

Quality Control Monitoring & Evaluation of Triglycerides, Cholesterol And Inter-Laboratory Assessment of Two Hospitals In Central Nigeria.

Stephen I. Audu^{1*}, Tukura B.W¹, Abah J.², Inyanda D³, Ojile J.O.⁴,
Isah J¹ and Alao B⁵.

¹Department of Chemistry, Nasarawa State University, Keffi, Nigeria, ²Department of Chemistry, University of Namibia, ³Department of Science Laboratory Technology, Federal Polytechnic Nasarawa ⁴Department of Reproductive Health, Smith & Nephew Inc., ⁵Department of Haematology, Benue State University Teaching Hospital, Makurdi, Nigeria.

Corresponding Author: Stephen I. Audu

Abstract: Quality control (QC) is one of the most important impacts on laboratory testing—it ensures both precision and accuracy of patient sample results. The integrity of quality control samples is important to both management of overall quality as well as to meeting requirements of proficiency testing. The study was done to assess the quality control monitoring and evaluation of triglycerides & Cholesterol and also for inter-laboratory studies of some haematological parameters [White blood cell (WBC), Red blood cell (RBC) and Haemoglobin (Hgb)], in Nisa Premier Hospital Abuja and the Benue State University Teaching Hospital Makurdi, North central Nigeria. The quality control monitoring & evaluation of Triglycerides and cholesterol were conducted for two consecutive months using the spectrophotometer (kenza240 TXbiolabo France), and the inter-laboratory studies was done in blood samples at both locations. At both locations determination was performed directly using the Automated Haematology Analyzer ((KX-21N Sysmex, USA)). The results of quality control showed that Trends (loss of reliability) and shifts (abrupt changes in the control mean) were noted in the results of this study, there were two major trends in July and more shifts in the month of August than in the month of July for triglycerides and a major trend and two violations of the Westgard Ruler for cholesterol. The results of inter-laboratory assessment of the haematological parameters shows that both imprecision and bias exist between both laboratories. Although imprecision values were generally in the range: $1 < CVR < 1.5$, indicating “Acceptable to marginal performance”, the overall bias between laboratories was in the range: $SDI > 1.5$ or $SDI < -1.5$; In this study, Warning signs were recorded but there was no cause for rejection of the analytical run. These violations typically identify smaller systematic error or analytical bias that is not often clinically significant or relevant. .

Keywords: Quality Control, Triglycerides, Cholesterol, Inter-laboratory, Westgard Rule

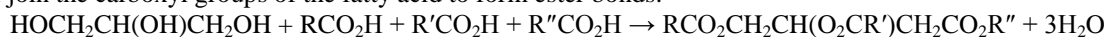
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I. Introduction

Quality control (QC) covers the part of quality assurance which primarily concerns the control of errors in the performance of tests and verification of test results. Quality control must be practical, achievable and affordable. The primary aim of quality control is to do the test reliably. The broad aim of quality control is that results from one laboratory should be comparable with that from any laboratory in the world provided the same method is followed [1]. Collaborative trials or method performance studies are used to test the performance (generally the precision) of a single analytical method. A standard method, which is routinely used in several laboratories, can also be examined collaboratively to test for a possible bias of either the method (method bias) or the laboratories (laboratory bias) that routinely use it [2]. Quality control (QC) is one of the most important impacts on laboratory testing—it ensures both precision and accuracy of patient sample results. The integrity of quality control samples is important to both management of overall quality as well as to meeting requirements of proficiency testing [3]. Accuracy can be defined as the agreement between value found and an accepted reference value. This requires a “gold” standard or method but in the absence of a gold standard or method, comparison to established reference labs may substitute. It is usually expressed as a percentage. Accuracy and precision together determine the total error of the analysis [3], whereas precision is a measure of reproducibility. Daily quality control chart preparation is mandatory. Control value within $\pm 2SD$ is good sign and patient’s results obtained are reliable and can be reported. If control value is beyond $\pm 2SD$ the result must not be reported and fresh control serum should be measured together with few patients sample. If the result is now

