QUALITY CONTROL ASSESSMENT IN THE CLINICAL CHEMISTRY LABORATORY- A GHANAIAN STUDY

By

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DECLARATION

I hereby declare that this submission is my own work towards the MSc. and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Quality control is essential in the clinical laboratory in order to ensure reliability of results of tests, with acceptable levels of accuracy and precision. There is also the increasing awareness of the need for the exchange of accurate data between laboratories, whether for research and collaborative studies or for patient care. The objective of the study was to assess the level of quality control in the clinical chemistry laboratories in the Kumasi metropolis. Validation was made for 5 analytes (total cholesterol, urea, glucose, sodium and potassium). The study included determination of within-day (N = 20) and between-day imprecision (N = 20), inaccuracy (N = 20) and method comparison (with the acceptable WHO manual methods for each analyte and among the laboratories) for three laboratories KATH, Medilab and SDA using ethylene glycol stabilized bovine and human sera as well as pure analytes with known concentration. Structured questionnaires were also administered to assess the practice of quality control. For validation of complete analytical process total error (TE) was calculated. Results were assessed according to quality specification criteria given by European Working Group. Within-day imprecision CVs were all below 3% except for potassium from KATH and glucose from SDA (bovine serum). Between-day CVs for all analytes were below 9%, except for glucose (bovine serum) from SDA. Short-term (Within-day) performances as assessed with the capability index were all ≥1 except for urea from SDA with capability index < 1. However the autoanalysers were generally unstable in the long term with capability indices for most analytes recording a value less than 1. Inaccuracy was generally low but total error was high for most of the between-day results from the three laboratories which was due to the high imprecision. Total error assessment for cholesterol and urea measurement from both KATH and Medilab were acceptable per quality recommendation. Passing-Bablok regression analysis provided linear equation and 95% confidence interval for intercept and slope and this was used to assess analytical differences or agreement between the manual methods and the autoanalyzers. Complete accordance with WHO manual method was shown by total cholesterol, urea, sodium and potassium hence both methods can be used intercheangeably. However constant and proportional differences or both were shown by some results (glucose) from KATH, Medilab and SDA. A comparison of methods between the three laboratories showed a complete agreement between KATH and Medilab for all analtytes (with the exception of glucose for bovine serum). There were proportional differences between KATH and SDA for urea and glucose, whiles both constant and proportional differences were shown in results between Medilab and SDA. Financial constraints and knowledge of the use of control charts were among the major challenges laboratories faced in quality control practice. Analytes that do not fulfil quality specification criteria require more frequent quality control protocol which includes several runs of control material in series. Also since instrumental inexactness has been sighted as possible cause of errors, it is important that routine maintainance is carried out on the autoanalyzers. It is also essential that an oversight body is established that will regulate the continuous practice of method comparison studies in order to ensure between laboratory methods agreements.

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LIST OF ABREVIATIONS

BUN	Blood Urea Nitrogen
CLSI	Clinical and Laboratory Standards Institute
CP	Capability Index
CT	Cusum Test Of Linearity
CV_{bd}	Between-Day Coefficient of Variation
CV_{wd}	Within-Day Coefficient of Variation
EQA	External Quality Assessment
GH	Glucose high
GL	Glucose low
IFCC	International Federation For Clinical Chemistry
IQC	Internal Quality Control
ISE	Ion Selective Electrode
ISO	International Standards Organization
K+	Potassium
KATH	Komfo Anokye Teaching Hospital
MEDILAB	Medilab Diagnostics Limited
Na+	Sodium
QA	Quality Assessment
QC	Quality Control
QI	Quality Improvement
QLP	Quality Laboratory Processes
QP	Quality Planning
RE	Random Error
SDA	Seventh Day Adventist Hospital
SE	Systematic Error
TE	Total Error
TQM	Total Quality Management
WHO	World Health Organization

Chapter 1

INTRODUCTION

1.1 BACKGROUND

Clinical chemistry laboratories are increasingly playing crucial roles in medicine as clinicians rely heavily on clinical chemistry tests to make proper diagnosis. Quality in clinical chemistry laboratories therefore cannot be compromised, especially in today's world where the emergence of sophisticated instrumentation and analytical methods has made laboratory tests so simple and easy to perform. Such quality standards maintained by any laboratory if acceptable will not only benefit patients directly but also be an important boost for the laboratory (Cooper, 1997).

Clinical chemistry quality control has come a long way, with significant contributions by Levy and Jennings, Barnett and others (Levey and Jennings, 1950; Barnett, 1968). The term total quality management (TQM) has been coined today to be the embodiment of all quality requirements comprising quality planning (QP), quality laboratory processes (QLP), quality control (QC), quality assessment (QA) and quality improvement (QI) (Westgard and Burnett, 1990). TQM is sometimes considered a part of the whole quality assurance process, and has further been stratified into three main components: pre-analytical (QP), analytical (QLP and QC), and post-analytical (QA and QI) quality. In the 1990s a popular view that surfaced was that laboratories should concentrate only on pre-analytical and post-analytical quality as analytical quality was no longer a problem (Burtis and Ashwood, 2001). Describing this idea as a myth that was never true, Westgard argues that to properly monitor and manage errors in the whole clinical laboratory testing process, it must start with analytical quality control(Westgard, 2008a).

The unchallenged role played by analytical laboratory quality control is further made evident by considering the two main areas it encompasses: Internal Quality Control (IQC) and External Quality Assessment (EQA) (Boyle *et al.*, 2008). Precision and accuracy control are the main foci of IQC, their purpose are to ensure that analytical results are reliable (Anderson *et al.*, 1991). IQC should be practiced periodically both during normal analytical runs and most importantly when a new method or equipment is to be used or tested. Simple IQC practices may include calibration, control and reference material measurements, within-run precision measurements, use of control charts, and use of simple statistics to interpret results. More complex procedures require more resources and they may include between-run precision measurements, commonly called the replication study, and the comparison of methods study. Upon the introduction of a new method, these later practices becomes mandatory to ensure acceptance of the method; in which case other important quality control experiments like detection limit, reportable range, recovery and interference must all be performed too (Boyle *et al.*, 2008).

The strength of a laboratory's IQC practices will determine how well it will fare on an EQA study (Anderson *et al.*, 1991). EQA, also called inter laboratory comparison provides the perfect platform for the monitoring of laboratories, and it is a good marker of laboratory performance, competence and proficiency. Other goals of EQA include identification of inter laboratory differences, and assessment of how suitable a reference material may be for use in a test or procedure; all these aimed at establishing a between-laboratory and between-methods agreement. Depending on the scope of the EQA scheme and the particular objectives chosen, one or more of four kinds of EQA studies can be employed: method evaluation, competence, certification and proficiency testing studies (CLSIEP15-A2., 2005).

Proficiency testing is the most common and frequent type of EQA study and it is a test of a laboratory's achievements and performance of both the specific analytical

procedure and method underutilization in the laboratory, and most importantly of the analyst operating the procedure(Westgard and Burnett, 1990).

A standard requirement of all laboratories is to ensure the traceability of analytical results; something that cannot be achieved without good IQC and EQA practices (ISO15189, 2007). It is not surprising therefore that the International Standards Organization (ISO) has chosen both IQC and EQA as mandatory requirements for any laboratory rendering testing services (ISO15189, 2007). In the developed world where at least one institution exists to keep laboratories on their toes on quality issues, IQC and EQA are very common practices in laboratories(ISO15189, 2007). Most African countries are however yet to attain this level of organisation and Ghana is no exception. A very recent publication by the WHO's African Health Monitor (2010) identified limited quality assurance and control protocol as one of the challenges faced by African clinical chemistry laboratories, and even proposed the establishment of a 'National Public Health Reference Laboratory' as a way out.

Poor quality control practices has been observed in our laboratories, as both IQC and EQA were very limited(USAID, 2006). Interestingly, more and more laboratories keep springing up by the day and it is important that systems are put in place to assess the analytical results coming from these laboratories. Some imprecision have been reported in the measurement of liver function (LFT) assays by the ATAC 8000 autoanalyser at the Komfo Anokye Teaching Hospital in the Ashanti Region(Owiredu *et al.*, 2007b). A similar study conducted with sodium and potassium, the most commonly measured electrolytes generated similar imprecision with the same autoanalyser (Owiredu *et al.*, 2007b). An EQA study by (Opoku-Okrah *et al.*, 2008) involving haematology laboratories established that a continuous monitoring of haematology laboratories was very crucial for quality results.

1.2 AIM

To assess the level of quality control in the clinical chemistry laboratories in the Kumasi metropolis

1.2.1 Specific objectives

a) Validate, precision and accuracy of the clinical chemistry analyzers used by the selected laboratories.

b) Evaluate the acceptability (capability) of the clinical chemistry analyzers used by the selected laboratories with reference to medically allowed tolerance limits.

c) Collect information from laboratory managers and analysts on the practice of quality control in their laboratories using a structured questionnaire.

d) Compare results to International requirements to assess the current state of quality standards in the selected clinical chemistry laboratories.

1.3 JUSTIFICATION

The goal of any clinical laboratory investigation has always been to obtain reliable findings but this cannot be achieved without reliable analytical results. Quality control is a prerequisite for reliable analytical results and the need for this has been established (Westgard and Burnett, 1990). The crucial roles played by IQC and EQA in ensuring reliability of analytical results have also been established (Westgard, 2008c).

The estimation of glucose, BUN, TC, sodium and potassium in blood play very important roles in the diagnosis of various disease states including diabetes, kidney disease and liver disease, etc. The Komfo Anokye Teaching Hospital, being a referral and specialist hospital for these diseses above, has a lot of such patients being referred from municipal and district hospitals as well as health centres; the resultant effect being the flooding of its Clinical Biochemistry Diagnostic Unit with various laboratory

requests from clinicians to aid in both diagnosis and prognosis of ailments. Quality control practices in this section of the diagnostic unit therefore cannot be compromised and the reliability of equipment used to provide results must be of concern.

The increased patient test loads at the unit sometimes results in some patients patronizing private laboratories in the metropolis. Considering the alarming rate at which these laboratories are springing up, it is important that quality issues are a concern to us. However in Ghana, there exists no such system or institution with the mandate to oversee the quality control issues in laboratories, though the Ministry of Health has a section under it called 'The Clinical Laboratory Unit' which seeks to train and update laboratory scientist on the current issues in laboratory medicine, there are no clear cut structures that enforce the inter laboratory comparison of analytical process and procedures. It is important that studies such as this present one are frequently carried out to help in the improvement of interlaboratory comparison. Medilab diagnostics (Bantama branch-Kumasi) limited is a private laboratory with several branches in Ghana. It is a reputable organisation with qualified and trained technologist. The laboratory is well patronised due to its reputation and perhaps the fact that it is located close to the KATH. It is therefore important they are included in any quality assessment program. SDA is also a government assisted hospital situated in the Kumasi metropolis at a few kilometres fro the KATH. Its laboratory also provides valuable services to the people in and around Kumasi and hence its inclusion will serve help to compare with KATH which is also government assisted.

