values may be considered, in this study the ± 10% difference was selected. For every analyser being tested, a difference greater than ± 10% from the reference was considered as probably misleading. In this study, it translates to a deviation of not more than 0.62g/dl for Sample A and not more 1.3g/dl for sample B which represents the low (critical) range and normal Hb concentration respectively. Therefore, applying this criterion some analysers surpassed this allowable error gap, with some showing a negative bias while others showed a positive bias. Important to point out is that that these deviations may not be substantial at the normal range of Hb but very significant at the critical ranges of 6g/dl to 10g/dl where decision is made on whether to or not to transfuse.

Generally, Hb results acquired from Diaspect and Sahli showed negative bias where both devices underestimated hemoglobin values by more than 2g/dl across the three levels of haemoglobin concentration. The exception was Sahli which at low hemoglobin levels had a tendency to give values comparable to the reference method. The implication of this biases is that underestimation of Hb concentrations may classify healthy individuals as anaemic and could lead to unnecessary clinical interventions in patients, overtreatment and incurring of additional costs.

The results of this study agree with those of others that have reported underestimation of hemoglobin by Sahli[17, 18]. The considerable variability in the Hb values obtained with the Sahli could be due to its inbuilt errors, subjective visual colour comparison, inaccuracy in pipetting of blood, fading of comparator after prolonged use and poor sensitivity and reliability. Contrary to the results presented in this study, Robertson, Lewis, and Osei-Bimpong[19] reported that the Hb values obtained with Diaspect were comparable with the reference analyser.

On the other hand, this study results show that Hemocue, Urit and Mission consistently overestimated the Hb values when compared to the reference values. Hemocue overestimated Hb values by more than 2g/dl and this overestimate appears to increase with increase in the actual Hb value. This study findings agree with others which have reported overestimation of Hb values by Hemocue[20, 21]. However, other studies have reported that Hemocue is accurate and reliabl [22, 23]. On the contrary, Neufeld et al [24] reported that Hemocue underestimated Hb values. Due to these contradicting findings on the accuracy and reliability of Hemocue, further studies evaluating the accuracy of Hemocue need to be done. It is important to point out that Hb values overestimation may result in missing out of the true anemia in a patient where an anemic patient is classified as healthy. This can lead to delays in transfusion, under treatment and consequently increased morbidity.

The findings in this study about Urit agree well with those of Jitthai[25]who reported that Urit gave significantly higher Hb values than the automated blood analyser. The biggest variation with Urit seen at low and high Hb concentrations may imply that, laboratories use calibrators for the normal Hb concentration only without including calibrators for the low and high Hb levels.

This study findings showed there is considerable variation of Hb values when using manual

methods in comparison with automated methods and that automated methods have higher precision than the manual methods. This study results agree with those of Fink *et al*[26]. Inherent errors in the manual methods such as inaccuracies in dilutions, pipetting and improper mixing of samples, and errors caused by the observer would be the causes of this great variation. These apparent differences in Hb results obtained by different laboratories using different analysers/methods can be addressed by validation of analysers and harmonization of methods for Hb measurement in order to achieve inter-device and inter-method comparison of Hb results.

Conclusion

Celltac, Humalyzer Junior, Medonic, Mindray, Colourimeter, Hemocontrol and Sysmex produced results that are reasonably accurate, however Diaspect and Sahli underestimated Hb while Hemocue, Urit and Mission overestimated Hb when compared to the reference values. The manual methods generally showed lower precision when compared to automated methods. There is need for policy guidelines on validation, standardisation and approval of all analysers of Hb measurement being used in the Kenyan clinical laboratories in order to achieve inter-laboratory and inter- methods comparability of results. Laboratories should gradually replace manual methods with automated methods of Hb measurement which are more accurate and reliable.

Inter-laboratory quality assurance programs should be encouraged by all the relevant bodies and authorities such as KMLTTB and the Ministry of Health. In addition, government at all levels that is national and county governments should be primarily responsible for instituting formal EQAs across the country in order to continually improve laboratory performance and strengthen health care services.

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