within $\pm 2SD$, the result can be reported. The type of material used for quality control are frozen, pooled serum; commercially available lyophilized freeze dried pool serum; commercially available low temperature liquid serum pools [4]. For hematology, standardization of staining procedures, preparation of proper blood film, bone marrow smear, special staining techniques flow cytometry, chromosome analysis are the mainstay of quality control [4]. Interpretation of quality control data involves both graphical and statistical methods. Quality control data is most easily visualized using a Levey-Jennings charts. The dates of analyses are plotted along the X-axis and control values are plotted on the Y-axis. The mean and one, two, and three standard deviation limits are also marked on the Y-axis. Inspecting the pattern of plotted points provides a simple way to detect increased random error and shifts or trends in calibration [5]. Rules, such as the Westgard rules can be applied to see whether the results from the samples when the control was done can be released, or if they need to be rerun. The formulation of Westgard rules were based on statistical methods. Westgard rules are commonly used to analyse data in Shewhart control charts. Westgard rules are used to define specific performance limits for a particular assay and can be used to detect both random and systematic errors. Westgard rules are programmed in to automated analyzers to determine when an analytical run should be rejected. These rules need to be applied carefully so that true errors are detected while false rejections are minimized. The rules applied to high volume chemistry and hematology instruments should produce low false rejection rates [6,7,8]. Triacylglycerols (triglycerides) are lipids, a type of fat, and their level in the blood is considered to be a measure of an individual's heart health. The dietary fat is synthesized in the liver, and moreover, can be obtained from food, particularly that which is derived from animal-based sources. Monounsaturated, polyunsaturated, and saturated fats that we get from our diet are also considered as triglycerides. They are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver, and are a major component of human skin oils. [9] Triglycerides are chemically tri esters of fatty acids and glycerol. Triglycerides are formed by combining glycerol with three fatty acid molecules. Alcohols have a hydroxyl (HO-) group. Organic acids have a carboxyl (-COOH) group. Alcohols and organic acids join to form esters. The glycerol molecule has three hydroxyl (HO-) groups. Each fatty acid has a carboxyl group (-COOH). In triglycerides, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds:



The three fatty acids (RCO₂H, R'CO₂H, R''CO₂H in the above equation) are usually different, but many kinds of triglycerides are known. The chain lengths of the fatty acids in naturally occurring triglycerides vary, but most contain 16, 18, or 20 carbon atoms. Natural fatty acids found in plants and animals are typically composed of only even numbers of carbon atoms, reflecting the pathway for their biosynthesis from the two-carbon building-block acetyl CoA. Bacteria, however, possess the ability to synthesise odd- and branched-chain fatty acids. As a result, ruminant animal fat contains odd-numbered fatty acids, such as 15, due to the action of bacteria in the rumen. Many fatty acids are unsaturated, some are polyunsaturated (e.g., those derived from linoleic acid) [10]. The pancreatic lipase acts at the ester bond, hydrolysing the bond and "releasing" the fatty acid. In triglyceride form, lipids cannot be absorbed by the duodenum. Fatty acids, monoglycerides (one glycerol, one fatty acid), and some diglycerides are absorbed by the duodenum, once the triglycerides have been broken down. In the intestine, following the secretion of lipases and bile, triglycerides are split into monoacylglycerol and free fatty acids in a process called lipolysis. They are subsequently moved to absorptive enterocyte cells lining the intestines. The triglycerides are rebuilt in the enterocytes from their fragments and packaged together with cholesterol and proteins to form chylomicrons. These are excreted from the cells and collected by the lymph system and transported to the large vessels near the heart before being mixed into the blood. Various tissues can capture the chylomicrons, releasing the triglycerides to be used as a source of energy. Liver cells can synthesize and store triglycerides. When the body requires fatty acids as an energy source, the hormone glucagon signals the breakdown of the triglycerides by hormone-sensitive lipase to release free fatty acids. As the brain cannot utilize fatty acids as an energy source (unless converted to a ketone) [11]. The glycerol component of triglycerides can be converted into glucose, via gluconeogenesis by conversion into dihydroxyacetone phosphate and then into glyceraldehyde 3-phosphate, for brain fuel when it is broken down. Fat cells may also be broken down for that reason, if the brain's needs ever outweigh the body's. [12] In the human body, high levels of triglycerides in the bloodstream have been linked to atherosclerosis and, by extension, the risk of heart disease [13] and stroke. [12] However, the relative negative impact of raised levels of triglycerides compared to that of LDL:HDL ratios is as yet unknown. The risk can be partly accounted for by a strong inverse relationship between triglyceride level and HDL-cholesterol level. But the risk is also due to high triglyceride levels increasing the quantity of small, dense LDL particles [13]. Cholesterol is a compound of the sterol type found in most body tissues. Cholesterol and its derivatives are important constituents of cell membranes and precursors of other steroid compounds, but a high proportion in the blood of low-density lipoprotein (which transports cholesterol to the tissues) is associated with an increased risk of coronary heart disease [3]. Cholesterol is susceptible to oxidation and easily forms oxygenated derivatives known as oxysterols. Three different mechanisms can form these: autoxidation, secondary oxidation to lipid peroxidation, and