Chapter 2

LITERATURE REVIEW

A properly functioning quality control program is one of the most effective safe guards available to ensure high quality of laboratory work, particularly in clinical chemistry (Shi *et al.*, 2008). No longer is it sufficient to presume that laboratory results are satisfactory. It has become necessary to ascertain that they are satisfactory by using tangible evidence of competence. Quality control measures provide that knowledge and evidence (Newell *et al.*, 1962; Fisher and DeLisa, 2008).

Many laboratories now have good quality control programs in clinical chemistry and these my have been developed locally or have been based on the suggestions of others who have described various aspects of or devices employed in their programs that can be used for quality control(Fisher and DeLisa, 2008). Sufficient experience has been gained to allow us to appreciate that quality control programs are very much worthwhile, that some form of quality control constitutes part of the good practice of clinical chemistry, and that quality control should be regarded as essential as the sterilization of instruments for surgery(Westgard and Burnett, 1990).

In the clinical laboratory, both accuracy and precision are maintained by internal quality control (IQC) and complemented by external quality assessment scheme (EQAS) (England *et al.*, 1998). External quality assessment is a system of retrospective and objective comparison of results from different laboratories by means of proficiency testing organized by an external agency (England *et al.*, 1998). IQC is a set of procedures undertaken by laboratory staff for the continuous monitoring and the operation of the results of measurements in order to decide whether results are reliable enough to be released (England *et al.*, 1998). The main purpose of EQA is to establish between laboratories and between methods including between instrument comparability and agreement with reference standards as well as detecting systematic errors (Cheesbrough, 2000). Thus an acceptable level of quality is necessary for ensuring that clinical laboratory results are reliable and would therefore require both

IQC and EQAS (Cavill *et al.*, 1981; Whitehead and Woodford, 1981; Gulati and Hyun, 1986).

2.1 OVERVIEW OF LABORATORY SERVICES IN DEVELOPING COUNTRIES

The quality of clinical laboratories in developing countries increased steadly with introduction various methods and analyzers (Keel, 2002). Currently there are top-of-the-line laboratories in these countries which compare favourably with those in developed nations. However the vast majority of patients do not have routine access to such laboratories (Preston, 1998).

In many developing countries it is very easy to set up a clinical laboratory. The factors determining the performance of a clinical laboratory include good equipment, reliable reagents and trained, conscientious staff, but many laboratories compromise on such vital prerequisites (Preston, 1998). In many cases, retired laboratory technicians and others, often entirely unqualified people, establish small clinical laboratories, where standards are not maintained since their knowledge is limited (Rickon, 2000). The managers earn money by using antiquated machines, compromising on the reagents and by employing unqualified technicians to interpret the test results. Yet the medical laboratory should provide a vital part of the management of any patient – any lapse or mistake in the performance of tests can lead to serious harm whether at the diagnostic stage or in the course of treatment (Rickon, 2000).

2.2 CAUSES OF POOR LABORATORY SERVICES IN DEVELOPING COUNTRIES

There are numerous causes of poor services, the primary one being failure to follow regulations, or in some cases, to develop relevant regulations. The reasons for these are many and varied but professional laboratory staff as a whole must share the

responsibility for tolerating inefficient or obviously incorrect practices. The following factors are especially relevant: (Preston, 1998).

Low Budget

In most developing countries including Ghana, health care is primarily funded from general government revenue without charging the consumers. (Newell, 1960; Newell *et al.*, 1962).

The expenditure on health is very low to begin with. It is less than one quarter of what developed countries spend and often very much less. Moreover its distribution amongst the various sectors is inequitable. Most funding is spent on high-profile projects in teaching institutions in large urban centres. Laboratory services do not command a high priority(Freder, 2005).

2.2.1 Scarcity of Laboratory Staff

The tremendous progress in the field of laboratory medicine has made accurate assessment and monitoring the progress of an ailment much easier. The result has been a great rush in demand for laboratory services. It is unfortunate that the availability of laboratory personnel has lagged far behind (Paramasivan *et al.*, 2010). In addition, there is significant migration of trained manpower to more profitable markets abroad. The exact number of clinical laboratory staff working in most developing countries, their background and qualifications has not been determined (Rickon, 2000). However, according to one estimate, there are no more than 1500 clinical pathologists, or about 10 per million of the Pakistan population (Rickon, 2000) 2002). By contrast, a developed country, such as England, has 109 pathologists per million. Support staff, such as technicians, is in even shorter supply. In Afghanistan there are hardly any at all. In Brazil, until a few years ago, pathologists outnumbered technologists. There is no doubt that a huge gap exists between supply and demand (Freder, 2005).

2.2.2 Poor Training

The training of technicians leaves much to be desired (Freder, 2005). Only in recent years has training been improved to acceptable standards. Similarly, many technicians receive only on-the-job training, with little formal education. The quality of the work of such a body of inadequately trained personnel is bound to be substandard (Freder, 2005).

2.2.3 Lack of Appropriate Equipment and Infrastructure

Laboratory equipment is mostly manufactured in industrialized countries. It has become increasingly more sophisticated. The procurement officials in developing countries usually buy such fancy gadgetry for purposes of prestige rather than to make full use of its capabilities. There is no infrastructure for maintenance, or even an assured supply of electricity. It takes less than one year for some machines to break down in some way and approximately 60-80% of laboratory equipment is estimated to be non-functional (Preston, 1998).

2.2.4 Lack of Regulatory Mechanisms

There is no license required to establish a clinical laboratory in many developing countries. In Southeast Asian regions of the World Health Organization (WHO), only two out of seven countries have accreditation programmes (Steigstra *et al.*, 1991).

2.3 STEPS NECESSARY FOR IMPROVEMENT OF *IN VITRO* DIAGNOSTICS IN DEVELOPING COUNTRIES

A number of steps are required of governments as well as professional associations to improve on the current situation: this include external quality assessment, training larger numbers of qualified laboratory staff, the use of telecommunication, the application of suitable technologies for developing countries, and establishment of national quality standards as well as implementing major laboratory service programs (Rickon, 2000).

2.4 OBJECTIVES AND LIMITATIONS OF QUALITY CONTROL

The main objective of quality control is to ensure good overall performance and thus enhances the confidence that can be accorded to any single estimation. Quality control programs stress accuracy at the bench level. This approach in itself is praise worthy, for if the estimation is in error, the whole report is in error(Middle, 1998).

While many of the concepts and principles of quality control have been borrowed from industry, only certain features of industrial programs of quality control can be utilized in the clinical laboratory. An important aspect of quality control in industry is to schedule all production. In contrast, because the workload of the clinical laboratory cannot be scheduled, peak loads cannot be forecast accurately. A second important difference is that with the finished product, the written report, cannot be subjected to any physical measurements (colour comparisons, taste, weight, size, etc(Boyle *et al.*, 2008).

While it is true that certain constituents of body fluids must fall within a specified range to be compatible with life, the true value is never known in any particular instance (Levey and Jennings, 1950). Furthermore, the control limits which are used are based on past performance. The data upon which the calculations are made include the best as well as the worst results of the whole group of analysts. Nevertheless, these control limits stress that any scientific measurement is not absolute. In any measurement there are inherent unavoidable errors which are beyond the control (Levey and Jennings, 1950).

2.5 INTERNAL QUALITY CONTROL

Internal quality control (IQC) ensures that factors determining the magnitude of uncertainty do not change during the routine use of an analytical method over long periods of time. Together with validation, IQC forms the mainstay of quality practice in chemical analysis. Broadly speaking, validation comprises the estimation of the

uncertainty of results resulting from the use of method under given conditions. Analysts can then judge whether the method is fit for purpose by comparing that uncertainty with the end-users' requirements. Internal quality control (IQC) is a process for checking that the uncertainty at validation does not deteriorate after the validation process, that is, when the method is in routine use (Zuvela *et al.*, 2010). IQC is conducted by inserting one or more control materials into every run of analysis. The control materials are treated by an analytical procedure identical to that performed on the test materials. The results are plotted on control charts, which are interpreted using control rules. There are factors that need careful consideration if the IQC system is to represent the routine analytical operation adequately. IQC is already traceable via the validation process and as stated earlier its only purpose is to check that the analytical system has not changed since validation. Like all statistical control, IQC operates on the basis of the mean result and standard deviation of the analytical process (Zuvela *et al.*, 2010).

2.5.1 Within-run precision

A run is a set of test materials that is analysed under repeatability conditions, that is, within a 'short time'. Within a run, there should be no changes in the magnitude of errors. However, repeatability in that sense is an ideal that is never realised. There are always systematic changes within a run, however short the time span from the first to the last analysis. So in practice we have to settle for 'negligible change' rather than 'no change'. This can be done by treating 'repeatability conditions' and 'run' as mutually defining. For example, a run could comprise a sufficient number of test materials to provide three hour's continuous analysis. We then treat the variations within the run as random and attribute them to repeatability (Plusa *et al.*, 1995; Zuvela *et al.*, 2010).

2.5.2 Between-run ('intermediate') precision

Internal quality control, however, is based on between-run precision, closeness of agreement between results obtained in separate runs of analysis. This necessarily has a

greater dispersion than within-run precision, owing to an additional source of error affecting individual runs differently. This additional source is introduced by uncontrolled changes such as those brought about by a change of analyst, new reagents, recalibration and changes in the laboratory environment (Howarth, 1995).

To estimate between-run standard deviation in an unbiased manner, the control materials have to be placed at random positions in the analytical sequence of the run. If for example the control materials were always first in the sequence, they would be analysed just after the instrument had been calibrated, with little time for systematic changes to manifest. The replicated results would consequently tend to underestimate the between-run standard deviation (Zuvela *et al.*, 2010).

2.6 PROCESS CAPABILITY (CP) AND PROCESS PERFORMANCE (PP) INDEXES

The quality of a manufactured product is its uniformity about a target. There is no unique target in clinical chemistry but a true concentration for each sample. Therefore analytical quality is uniformity of assayed concentrations about true concentrations. Tolerance limits tell us how much variation can be accepted. They are laid down by national regulations or derived from clinical needs, from biological variation, or from the state of the art (MultiQC, 2008).

The capability (Cp) of a method relates the tolerance interval to the inherent analytical variability. The capability is equal to the ratio of the width of the tolerance interval (**U**pper **T**olerance **L**imit – **L**ower **T**olerance **L**imit) to the spread of the natural variations of the analytical process (6 standard-deviations). The factor SD of the denominator is the *short-term* standard deviation which measures the lowest possible variability of the process when it is permanently in control. In summary, Cp is the ratio of what must be done (the allowed tolerance) to what the process is able to do (the expanded uncertainty of the assay). It informs about the inherent capability of the analytical method provided it were operating at a stable average. The higher the

capability, the lower is the risk of jumping the tolerance limits and therefore the higher the quality (MultiQC, 2008).

2.7 EXTERNAL QUALITY ASSESSMENT (EQA)

At present, external quality assessment schemes (EQAS) exist in the field of laboratory medicine in many countries. Most of these are intended to assist individual laboratories to continuously monitor their performance and to compare it with that of other laboratories, whereas others may be primary intended for accreditation or licensing purposes. Additionally, EQAS may monitor the quality of the commercial analytical systems, reagents and test kits, and they help manufacturers to achieve a better harmonization of results from these different analytical techniques(Ricos *et al.*, 1996).

The two principal aims of EQAS are to define target values, and to define limits for acceptance. The target values should be assigned from reference methods, but as only a few schemes follow these principles, target values are derived from the statics of each survey (Ricos *et al.*, 1996). Continual participation in EQA is an effective means for identifying and ameliorating variables that influence the reliability of analytical assays for predictive markers, thereby assisting in technical validation and standardization. The concept of external quality assurance for the national health laboratories network is useful, as it identifies problems in the comparability of laboratory results and initiates a process towards solving these problems thus improving the quality of service at the level of each individual laboratory and the network level (Olafsdottir *et al.*, 1994).

The implementation of a quality assurance policy in a developing country requires a commitment from the government, the professional societies and the laboratory workers. It is important to recognize that a policy towards improving health care should include an external surveillance system for health laboratories. An EQA scheme will have the greatest impact when it is linked to a quality assurance

programme, which also includes internal quality control as an equally important component (Deom *et al.*, 1999).

External control materials serve to assess the matrix and concentration sensitivity of the analytical processes and mimic the analysis of individual unknown patients 'samples. They assess the quality of the internal-control procedures (Steigstra *et al.*, 1991).

2.8 OBJECTIVES OF EQA SCHEMES

An EQA scheme provides surveys at regular intervals, in which identical material, or in some instances similar material, is distributed to participating laboratories, who determine specified analytes using a variety of routine analytical methods. The survey may also include an assessment of the ability of laboratories to validate the clinical relevance of their observations. A number of EQA schemes aim at improving the performance of laboratories by ways of education, metrological recommendations and standardization (Di Giovine *et al.*, 2008)

The objectives of EQA schemes for health laboratories may be summarized as follows:

1. Raising awareness towards possible deficiencies in laboratory practice and guiding participants in corrective action towards improvement.
2. Collecting information on performance of measurement principles in order to guide professionals and or government bodies towards achieving harmonization.
3. Collecting information on the reliability characteristics of particular methods, materials and equipment and taking corrective actions as appropriate.
4. Identifying laboratories of excellent performance for their involvement in training and education.
5. Collecting information for the purpose of licensing or accreditation of laboratories.

6. Assessing and monitoring the impact of training.

Surveys give the organizing centre means to assess the performance of an analytical system, the performance of individual laboratories, and a group of laboratories as a whole. This evaluation requires a statistically sufficient number of results on clinically relevant samples from the participants. Quality control materials that challenge the specificity of a method must be included to study feasibility of a particular diagnostic test; however, such materials should not be used for the assessment of laboratory performance (Deom *et al.*, 1999).

2.9 ANALYTICAL ERRORS

The quality assurance system in clinical chemistry allows for identification of errors and control actions to correct them. Laboratory errors can be classified into pre-analytical, analytical and post-analytical.(Irjala and Gronroos, 1998). While pre-analytical and post-analytical errors are difficult to identify, the analytical variability (both imprecision and inaccuracy) can be monitored with internal quality control (IQC) programs and external quality assessment (EQA) schemes (Lalani *et al.*, 1988; Ohman, 1997; Middle, 1998; Dastugue, 2000). These errors are variously classified as random error or imprecision, systemic error or inaccuracy, constant error, proportional error, and total error (Westgard, 2008c).

2.9.1 Random Error or Imprecision (RE)

This is described as an error that can be either positive or negative, whose direction and exact magnitude cannot be predicted. Imprecision is usually estimated by calculating the standard deviation (SD) from the result of a set of replicate studies. The standard deviation often increases as the concentration increases. Therefore it is often useful to calculate the coefficient of variation (CV) to express the standard deviation as a percentage of the mean concentration from the replication study (Westgard, 2008c). The maximum size of a random error is commonly expressed as a 2SD or 3SD estimate to help understand the potential size of the error that might occur. This is

because information about the size of analytical errors is more useful for judging the performance of a method (Westgard *et al.*, 1974).

2.9.2 Systematic Error or Inaccuracy (SE)

This is an error that is always in one direction (Westgard, 2008b). In contrast to random error that may be either positive or negative and whose direction cannot be predicted. Systematic errors are in one direction and cause all the test results to be either low or high. How high or how low can be described by the bias. This is calculated as the average difference, or the difference between average of the values by the "test" method and a 'comparative" method in a comparison of methods experiment (Westgard, 2008c; Westgard, 2010). Alternatively the expected systematic difference may be predicted from the equation of the line that best fits the graphical display of test method values on the y-axis versus comparative method on the x-axis. SE may stay the same over a range of concentrations, in which case it can also be called constant error (Westgard, 2010).

2.9.3 Total Error (TE)

Historically, decisions on method performance were based on statistical tests of significance (such as the t-test and F-test) and the correlation coefficient (Barnett and Youden, 1970). In the 1970s Westgard and others studied the application of these statistics and found they had limited value for estimating the random, proportional and systematic errors that are the focus of method evaluation studies as well as development of guidelines for obtaining more reliable estimates and finally recommended that these estimates be compared to the amount of error allowable in the medical use of the test (Westgard *et al.*, 1974). It was proposed that the allowable error be defined in the form of a total error (TE) that reflected the combined effects of imprecision and inaccuracy on the test results (Westgard and Hunt, 1973).

Again Westgard and co argued that it made little difference to the user (physician) and consumer (patient) whether a test result was inaccurate or imprecise; in their

estimations, what was important was how far wrong it could be; the total error that includes both inaccuracy and imprecision. Total error is defined as the net or combined effect of random and systematic errors. (Westgard, 2008c). It represents a "worst case" situation or just how far wrong a test result might be due to both random and systematic errors. Because Laboratories typically only make a single measurement for each test, that measurement can be in error by the expected SE, or bias, plus 2 or 3SD, depending on how you quantitate the effect of RE (Westgard, 2008c). According Westgard while laboratory technologist like to think about imprecision and inaccuracy as separate errors, the physicians and the patients experience the total effect of the two, or the total error. The total error provides the customer or consumer-oriented measure of test performance, which makes it the most important parameter for judging the acceptability of analytical errors (Westgard, 2008c).

2.10 WESTGARD MULTI RULE

Dr. James Westgard of the University of Wisconsin in 1981 published an article on laboratory quality control that set the basis for evaluating analytical run quality for medical laboratories. The elements of the Westgard system are based on principles of statistical process control used in industry since the 1950s (Westgard *et al.*, 1981). Basically it involves six basic rules which are used individually or in combination to evaluate the quality of analytical runs. The rules are expressed in short hand notation in the form of N_L where N represents the number of control observations to be evaluated and L represent the statistical limit for evaluating the control observations. Thus 1_{3s} represents a control rule that is violated when one control observation exceeds the ±3SD control limits.

The first rule (1_{2s}) is a warning rule that is violated when a single control observation is outside the ±2SD limits. In the absence of added analytical error, about 4.5% of all quality control results will fall between the 2SD and 3SD limits. This rule merely

warns that random error or systematic error may be present in the test system. The relationship between this value and other control results within the current and previous analytical runs must be examined. If no relationship can be found and no source of error can be identified, it must be assumed that a single control value outside the ±2SD limits is an acceptable random error. Patient results can be reported. The second rule (1_{3s}) identifies unacceptable random error or possibly the beginning of a large systematic error(Westgard, 2008c). This implies that Any QC result outside ±3SD violates this rule and thus other rules must be taken into consideration before reporting patient's results. The third rule (2_{2s}) identifies systematic error only. The rule is violated when two consecutive QC results are greater than 2SD on the same side of the mean. (Cooper, 2008). The fourth rule (R_{4s}) identifies random error only, and is applied only within the current run. If there is at least a 4SD difference between control values within a single run, the rule is violated for random error.

A violation of any of the following rules (3_{1s} and 4_{1s}) does not necessarily require rejection of the analytical run(Cooper, 1997). These violations typically identify smaller systematic error or analytical bias that is not often clinically significant or relevant. The 3_{1s} rule is violated when three consecutive results greater than 1SD fall on the same side of the mean whiles the 41s rule is violated when four consecutive results greater than 1SD s fall on the same side of the mean. Analytical bias may be eliminated by performing calibration or instrument maintenance(Cooper, 1997).

2.11 QUALITY CONTROL CHARTS

Quality-control samples are widely used in clinical chemistry laboratories to assess the quality and stability of routine analytical methods. Different laboratory managers have different views as to how many quality control samples should be inserted into a given analytical run and where these samples should be placed. Whatever the regime in operation, it is standard practice to plot the quality control values on Shewhart-type

or Levey-Jennings control charts (Levey and Jennings, 1950; Shewhart, 1931). These charts are well understood and they give rise to a wide range of possible control charts that could be used. For example, one rule might be to declare an out-of-control situation as soon as one quality-control value is more than two standard deviations (>2s) away from the target value. A second example might be to conclude that the process is out of control on the first occasion of seven consecutive observations falling on the same side of the target value. A third scheme might be to run both of the schemes simultaneously and conclude that the system has gone out of control when at least one of the component schemes has gone out of control (Zuvela *et al.*, 2010).

2.11.1 Shewhart's P Control Charts

Shewhart's p control charts have been applied in external quality-assurance program to monitor the long-term performance of laboratory's analytical quality. The p control charts have been able to detect long-term changes in laboratory's analytical performance that would have been difficult to detect by more-conventional techniques (Chesher and Burnett, 1996). These charts may not be only a simple method for the long-term monitoring of analytical performance of a laboratory, but also of use to the area of external quality-assurance programs.

Shewhart p control charts are graphical tools for monitoring the proportion of samples that does not meet some predetermined specification limits (the so-called rejectable proportion or defective fraction). In using the Shewart's p control charts it is a practice to choose a maximum allowable limit of error (ALE), which may be expressed as either "± concentration' or "± percentage" of the target value. The charts may be applied to characteristics that can be observed only as attributes or characteristics that can be considered as attributes. In this case, the proportion of analyses (measured as continuous variables) exceeding the ALE can be considered as an attribute. For independent trials the fraction rejected will follow a binomial distribution with:

$$mean = \dot{p}$$

$$varianc(\alpha^2) = \dot{p}\frac{(1-\dot{p})}{n}$$

Where p = average fraction rejected and n = number of trials (sample size). The 3o-control limits (UCL, upper control limit; LCL, lower control limit) depend on the average value of p and the sample size n and are calculated with the following equations (Grant and Leavenworth, 1988)

$$UCL = \dot{P} + 3\sqrt{\frac{\dot{P}(1-\dot{P})}{n}}$$

$$UCL = \dot{P} - 3\sqrt{\frac{\dot{P}(1-\dot{P})}{n}}$$

The use of Shewart's p charts is helpful in achieving the following in a quality assessment program (QAP): The monitoring of long term analytical performance, monitoring of specific analytical performance, and analytes with relatively few measurements. In 1996, a study conducted by Chesher and Burnett in New Zealand concluded that Shewarts p control charts provides a simple way to monitor overall laboratory analytical performance over an extended period(Chesher and Burnett, 1996).