cholesterol-metabolizing enzyme oxidation. A great interest in oxysterols arose when they were shown to exert inhibitory actions on cholesterol biosynthesis [14]. This finding became known as the “oxysterol hypothesis”. Additional roles for oxysterols in human physiology include their participation in bile acid biosynthesis, function as transport forms of cholesterol, and regulation of gene transcription.[15] According to the lipid hypothesis, since cholesterol (like all fat molecules) is transported around the body (in the water outside cells) inside lipoprotein particles, elevated cholesterol concentrations refers to hypercholesterolemia is associated with cardiovascular disease because LDL particles promote atheroma development in arteries (atherosclerosis)[16]. This atherosclerotic disease process, over decades, leads to myocardial infarction (heart attack), stroke, and peripheral vascular disease[16]. Abnormally low levels of cholesterol are termed *hypcholesterolemia*. Research into the causes of this state is relatively limited, but some studies suggest a link with depression, cancer, and cerebral hemorrhage[17].

II. Materials And Methods

2.1. Study Setting and Design

The study was carried out at the Haematology Laboratory of the Benue State University Teaching Hospital (BSUTH), Makurdi, and the biosciences laboratories of Nisa Premier Hospital, Abuja, Nigeria. These laboratories provide services to inpatients, outpatients and patients on emergency. The study was approved by the Health Research and Ethics Committee of the Hospitals.

2.2. Quality Control

Quality control monitoring and evaluation of triglycerides and cholesterol were conducted using Biolabo Extrator-N (Normal) and Biolabo Extrator-P (Pathological) by gently mixing 5mL of Extrator-N buffer with lyophilized Bovine Serum and inserting into the spectrophotometer KENZA 240 TX and results collated for two consecutive months of July and August 2016. The quality control product which is a patient-like material ideally made from human serum, urine or spinal fluid. The control products were in the form of liquid or freeze dried (lyophilized) material and are composed of one or more constituents (analytes) of known concentration [3]

2.3 Determination of haematological parameters:

The following haematological parameters [White blood cell (WBC), Red blood cell (RBC) and Haemoglobin (Hgb)] were determined in blood samples collected randomly from 410 patients at both locations. At both locations determination was performed using the same instrumentation; Automated Haematology Analyzer ((KX-21N Sysmex, USA). The blood samples of patients were collected into bottles containing EDTA anticoagulant and inserted directly into the Automated haematology Analyzer and concentrations of the haematological parameters were read. The Automated Haematology Analyzer are used widely in patient and research settings to count and characterize blood cells for disease detection and monitoring. The Automated haematology Analyzer used the Coulter principle, which blood is passed between two electrodes through an aperture so narrow that only one cell can pass through at a time. The change in impedance is proportional to cell volume resulting in a cell count and measure of volume.

2.4 Statistical analysis

Data collected were collated on Microsoft Excel spreadsheet and analysis was done using Analyse-it® Version 4.6, (Analyse-it Leeds, UK) [18]. The two of the most important metrics of an interlaboratory program are the coefficient of variation ratio (CVR) and standard deviation index (SDI), which are consensus-based metrics of imprecision and bias, respectively were used, and the Levey-Jeney's chart was used for quality control [18].