2.11.2 Multi-Rule Shewhart Chart

This type of control procedure is for applications where stable control materials are available and are analysed repeatedly over long periods of time. This kind of control procedure was initially described by Shewhart (Shewhart, 1990). It was later introduced in clinical chemistry by Levey and Jennings (Levey and Jennings, 1950). Control data are displayed on control charts, which are sometimes referred to as "Shewhart charts" and other times as "Levey-Jennings charts" (Westgard *et al.*, 1981). Control charts of this kind are now in use in most clinical laboratories. The applications from laboratory to laboratory differ primarily with (a) the use of single measurements or replicate measurements and (b) the criteria used in deciding

whether the data indicate the analytical run is in or out of precision(Westgard and Westgard, 2006).

The decision for most laboratories to use the multi-rule Shewhart procedure, has been informed by the need to have (a) a simple data analysis and display via control charts, such that computerized data handling is not necessary, (b) an easy adaptation and integration into the existing control practices in clinical laboratories; (c) a low level of false rejections or false alarms; (d) an improved capability for detecting analytical errors; and (e) some indication of the type of analytical error occurring when a run is rejected, to aid in problem solving(Westgard *et al.,* 1981).

In principle the analytical method to be controlled is first studied, to characterize its analytical performance. Measurements are made on control materials, which are assumed to be stable and to vary little in concentration from aliquot to aliquot, or vial to vial. Repeated measurements, therefore, characterize the imprecision or random errors of the analytical method. It is assumed that the distribution of these errors is Gaussian and can be described by its mean (x) and standard deviation (SD)(Zuvela *et al.*, 2010). These statistics are calculated from a replication study, generally over a 20-day period, with one measurement on each control material per analytical run and one analytical run per day(Westgard, 2011). A control chart is prepared for each control material. The chart displays concentration on the y-axis vs. time on the x-axis. Horizontal lines are drawn for the mean, and for upper and lower control limits, which are calculated from the standard deviation. Several sets of control limits are included on the control chart recommended here, to permit the use of several different decision criteria or control rules (Westgard *et al.*, 1981).

The term "control rule" is used to indicate a criterion for judging whether the observed control measurements (or observations) represent typical or atypical (stable or unstable) performance of the analytical method. Many different control rules could be used, but they all attempt to signal when the control measurements no longer represent the expected or previously observed error distribution. Simultaneous use of

several control rules can improve the performance of a control procedure. Individual rules have different capabilities for detecting different types of analytical errors. At least two control rules need to be selected; one that detects random analytical error and another that detects systematic analytical error (Westgard, 2011). When the control procedure signals that an analytical run should be rejected, the particular control rule providing the signal gives some indication of the type of analytical error that is occurring. This in turn may suggest certain sources of the error, and so aid in problem solving (Westgard and Westgard, 2006). Control rules should be chosen to provide a low probability for false rejection and a high probability for error detection. In the daily operation of the control procedure, samples of control materials are included in each analytical run. When any one of the control rules is violated, a decision is made to reject that analytical run. A decision to accept the analytical run requires that there be no violations of any of the control rules(Westgard, 2011).

Control limits – mean and standard deviations are calculated from at least 20 replicate observations from a particular control material. And the control limits are calculated from the mean and standard deviation as follows

$$3s\ control\ limit = x \pm 3s$$

$$2s\ control\ limit = x \pm 2s$$

$$1s\ control\ limit = \pm$$

2.12 COMPUTER ASSISTED QUALITY CONTROL

A MUMPS computer program, which stores and retrieves quality-control data from all automated and manual work-stations in the laboratory, has been developed as part of a laboratory information system. Tabular displays, Levey-Jennings charts, and summary statistics are available on a real-time basis. Significant economy over previous manual methods has been observed, and the total quality-control program in the laboratory has become a more active and timely process (Undrill, 1980).

For effective quality control programs in the clinical laboratories, whether through the use of pooled serum or commercially prepared control sera, the objective remains the same: to provide information about the accuracy and precision of the tests performed in the laboratory. The classical approach to this problem has been the daily use of controls and graphic representation on Levey and Jennings (Levey and Jennings, 1950) charts, with the calculations of mean, standard deviation, and coefficient of variation. Automatic data-processing equipment has altered our approach to classical quality control. It has allowed the laboratory to bring quality control into the "real-time" environment, rather than maintaining it as a bookkeeping function with tedious collection and charting of data and its associated statistical analysis. Previous attempts at utilizing data processing involved batch result entry with punched cards and did not take advantage of automatic data acquisition(Riddick, 1970).

The earliest benefit of this system was the ease with which the laboratory supervisor could review quality-control data. Delays in computing monthly statistics and charting results were eliminated. Result charting became unnecessary, because the graphic displays are displayed on a cathode ray tube or printed on demand for review. The ease of collecting and analysing quality control data allows for more frequent use of quality control material(Muser *et al.*, 2001). Multiple daily results are stored for review of within-day variances. Potential problems are spotted and analysed more quickly. Maintaining quality control cross-reference to work lists allows the separation of quality control files for the evening and night shifts, as well as for separate work stations. Because separate instrumentation is used for these shifts, consistency of results between different instruments is evaluated daily.

This approach saves a lot of time and there is no need manually to maintain quality control records and chart results(Muser *et al.*, 2001). The system detects and reports quality control values that are outside pre-set limits, and on a continuous basis calculates the mean, standard deviation, and coefficient of variation of all control values (Undrill, 1980).

Chapter 3

MATERIALS AND METHODS

3.1 STUDY DESIGN

The study lasted a period of 12 months (Between January and December 2012) and was conducted in three (3) main phases. All protocols and experiments in the study followed guidelines set by the Clinical and Laboratory Standards Institute (CLSI), the International Federation for Clinical Chemistry (IFCC) and International Standards Organization (ISO) and in this regard various evaluation protocol documents and publications by these bodies, especially the CLSI was used(CLSIEP9-A2-IR., 2010).

3.1.1 Phase I

The Setting for this phase was the Clinical Biochemistry Diagnostic Unit of the Komfo Anokye Teaching Hospital, and the phase involved the internal quality control studies on the Cobas Integra 400 Analytical System. Activities of this phase involved three main stages and the main activities undertaken in each stage are illustrated by Figure 3-1 below.

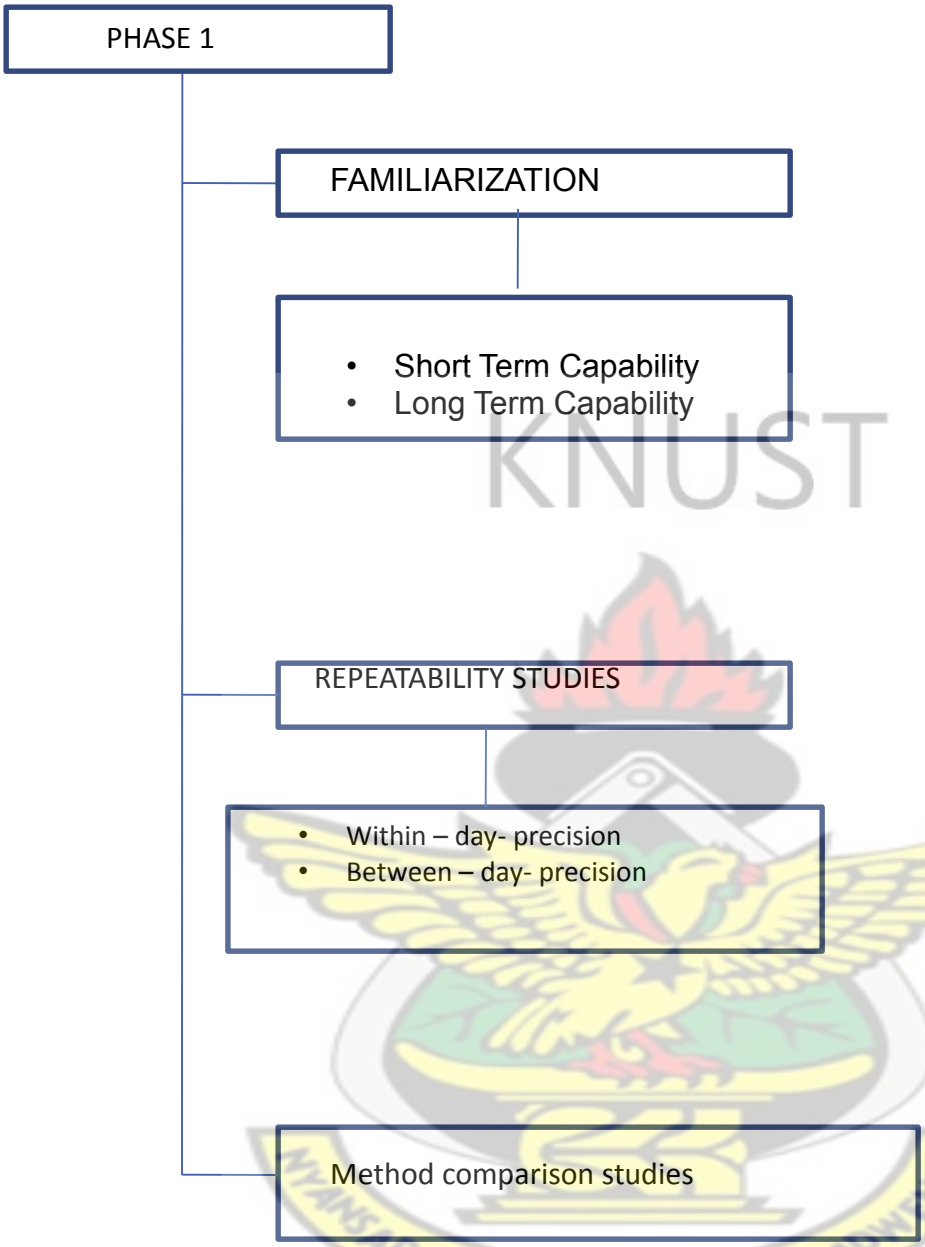

Figure 3-1Chart showing the various experiments to be conducted in Phase I of the study

3.1.1.1 Stage I – Familiarization

This stage considered the establishment of the working procedure, checking the capability of the analytical systems. The capability of the Cobas Integra at Komfo Anokye to measure analytes for both short and long terms was assessed and compared to the WHO manual methods as well as the EQA laboratories.

3.1.1.2 Stage II –Repeatability studies

This stage involved two kinds of replication studies: within-day and between-days studies to estimate the level of imprecision in the measurement of each analyte. Both within-day and between-day replication study were done using bovine and human serum as well as pure analytes. The within-day precision study was performed for each analyte, 20 replicate measurements were made for each sample using the Cobas Integra 400. For the between-day replication study, quality control material (bovine serum) as well as pooled patient serum and pure forms of the analytes were used. For each day, each sample was measured twice and the mean taken for 20 days. The repeatability experiments was conducted according to guidelines set by the CLSI (CLSIEP07-A2., 2005); (CLSIEP15-A2., 2006).

3.1.1.3 Stage III – Method comparison studies

This was performed according to CLSI procedures (CLSI EP09-A2-1R, 2010) as follows:
For each analyte, bovine and human sera as well as pure analytes (total cholesterol, urea glucose low and glucose high) were assayed each day in duplicates by both the Cobas Integra 400 and the manual method.

3.1.2 Phase II

This phase involved the EQA scheme. Two laboratories (Medilab and SDA) that representing both the private and public sectors were selected and recruited as recommended by the World Helath Organisation (WHO, 1999) and were assessed for both precision and accuracy of analytical methods. Medilab diagnostics uses the Mindray BS 380 auto analyser whiles SDA uses ABBOT spectrum Chemistry analyser.
The general features of the EQA scheme followed in the study, was in agreement with CLSI guidelines (CLSI GP27-A2, 2007) as shown in Figure 3-2 below.

All selected laboratories were made to sign consent forms agreeing to partake in the scheme and to play by the rules of the scheme. Each laboratory was sent a package that includes the following items:

- An instruction sheet that provides details on each specimen and guidelines on handling, result recording, and instructions on how and where results should be returned.
- A protocol form on which results will be recorded
- The various samples to be analysed.

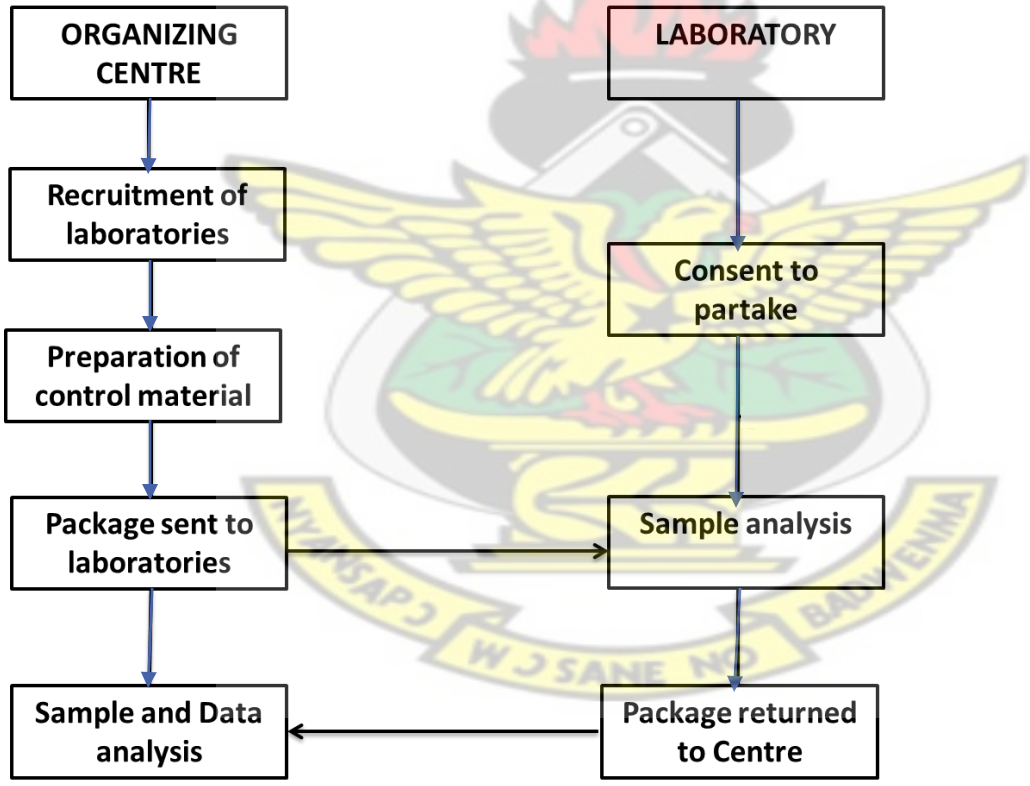

Figure 3-2 The EQA scheme to be followed in Phase II of the Study

For each laboratory, procedures that were followed are as follows:

- Replication studies (between-day run and within-day run) were performed as described above with control material.

- For each laboratory various errors (random, systematic and total errors) were measured. Pure glucose with two different concentrations (low and high) approaching significant medical decisions was analysed for 20 days. Pure urea and cholesterol were also measured for 20 days. Comparison of the laboratory's method with the manual methods for each analyte was done using control material.

3.1.3 Phase III

Laboratory analysts or managers of clinical laboratories in the Kumasi metropolis were recruited to partake in this phase of the study. Structured self-administered questionnaires was provided for them after consenting to partake, this sought information on various quality control practices in their laboratories. A total of 20 laboratories were recruited in this phase of the study. 12 from government hospitals and 8 from private laboratories.

3.2 PREPARATION OF CONTROL MATERIALS

All control materials used for the various studies was prepared using human serum, bovine serum and pure forms of the analytes (glucose, urea, potassium as potassium chloride, sodium as sodium carbonate, and cholesterol) and preparation followed guidelines set by the WHO (de Jonge *et al.*, 2004).

3.2.1 Bovine Serum

Clearance was sought from the Cattle Research Institute -KNUST and about 500 ml of blood was obtained from 10 different cattle into plain vacutainer tubes. After clot formation at 37^{o}C, the serum was separated and pooled together in a graduated conical flask. In all 250 ml of bovine serum was obtained. It was then kept frozen between -15 to -20^{o}C for twenty-four hours. After allowing thawing at room temperature without disturbance, 15% (37.5ml) of the clear top layer consisting mainly of water or very dilute

serum was gently pipetted out and an equivalent volume of ethylene glycol, as preservative and antifreeze agent was added to replace the volume removed. The serum was then mixed thoroughly with the ethylene glycol and filtered through non-absorbent cotton wool to remove any large aggregates. 1.5ml polystyrene capped tubes were labelled as BSC (Bovine serum control) with date. 1ml aliquot of the ethylene glycol stabilized QC serum was then pipetted into each tube. A total of 200 aliquots was made and the rest together with the aliquots were stored in a deep freezer (-15 to-20^{o} C) until analysed.

3.2.2 Pooled Human Serum

The liquid human quality control serum stabilized with ethylene glycol was prepared with a modification of the WHO recommended protocol. (Premachandra *et al.*, 1987; Browning *et al.*, 2004) and (Deom *et al.*, 1999). 5 mls each of whole blood was taken from a total of 100 patient samples from the clinical chemistry department of Komfo Anokye Teaching Hospital. Sera with apparent turbidity, excessive bilirubin, or haemolysis were excluded from pooling. The individual concentrations of each of the five analytes were fairly constant (TC=3.8-4.2 mmol/L, Urea=5.2-5.52 mmol/L, glucose =5.1-5.42 mmol/L, potassium =5.0-5.42 mmol/L, sodium 152-155 mmol/L). The samples were pooled together and screened for HBsAg, and Anti-HIV antibodies (Simon *et al.,* 1973; Sugimoto *et al.*, 1988). After mixing thoroughly to ensure homogeneity, the conical flask was frozen at-15 to -20^{o}C for twenty-four hours after which the procedure used for the preparation of the bovine serum was followed. A total of 200 aliquots labelled as human serum control (HSC) was made and the remaining serum and aliquots were kept frozen at -15 to-20^{o} C until analysed.

3.2.3 Preparation of Pure Analytical Reagents

An amount 0.44g of pure glucose was accurately weighed with an analytical balance (Scientech ZSA80). It was then dissolved with a small amount of distilled water in a beaker and then transferred into a 200 ml flat bottom flask. Distilled water was added to the 200ml mark to give a concentration of 12.2 mmol/l. A 100ml portion of this solution was pippeted into a clean beaker. 1.5 ml polystyrene capped tubes were labelled as GH (GH). 1ml aliquot of the pure glucose solution was then pipetted into each tube making a total of 100 aliquots. These aliquots were stored in a freezer until analysed. To minimize weighing error, 20mls portion of the remaining 100 ml was pipetted into a graduated measuring cylinder. It was then topped up to the 100 ml mark with distilled water to make a concentration of 2.24mmol/l (dilution factor of 5). 1.5ml polystyrene capped tubes were labelled as GL (Glucose (low)) and 1ml aliquots of this pure glucose solution was then pipetted into each tube making a total of 100 aliquot. These aliquots were stored in a freezer until analysed. For total cholesterol and urea, 4.2 and 5.3 mmol solution was prepared respectively following the same procedure used for the glucose solutions.

3.3 ASSAY OF THE ANALYTES

Reagent and standards for each analyte was obtained from Roche Diagnostics- Germany and assay of each was done by the Cobas Integra Analytical System. The Cobas Integra 400 chemistry analytical system (Roche Diagnostics Basle, Switzerland) is a random-access analytical system which employs four main methods for its measurements: absorbance photometry for enzymes and substrates, turbidimetry for specific proteins and drugs of abuse, fluorescence polarization for therapeutic drugs and thyroid tests, and ion selective electrode potentiometry for the electrolytes (sodium, potassium, chloride, and lithium) (Muser *et al.*, 2001). The cobas integra 400 was calibrated using commercially prepared standard (C.E.F.A.S) from Roche diagnostics. Standards were runned to

generate factors for the estimation of analytes on samples. Control sera with analyte concentrations falling within the normal and extreme ranges were also runned and used to check the quality of the calibration

Medilab diagnostics uses Mindray 380 BS for their chemistry analysis. It is a fully automated, discrete, random access analyser for routine, STAT, Urine and homogeneous immuno assays. It has a throughput of 300 tests / hour, and up to 450 tests / hour with ISE. Principles and methods used by the analyser include absorbance photometry, turbidimetry, end-point, fixed-time, kinetic, and an optional ISE. Control rules that are employed are the Westgard multi-rule, Cumulative sum check, and Twin plot (www.mindray.com)

The SDA hospital uses the ABBOTT Spectrum® Clinical Chemistry Analyser (Abbott Laboratories, Abbott Park, Illinois 60064). The system uses Spectrophotometric (mono, bi- and polychromatic) measurements for serum, plasma, urine, CSF. In most samples no specimen pre-treatment is required (except urine).The System can operate in batch, tandem, random and stat sampling modes. The analyser has a throughput of 350 tests/hour including ISE.

3.4 MANUAL METHODS OF ASSAY

Reagent and standards for each analyte was obtained from Fortress Diagnostics limited-Germany. Manual methods for determination of each analyte were performed for both the IQC and EQA method comparison studies. After appropriate procedures for each analyte were followed, using appropriate diagnostic kits, measurement was done by a spectrophotometer or flame photometer where appropriate.