III. Results

The results of quality control showed that Trends (loss of reliability) and shifts (abrupt changes in the control mean) were noted in the results of this study as presented in figure 1 to figure 4, there were two major trends in July and more shifts in the month of August than in the month of July for triglycerides and a major trend and two violations of the Westgard Rule for cholesterol. Two control values were out of control and violated the 1_{2s} Westgard rule for both triglycerides and cholesterol in the month of July and August. The result of interlaboratory study shows that imprecision and bias exist in both laboratories, but imprecision was within Acceptable to marginal performance as presented in table 1.

Table :1 Results of Haematological analysis of inter-laboratory study

Haem. Parameter	Laboratory	Mean	SD	CV	CVR_Nisa	CVR_BSUTH	Average CRV	SDI_Nisa	SDI_BSUTH	Average SDI
WBC	Nisa	3.28	1.86	0.057						
	BSUTH	3.22	1.57	0.049	1.161	0.862	1.011	1.23	1.453	1.344
RBC	Nisa	5.09	2.93	0.048						
	BSUTH	5.68	3.31	0.058	0.987	1.013	1.000	3.379	3.942	3.661
Hgb	Nisa	12.70	3.05	0.024						
	BSUTH	14.42	3.92	0.027	0.884	1.131	1.008	9.025	10.263	9.644

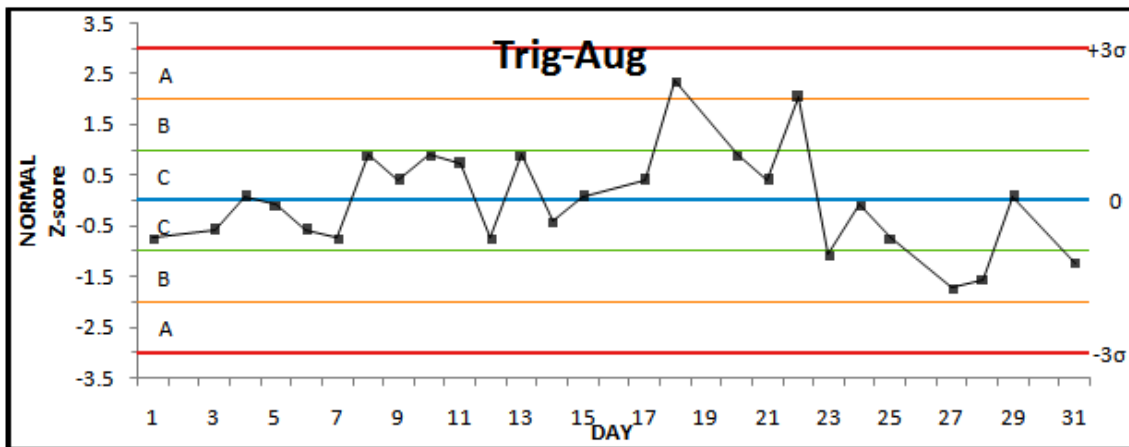
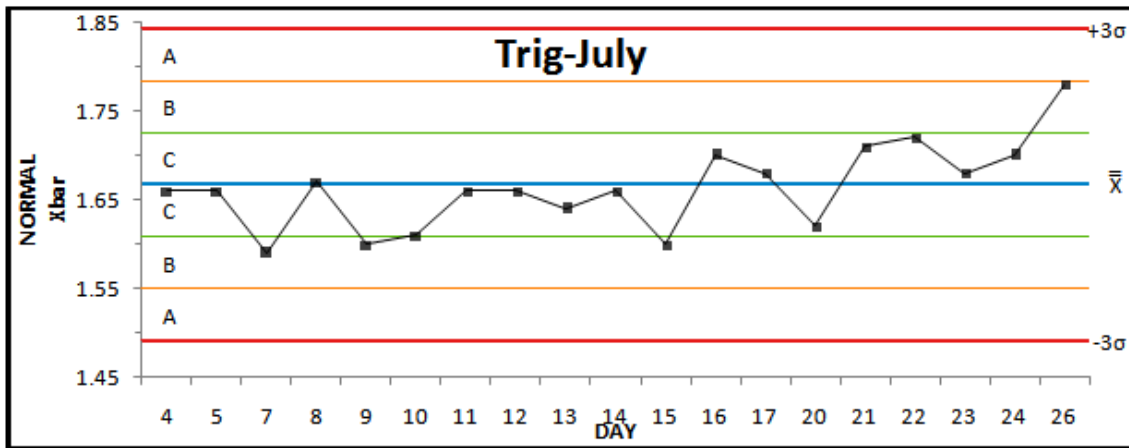