3.4.1 Principle and use of the flame photometer

The sample is diluted in lithium diluents and aspirated into a propane air flame. Sodium or Potassium when excited emit a spectrum which is selected using a filter and then detected on a photosensitive detector system. The amount of light emitted is proportional to the concentration of metallic ions present in the sample.

The flame photometer used for the analysis of sodium and potassium was a Jenway PFP7 flame photometer. Sodium standard solution – 1000 ppm (Jenway Part Number 025 021) and potassium standard Solution – 1000 ppm (Jenway Part Number 025 023) was obtained from the manufacturer. 5ppm sodium standard solution was prepared by diluting 5ml of the 1000 ppm sodium standard solution to 1000 ml with deionised water and stored in a polythene bottle. 10ppm Potassium standard solution was prepared by diluting 10ml of the 1000ppm potassium standard solution to 1000 ml with deionised water. 4, 3, 2 and 1 ppm sodium solutions and 8, 6, 4 and 2ppm potassium solutions were also prepared by serial dilution with deionised water. A blank control (deionised water) was aspirated and the readout was set to 000. The highest standards (5ppm for sodium and 10 ppm for potassium) were aspirated and the readout set to the appropriate reading using the fine and coarse sensitivity controls. The remaining standard solutions were then read and the results used to construct the calibration curve (Appendix). The blank and standards were periodically aspirated to check the calibration.

3.4.2 Calibration of the spectrophotometer

The spectrophotometer used for the analysis is an Optima SP- 300(Optima USA). For each analyte serial standards were prepared by serial dilution of the commercial standards that comes with the reagent. From this calibration curves (Appendix) were drawn for each of the analytes

3.4.3 Glucose

The enzymatic indicator test based on the Trinder reaction quantified by the formation of a pink quinomined dye. In this reaction, glucose is determined after the enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed is catalysed by peroxidase and react with phenol and 4-aminoantipyrine to form the dye indicator.(Whitehead and Woodford, 1981)

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{\text{POD}} \text{quinonemine} + 4H_2O$$

Table3-1 procedure for glucose analysis

	Reagent Blank	standard/Sample
standard/ sample	-	10µl
Reagent	1ml	1ml

The preparation (as sumerised on the table 3-1) was then mixed and allowed to incubate for 5 minutes at 37°C. The absorbance of the standard and sample were measured against the reagent blank using a wavelength of 500nm.

$$\text{Glucose Concentration} = \frac{\text{sample absorbance} \times \text{Standard concentration}}{\text{Standard absorbance}}$$

3.4.4 Cholesterol

Cholesterol is present in the serum as cholesterol esters and free cholesterol. Elevated levels of cholesterol are primarily considered as an indication of increased risk of

cardiovascular diseases. The cholesterol esters present in the serum are hydrolysed by cholesterol esterase and the cholesterol is then measured by oxidizing with cholesterol oxidase to form hydrogen peroxide. The hydrogen peroxide intern react with phenol and 4- aminoantipyrine present to form the red quinomine dye. The intensity of the dye formed is directly proportional to the level of cholesterol present in the sample.(Whitehead and Woodford, 1981)

Table3-2procedure for cholesterol assay

	Reagent Blank	standard/Sample
DDH_2O	10µl	
standard/ sample	N	10µl
Reagent	1000µl	1000µl

The preparation (as summerised in table 3-2) was then mixed and allowed to incubate for 5 minutes at 37°C. The absorbance of the standard and sample were measured against the reagent blank using a wavelength of 546nm.

$$\text{Cholesterol Concentration} = \frac{\text{sample absorbance} \times \text{Standard concentration}}{\text{Standard absorbance}}$$

3.4.5 Urea

Urea is hydrolysed in presence of urease to produce ammonia and CO_2. The ammonia produced combines with 2-oxoglutarate and NADH in presence of GLDH to yield glutamate and NAD.

$$\text{Urea} + H_2O + 2H \xrightarrow{\text{Urease}} + 2\,NH_4 + CO_2$$

$$NH_4 + \text{2-Oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} H_2O + NAD^+ + \text{Glutamate}$$

The decrease in absorbance due to the decrease of NADH concentration in unit time is proportional to the Urea concentration. (Whitehead and Woodford, 1981)

Table 3-3 Procedure for urea assay

	Calibrator	Sample
Standard/R4	10µl	
Sample	N	10µl
Working solution	1000µl	1000µl

The preparation (as summerised in table 3-3) was then mixed and allowed to incubate for 15 minutes and the absorbance measured.

$$\text{Urea concentration} = \frac{\text{sample absorbance} \times \text{calibrator concentration}}{\text{Standard absorbance}}$$

Table 3-4, Methods used by the various laboratories for the selected analytes

ANALYTES	**MANUAL**	**KATH(Cobas Integra 400)**	**MEDILAB(Mindray BS 380)**	**SDA (ABBOT Spectrum; JS)**
GLUCOSE	GOD – POD (Optima SP- 300)	Hexokinase	Hexokinase	Hexokinase
UREA	Urease-GLDH(Opt SP- 300)	Urease-GLDH	Urease-GLDH	Urease-GLDH
CHOLESTROL	Chol. esterase – POD (Opt SP- 300)	Chol. Esterase-POD	Chol. Esterase -POD	Chol. Esterase -POD
SODIUM	Flame photometry	ISE(Rouch 9180)	ISE	ISE
POTASSIUM	Flame photometry	ISE(Rouch 9180)	ISE	ISE

GOD-POD= Glucose oxidase- peroxidase, GLDH=glutamate dehydrogenase, ISE= Iron Selective electrode, Chlo. Esterase-POD= Cholesterol esterase peroxidase

3.5 PATIENT SERUM AND ETHICS

Part of the method comparison studies and EQA employed serum of patients visiting the laboratory for various tests appropriate for their conditions. Ethical clearance was sought from the Committee for Human Research, Publications and Ethics (CHRPE) of the Komfo Anokye Teaching Hospital and patients were made to give their informed consent before partaking in the study. All the selected laboratories also give written consent for the work to be carried out in their laboratories.

Chapter 4

RESULTS

4.1 WITHIN-DAY PRECISION

Table 4-1 shows the within-day imprecision data for the five analytes, for each analyte, within-day imprecision was assessed as the coefficient of variation for the within-day measurements. Coefficients of variation (CV_{wd}) for within-day imprecision for most of the tested analytes from all the laboratories as well as the manual methods were below 3% except for potassium from KATH, glucose from SDA(bovine), and urea from SDA(human) with values 3.88%, 3.91% and 3.01% respectively. When compared with quality specification, potassium and sodium from KATH, sodium and total cholesterol from Medilab and glucose from SDA, all for bovine serum were higher than recommended. For the WHO recommended manual method, all the analytes met the quality specification. In general the level of variability in the results for the measured analytes (within-day) was low. With the exception of sodium (both bovine and human sera) from Medilab which recorded standard deviation values of ±1.17 and ±1.19 respectively, all other analytes measured recorded a standard deviation less than ±1. This is an indication of good precision when the autoanlyzers are used in a short term.

Table 4-1General characteristic of the five analytes from KATH, MEDILAB, SDA and WHO Manual methods using bovine and Human Sera for within-day run

ANALYTE	MANUAL		KATH		MEDILAB		SDA		QS(CV%)
BOVINE	**mean± SD**	**CV %**	**mean± SD**	**CV %**	**mean± SD**	**CV %**	**mean± SD**	**CV %**	
TC	3.25 ± 0.04	1.23	3.28 ± 0.05	1.52	3.31 ± 0.09	2.72	3.35 ± 0.09	2.68	2.7
UREA	4.68 ± 0.09	1.92	4.74 ± 0.14	2.95	4.61 ± 0.10	2.17	4.45 ± 0.10	2.25	6.9
GLUCOSE	2.06 ± 0.02	0.97	2.07 ± 0.02	0.97	2.05 ± 0.02	0.97	2.30 ± 0.09	3.91	2.2
K+	5.13 ± 0.03	2.53	5.15 ± 0.20	3.88	5.15 ± 0.09	1.74	X	X	2.4
Na+	142.8±0.41	0.29	143 ± 0.65	0.45	143.7 ±1.57	1.09	X	X	0.4
HUMAN SERUM									
TC	4.00 ± 0.05	1.25	4.01 ± 0.05	1.24	4.07 ± 0.06	1.47	4.02 ± 0.05	1.24	2.7
UREA	5.30 ± 0.08	1.51	5.32 ± 0.11	2.07	5.39 ± 0.15	2.78	5.30 ± 0.16	3.01	6.9
GLUCOSE	5.25 ± 0.11	2.10	5.28 ± 0.07	1.33	5.18 ± 0.10	2.04	5.28 ± 0.07	1.33	2.2
K+	5.05 ± 0.11	2.17	5.1 ± 0.11	2.16	5.19 ± 0.10	1.93	X	X	2.4
Na+	153.0±0.31	0.20	153.2±0.41	0.27	153.1±1.19	0.78	X	X	0.4

Data is presented as mean ± standard deviation (coefficient of variation) (SD (CV %)), QS = quality specification TC=total cholesterol, K+= potassium, Na+ = sodium, bovine=ethylene glycol stabilized bovine serum, human serum= ethylene glycol stabilized human serum

Table 4-2General characteristics of the five analytes from KATH, MEDILAB, SDA and WHO Manual methods using Bovine and Human Sera as well as pure analytes with known concentrations for Between-day run

ANALYTE	MANUAL		KATH		MEDILAB		SDA		QS(CV%)
	mean± SD	CV %	mean± SD	CV %	mean± SD	CV %	mean± SD	CV %	
BOVINE									
TC	3.25 ± 0.06	1.84	3.27 ±0.12	3.67	3.33 ± 0.11	3.30	2.93 ± 0.13	4.43	2.7
UREA	4.46 ± 0.17	3.81	4.59 ±0.28	6.10	4.71 ± 0.20	4.24	4.69 ± 0.20	4.26	6.9
GLUCOSE	1.90 ± 0.04	2.11	2.00 ±0.09	4.50	1.94 ± 0.11	5.67	1.98 ± 0.21	10.61	2.2
K+	5.03 ± 0.11	2.19	5.10 ±0.21	4.12	4.73 ± 0.23	4.86	X	X	2.4
Na+	145.4±0.56	0.39	145.8±2.31	1.58	148.2±5.47	3.69	X	X	0.4
HUMAN SERUM									
TC	3.97 ± 0.07	1.76	4.03 ± 0.14	3.47	4.06 ± 0.15	3.69	2.73 ± 0.21	7.59	2.7
UREA	5.10 ± 0.12	2.35	5.24 ± 0.33	6.29	5.42 ± 0.26	4.79	5.60 ± 0.32	5.71	6.9
GLUCOSE	5.10 ± 0.09	1.76	5.25 ± 0.27	5.14	5.28 ± 0.28	5.30	5.44 ± 0.41	7.54	2.2
K+	5.00 ± 0.11	2.20	5.10 ± 0.15	2.94	4.60 ± 0.17	3.69	X	X	2.4
Na+	159.8±0.62	0.39	160.6 ± 4.61	2.87	161.5±5.28	3.27	X	X	0.4
PURE ANALYTE									
TC	4.22 ± 0.11	2.61	4.54 ± 0.16	3.52	4.58 ± 0.17	3.71	4.51 ± 0.31	6.87	2.7
UREA	5.26 ± 0.14	2.66	5.41 ± 0.16	2.95	5.45 ± 0.27	4.95	5.30 ± 0.14	2.64	6.9
GL	2.36 ± 0.04	1.69	2.56 ± 0.05	1.95	2.36 ± 0.12	5.08	2.44 ± 0.13	5.32	2.2
GH	12.22±0.08	0.65	12.46±0.12	0.96	12.38±0.14	1.13	12.54±0.16	1.28	2.2

Data is presented as mean ± standard Deviation (SD), coefficient of variation (CV %)), QS = quality specification TC=total cholesterol, K+= potassium, Na+ = sodium, GL= low glucose concentration, GH = high glucose concentration, bovine =ethylene glycol stabilized bovine serum, human serum= ethylene glycol stabilized human serum

X= sodium and potassium form SDA where not reported because of the breakdown of the auto analyser.

4.2 BETWEEN-DAY PRECISION

Data For the between-day run analysis are shown on table 4-2. Variability of between-day measurements was generally good with standard deviations below ±1. However sodium for both bovine and human sera coming from KATH (±2.31 and 4.61 respectively) and Medilab (± 4.61and ±5.28 respectively) were greater than ±1. This implies that the dispersion around the mean for sodium and potassium results obtained from KATH and Medilab were higher when compared to the manual method. Coefficients of variation (CV_{bd}) for most of the analytes from the laboratories were below 9%, except for GL (pure analyte) from SDA which recorded a value of 10.61%. However when compared to quality specification few of the analytes from the various laboratories (WHO recommended manual method exclusive) met the recommended standard. Results for urea from all laboratories were relatively precise as compared to other analytes. The estimated CVs for urea were all within the recommended quality specification this may in part be due to the high allowable variability in the measurement of urea. Given that the recommended CV for urea is as high as 6.9%

Table 4-3Long Term (between-day) Capability and Short term (within-day) Capability Indices of the five analytes using Bovine and Human serum as well as pure analytes

ANALYTE	LONG-TERM				SHORT-TERM			
BOVINE	**MANUAL**	**KATH**	**MEDILAB**	**SDA**	**MANUAL**	**KATH**	**MEDILAB**	**SDA**
TC	1.3	0.9	1.0	0.3	2.6	2.2	1.4	1.0
UREA	1.5	0.9	0.9	0.4	1.8	1.4	1.0	0.6
GLUCOSE	1.5	0.7	0.7	0.9	3.5	1.1	3.3	1.0
K+	1.0	0.4	0.3	X	1.8	0.8	1.8	X
Na+	1.4	0.4	0.2	X	2.2	2.2	1.0	X
HUMAN								
TC	1.1	1.0	0.9	0.2	2.9	2.6	1.6	1.1
UREA	1.2	0.6	0.6	0.3	1.8	1.3	1.5	1.1
GLUCOSE	1.3	0.7	0.5	0.4	2.6	2.4	2.1	2.5
K+	1.0	0.7	0.5	X	3.3	1.1	1.3	X
Na+	1.3	0.6	0.6	X	3.2	3.2	2.3	X
PURE ANALYTES								
TC	1.4	1.0	0.9	0.7				
UREA	1.6	1.0	0.9	1.0				
GL	2.9	0.4	0.6	0.5				
GH	3.8	2.8	2.0	1.7				

Data is presented as long and Short-term capability indicess. Long-Term = Long Term capability index, Short-Term = short term capability index TC=total cholesterol, K+= potassium, Na+ = sodium, bovine =ethylene glycol stabilized bovine serum, human serum= ethylene glycol stabilized human serum. X= sodium and potassium form SDA where not reported because of the breakdown of the auto analyser.

4.3 PROCESS CAPABILITY

Table 4-3 indicates long term (between-day) capability and short term (within-day) capability indices of the five analytes using bovine and human serum

For the within-day capability indices the manual method recorded the highest capability for all analytes (both bovine and human sera). The short term capability indices for the manual methods ranged from 1.8 for urea (human serum) to 3.5 for glucose (bovine serum). For KATH even though within-day capability indices were greater than 1(with the exception of potassium-bovine (0.8) the values were consistently lower than those obtained from the manual methods). Medilab had within-day capability indices greater than 1 for all analytes whiles only urea from SDA recorded a short term capability indices of 0.6 which is lower than recommended.

For the long-term capability, these analytes were run in duplicates for 20 days by all three laboratories and the mean recorded for the value of the day. In general, between-day capability indices for the WHO manual methods, were relatively lower as compared to the within-day capability indices, but were not below the acceptable capability of 1.0 for all analytes. The values were within the range of 1.0 for potassium (both bovine and human sera) and 3.8 for GH. For KATH, GL (0.4) recorded the lowest long term capability index whiles GH (2.8) yielded the highest long term capability index. Total cholesterol results from KATH were relatively stable when compared to other analytes (bovine 0.9, human 1.0 and pure 1.0). A similar trend was followed by Medilab and SDA as both laboratories recorded GH as the analyte with the highest long term capability index with respective values of 2.0 and 1.7. The long term capability indices recorded by SDA were generally low when compared to both KATH and Medilab. A comparison of the results by control material shows that the capability indices for the pure analytes were

relatively higher than both the bovine and human sera for all laboratories including the manual methods.

Table 4-4 Comparing actual means with theoretical values for pure analytes with known concentration.

ANALYTE	MANUAL			KATH			MEDILAB			SDA			
	Mean	D(CID)	p-value	mean	D(CID)	p-value	Mean	D(CID)	P-value	mean	D(CID)	p-value	TM
TC	4.22	-0.02(-0.04-0.08)	0.45	4.54	-0.34(0.26-0.41)	<0.0001	4.58	-0.38(0.30-0.46)	<0.0001	4.51	-0.31(0.16-0.45)	0.0003	4.20
Urea	5.26	0.04(-0.11- 0.025)	0.21	5.41	-0.11(0.04- 0.19)	0.0051	5.45	-0.15(0.02-0.28)	0.0235	5.3	-0.08(0.01-0.14)	0.0323	5.30
GL	2.36	0.008(-0.02-0.00)	0.053	2.56	-0.45(0.34-0.56)	<0.0001	2.36	-0.18(0.12 -0.24)	< 0.0001	2.44	-0.21(0.09-0.33)	0.002	2.24
GH	12.22	-0.02(-0.02-0.06)	0.23	12.46	-0.26(0.21 -0.31)	< 0.0001	12.38	-0.31(0.11- 0.51)	0.0038	12.54	-0.34(0.27-0.41)	<0.0001	12.20
Na+	140.4	-0.40(-0.07-0.87)	0.08	142.8	-2.750(1.09- 4.41)	0.0025	143.4	-3.40(1.91 -4.89)	0.0001				140.0
K+	4.01	-0.01(-0.03-0.05)	0.58	4.380	-0.38(0.23-0.52)	< 0.0001	4.370	-0.37(0.23- 0.50)	< 0.0001				4.0

Data is presented as means, discrepancies; Mean= actual mean, D (CID) = discrepancy (95%confidenc interval of discrepancy), TM = theoretical mean

4.4 COMPARISON OF ACTUAL MEANS WITH THEORETICAL VALUES FOR PURE ANALYTES WITH KNOWN CONCENTRATION

Table 4-4 shows results from a one sample t-test comparing theoretical means of pure analytes with known concentration to the actual means measured by the laboratories. This was done to ascertain the stability of the manual methods used so that the mean values obtained using the manual methods can be used as the "target values" for all control materials. Comparison of the results reveal that there was no significant difference (P-value > 0.05) between the theoretical mean and the actual mean measured with the manual method. However results from all the three laboratories showed a significant differences between the manual methods and the auto anlyzers being used.

Table 4-5Total inaccurac and percentage Total Error for both within-day and Between-day replication studies all measured and calculated values are compared with given quality specifications

	BETWEEN- DAY						WITHIN- DAY							
ANALYTE	**KATH**		**MEDILAB**		**SDA**		**KATH**		**MEDILAB**		**SDA**			
BOVINE	**%BIAS**	**%TE**	**% BIAS**	**%TE**	**%BIAS**	**%TE**	**%BIAS**	**%TE**	**% BIAS**	**%TE**	**%BIAS**	**%TE**	**QS(%BIAS)**	**QS(%TE)**
TC	-1.0	6.86	-2.4	7.85	10.3	17.50	-0.9	3.40	-2.0	6.48	-7.9	12.32	4.0	8.50
UREA	-3.2	13.26	-5.3	12.30	-6.9	13.92	-1.4	6.26	-3.6	7.18	3.7	7.41	5.5	15.7
GLUCOSE	-4.1	12.33	-1.4	10.67	-4.1	28.12	-0.6	2.20	-0.24	1.84	-5.6	12.05	2.2	6.90
K+	-1.3	8.50	6.2	14.22	X	X	-2.3	8.70	-0.3	3.17	X	X	1.8	5.80
Na+	-0.2	2.81	-1.9	7.99	X	X	-1.7	2.44	-0.6	2.10	X	X	0.3	0.90
HUMAN SERUM														
TC	-1.3	7.02	-2.1	8.18	22.5	34.90	-0.3	2.35	-0.8	3.23	-0.4	2.45	4.0	8.50
UREA	-2.4	12.77	-5.9	13.80	-8.32	19.60	-0.5	3.92	-1.7	6.29	-0.1	5.06	5.5	15.7
GLUCOSE	-0.2	8.68	-0.8	9.55	-3.30	15.76	-0.7	2.89	-0.6	3.97	-0.6	2.79	2.2	6.90
K+	-2.3	7.15	-8.4	14.49	X	X	-0.4	3.96	-2.5	5.68	X	X	1.8	5.80
Na+	-0.4	5.14	-0.2	5.59	X	X	-0.1	0.55	0.04	1.33	X	X	0.3	0.90
PURE ANALYTES														
TC	-1.9	7.76	-3.7	9.82	-6.4	17.73							4.0	8.50
UREA	-2.9	7.77	-3.5	11.67	-1.2	5.56							5.5	15.7
GL	-16.3	21.17	-6.8	15.55	-7.5	16.27							2.2	6.90
GH	-3.20	4.78	-0.1	1.96	-3.9	6.01							2.2	6.90

Data is presented as percentage bias (reference –test) and Total Error. %BIAS=Percentage bias, %TE= total error. TC=total cholesterol, K+= potassium, Na+ = sodium, bovine =ethylene glycol stabilized bovine serum, human serum = ethylene glycol stabilized human serum

4.5 INACCURACY AND TOTAL ERROR

Table 4-5 shows results for total inaccuracy as deviation percentage of mean measured value vs. target value, total error (TE) as quality characteristics of validated method according to equation TE = bias + 1.65 x CV(Smolcic *et al.*, ; Smolcic *et al.*, 2011). This is the net or combined effect of random (total imprecision) and systematic (inaccuracy) errors.