Figure: 1 QC charts for Bilirubinfigure

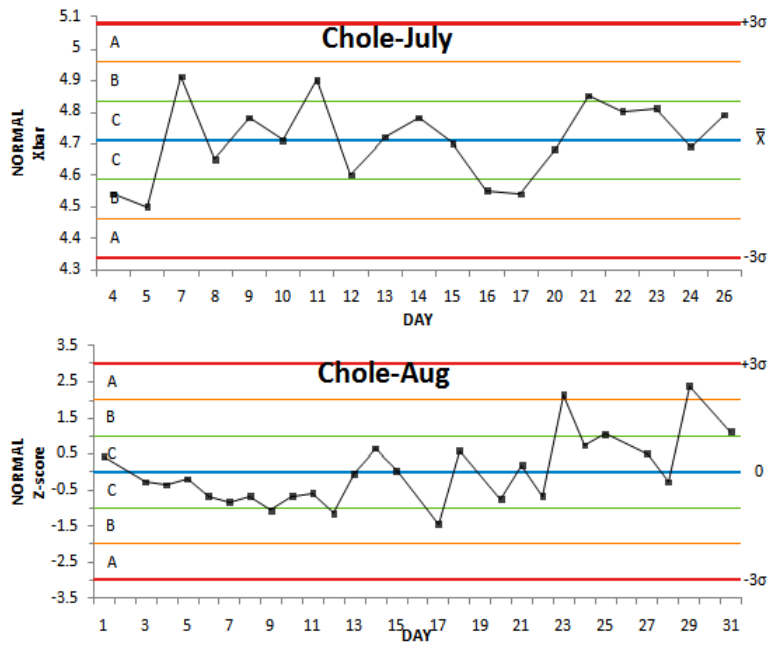


figure:2 QC charts for Cholesterol Chole- July/August 2016

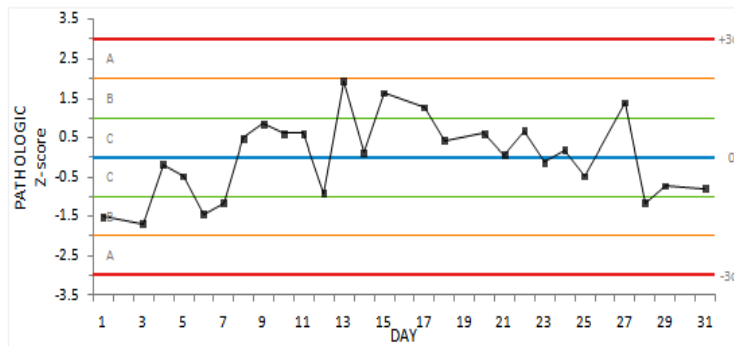


figure 3: Pathologic Triglycerides QC charts July/Aug 2016

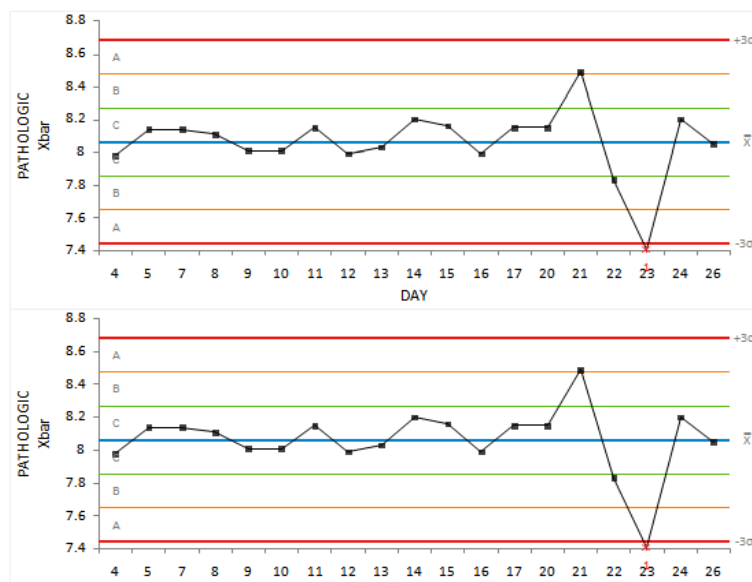


Figure 4: Pathologic QC of Cholesterol- July/August

IV. Discussion

The results of quality control showed that Trends (loss of reliability) and shifts (abrupt changes in the control mean)[18] were noted in the results of this study as presented in figure 1 to figure 4, there were two major trends in July and more shifts in the month of August than in the month of July for triglycerides and a major trend and two violations of the Westgard Rule for cholesterol. Two control values were out of control and violated the 1_2s Westgard rule for both triglycerides and cholesterol in the month of July and August. Two values were out of control and violated the 2_2s Westgard rule for cholesterol in the month of August and July for pathological controls and this indicates a systematic error. Control value within $\pm 2SD$ is a good sign and patient's results obtained are reliable and can be reported. If control value is beyond $\pm 2SD$ the result must not be reported and fresh control serum should be measured together with few patients sample. If the result is now within $\pm 2SD$, the result can be reported. The internal quality control is a key technical requirement through accreditation by the NF EN ISO 15189. This document presents recommendations to assist the medical laboratory to design, implement and operate daily and retrospectively an efficient system of internal control quality. It identifies the important issues attached to these different steps [19]. In this study some warning signs were recorded, it is not completely unusual but in the course of the study the laboratories had to introduce unusual attempts to bring their system in control. Analytical bias may be eliminated by performing calibration or instrument maintenance [18].

The result of interlaboratory study for haematological parameters (Table 1) shows that both imprecision and bias exist between both laboratories. Although imprecision values were generally in the range: $1 < CVR < 1.5$, indicating "Acceptable to marginal performance; may need to investigate test system imprecision", the overall bias between laboratories were in the range: $SDI > 1.5$ or $SDI < -1.5$; "Marginal performance; may need to perform corrective action" [17]. Steindel SJ. et al. conducted a quality control practices for calcium, cholesterol, digoxin and haemoglobin: a college of American pathologist Q-probes study in 505 hospital laboratories. The study was done to assess quality control (QC) practices and their impact on hospital laboratories using the College of American Pathologists (CAP) Q-Probes process. Retrospective and prospective QC failure rates compared with QC protocols and the corrective steps. Five hundred five hospital laboratories returned various components of the study. Median retrospective run rejection rates per 1000 runs: calcium, 4.3; cholesterol, 3.6; digoxin, 4.3; and hemoglobin, 2.4. Corresponding median prospective run rejection rates per 1000 runs: calcium, 5.8; cholesterol, 5.6; digoxin, 6.5; and hemoglobin, 3.6. Participants resolved most out-of-control events in less than 20 minutes, with no patient samples repeated. More than 95% of the time, participants resolved out-of-control events simply by repeating controls. Most participants used a single control rule based on a target mean plus or minus a multiple of the standard deviation. They recommended that current testing methods yield few out-of-control events, which usually are resolved rapidly, with little impact on laboratory operation. So modification and simplification of laboratory QC practices to decrease false rejection rates and to use modern instrumentation more efficiently was recommended. The results of this study is very vital for the patients and the laboratory staffs as it gives room for improvement and adequate corrective measures to be taken in diagnostic analysis of patient samples and also for the clinicians to be more comfortable with the results emanating from the laboratories as reliable, sensitive and are within the acceptable limit of precision.

V. Conclusion

In this study, some warning signs were recorded, it is not completely unusual these violations typically identify smaller systematic error or analytical bias that is not often clinically significant or relevant. The results of the study shows that both imprecision and bias exist between both laboratories, indicating corrective action to be taken for both laboratories in terms of measurement imprecision.

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