The bias from Bland-Altman plots (reference (manual) –field method) for each analyte between the laboratories and the WHO manual methods were used for the calculation of the total error. When the results (actual means) for pure analytes using the WHO manual methods were compared to the prepared concentration (theoretical means), there were no significant differences with P-value for all analytes > 0.05(Table4-4). Also the process capabilities for both within and between-day replication studies as well as the measured imprecision (CVs) from the WHO manual methods were all within quality recommendations. Therefore, the "target values" from the manual methods were used as the target values for the analysis.

KATH recorded negative biases for all the analytes measured for both within and between-day replication studies. For within-day inaccuracy, the results obtained from KATH fell within a range of -2.3% for potassium (bovine serum) and -1.7% for sodium (bovine serum). When compared to quality specification sodium and potassium (bovine serum) did not meet the specification. This may be due to the combine effect of both imprecision and inaccuracy since both the within-day CV (Table 4-1) and bias did not meet quality recommendation. Within-day run sodium from Medilab (both bovine and human sera) did not pass the recommended quality limits. Even though for human serum the bias was acceptable, a within-day imprecision (CV of 1.09%) was large enough to produce an unacceptable total error. Total cholesterol and glucose (bovine serum) from SDA did not meet quality specification. Both the level of within-day imprecision (3.91%) and inaccuracy (5.6%) were higher than recommended. However

the imprecision of total cholesterol on the other hand was acceptable but the inaccuracy was higher than recommended.

KATH and Medilab both consistently recorded an acceptable between-day total error for urea and total cholesterol. For urea both imprecision and inaccuracy were within the accepted standards for the two laboratories. For total cholesterol both laboratories recorded imprecision higher than recommended (Table 4-2). However the estimated biases (-1.0 and -2.4 respectively) were well within the acceptable recommendation. This actually influenced the acceptable total error. For SDA only urea (bovine serum and pure analytes) and GH recorded an acceptable total error. Total cholesterol for all three control material were out of range (17.5%, 34.9% and 17.73% for bovine, human and pure analytes respectively. This is further established by very low between-day capability indices for total cholesterol for all the three control materials. Glucose (both bovine and human serum) and GL for all three laboratories recorded an unacceptable total error. For both Medilab and KATH imprecision rather than inaccuracy was the major contributing factor to this irregularity. Both laboratories recorded acceptable biases for bovine and human serum glucose. In contrast both imprecision and inaccuracy contributed to the unacceptable total error for glucose from SDA.

Sodium and potassium results from both Medilab and KATH were also above the quality specification. Quality specification rules for potassium measurement are relatively strict, with the recommended total error of 0.9% and a bias of 0.3%. Total error result for potassium (both bovine and human) from KATH and Medilab were above recommendation. It is worth noting that apart from total cholesterol (both bovine and human serum) from SDA and potassium (bovine serum) from Medilab, all other analytes recorded a negative bias. This an indication of an overestimation of the manual methods by the autoanalyzers (Manual-field method)

Table 4-6 Results of method comparison between WHO manual methods and autoanlyzers from KATH, Medilab and SDA. Intercepts and slopes with 95% CI according to Passing and Bablock regression analysis and P-value for CUSUM test of linearity are presented.

ANALYTE	KATH			MEDILAB			SDA		
BOVINE	**INT.(CI)**	**SLOPE(CI)**	**CT(P-VAL)**	**INT.(CI)**	**SLOPE(CI)**	**CT(P-VAL)**	**INT.(CI)**	**SLOPE(CI)**	**CT(P-VAL)**
TC	2.7(1.51-3.00)	0.2(0.07-1.5)	0.14₦	2.9(-3.06-3.23)	0.1(0.0-1.9)	0.14¥	3.0(2.24-3.22)	0.1(0.0-0.3)	0.36≠
UREA	-0.7(-16.5-2.1)	1.1(0.5-24.7)	0.72¥	-0.7(-12.5-2.08)	1.2(0.5-4.4)	0.72¥	-4.6(-0.77-1.1)	1.9(0.71-9.8)	0.72¥
GLUCOSE	0.9(0.54-1.90)	0.5(1.1-1.30)	0.30≠	1.9(-1.0-1.900)	1.3(1.1-4.0)	0.13 Ᵽ	0.9(0.54-0.9)	0.5(0.0-0.91)	0.30≠
K+	1.4(-0.27-2.6)	0.7(0.5-1.00)	0.74¥	2.73(-9.0-5.10)	0.5(0.0-3.0)	0.36¥			
Na+	0.0 (-0.1-86.0)	1.0(0.4-1.00)	0.06¥	145(-71.7-145)	0.0(0.0-1.5)	0.34¥			
HUMAN SERUM									
TC	2.5(-2.7-3.90)	0.4(0.0-1.66)	0.72¥	2.5(-2.70-1.67)	0.4(0.0-1.67)	0.72¥	3.9(3.05-3.90)	0.0(0.0-0.29)	0.44≠
UREA	-5.2(-3.95-5.2)	0.0(0.0-1.22)	0.72¥	-5.2(-6.02-5.15)	0.0(0.0-1.17)	0.98¥	-5.15(-6.1-5.15)	0.0(0.0-1.19)	0.72¥
GLUCOSE	3.7(0.50-5.30)	0.3(0.0-0.90)	0.98≠	5.3(4.15-6.20)	0.0(-0.2-0.25)	0.40≠	5.3(3.93-5.76)	0.0(-0.1-0.24)	0.76≠
K+	1.7(-1.9-0.150)	0.6(0.1-1.00)	0.36¥	3.2(-0.34-4.90)	0.4(0.0-1.25)	0.72¥			
Na+	106(-64.0-125)	0.3(0.2-1.60)	0.36¥	127(-101.2-152)	0.2(0.04-1.36)	0.42¥			
PURE ANALYTES									
TC	1.5(-5.3-4.0)	0.6(0.1-2.10)	0.72¥	1.5(-4.92-3.51)	0.6(0.16-2.00)	0.10¥	3.29(1.34-4.45)	0.2(-0.1-0.65)	0.65≠
UREA	0.6(-6.6-93.8)	0.9(0.3-2.20)	0.98¥	4.3(-1.22-6.00)	0.2(-0.13-1.7)	0.36¥	0.23(-4.97-2.54)	0.9(0.5-1.9)	0.66¥
GL	2.1(1.9-2.20)	0.03(0.0-0.03)	0.98≠	1.9(1.26-2.22)	0.1(0.0-0.40)	0.10≠	2.11(1.90-2.22)	0.04(0.0-0.13)	0.98≠
GH	5.6(-6.5-12.2)	0.5(0.0-1.50)	0.98¥	7.2(-0.79-12.08)	0.4(0.01-1.05)	0.72¥	8.4(-3.42-12.22)	0.3(0.0-1.25)	0.36¥

Data is presented as intercept (confidence interval), (slope (confidence interval) and p-value for cu sum test of linearity.
INT. (CI) = intercept (confidence interval), CT (P-VAL) = p-value for cu sum test of linearity.

¥Intercept CI includes 0 as value and slope CI include 1 as a value (in accordance with WHO manual methods)

₦Intercept CI does not include 0 as value and slope CI includes 1 as value (constant difference)

Ᵽ Intercept CI includes 0 as a value and slope CI does not include 1 as value (proportional difference)

≠Intercept CI does not include 0 as value and slope CI does not include 1 as value (both constant and proportional difference)

4.6 METHOD COMPARISON BETWEEN THE MANUAL METHODS AND KATH, MEDILAB AND SDA

For the assessment of homogeneity between the autoanlyzers and the WHO manual methods for the tested analytes, Passing and Bablock regression was used to identify constant, proportional, both constant and proportional differences as well as the analytical processes that are in agreement with the WHO recommended manual methods. The results are indicated on table 4-6.

Total cholesterol (with the exception of TC from KATH-bovine) and urea measurements from both KATH and Medilab were consistently in accordance with the WHO recommended manual methods for all control materials. However total cholesterol from KATH (bovine) recorded a constant difference between the auto analyser and manual method. Urea measurements from SDA were also consistently in accordance with the WHO recommended manual method. Total cholesterol from SDA however consistently showed both constant and proportional difference for all three control materials.

Glucose measurement from all three laboratories were embedded with both constant and proportional differences for all three control materials (except GH). This is not actually surprising in that the total error results for glucose (Table4-5) from all three laboratories were mostly above the quality specifications.

Sodium and potassium measurements from both Medilab and KATH were in accordance with the WHO manual methods for both bovine and human sera. To determine if residuals are randomly distributed around the fitted line, test of linearity of the autoanalyzers with the WHO recommended manual methods were assessed using the CUSUM test. Results show that all the analytical processes assessed from the autoanalyzers were linear (P-value > 0.05 for all analytes) with the WHO recommended manual methods. Even though complete agreement was not achieved for some analytes, none of them deviated from linearity when compared with the WHO manual methods.

Table 4-7Data on general information gathered from the questionaire

Parameter	Frequency N=20	Percentage
Change Of Kits	13	65.00
Times Of Calibration		
Daily	20	100
Type Of QC Material		
Laboratory Prepared	2	10.00
Both	5	25.00
Commercial Prepared	12	60.00
IQC Practice		
Daily	8	40.00
Weekly	7	35.00
Monthly	5	25.00
Concentration Levels		
One	13	65.00
Two	7	35.00
Frequency Of A Failed IQC		
Occasionally	5	25.00
Rarely	15	75.00
Control Chart Used		
Westgard	5	25.00
Cusum	5	25.00
No Chart	9	45.00
Freq Of Method Comparison		
Occasionally	15	75.00
Monthly	3	15.00
IQC Data Management		
Internally	20	100
Use Of Special Statistical Package	4	20.00
Challenges In IQC Assessment	15	73.33

Data are presented as frequency and percentages

Table 4-8Factors affecting quality control assessment

Parameters	**Private Freq (%) N=8**	**Government Freq (%) N=12**	**Total Freq (%) N=20**
Cost Of Control Material	7(87.5)	0(0.00)	7(35.00)
Cost Of Reagent	8(100)	0(0.00)	8(40.00)
Cost Of Assessing QC Results	8(100)	1(8.33)	9(45.00)
Cost Of Investigating Error	8(100)	3(25.00)	11(55.00)
Time Cost	1(12.5)	5(41.66)	6(30.00)
Number Of Analytes	5(62.5)	6(50.00)	11(55.00)
Ease Of Handling Control Chart	8(100)	8(66.66)	16(93.33)
Limited Personnel With QC Training	4(50.00)	5(41.66)	9(45.00)
Handling Of QC Statistic	4(50.00)	6(50.00)	10(50.00)

Data is presented as frequency and percentages. Freq (%) = frequency

4.7 FACTORS WHICH INFLUENCE QUALITY CONTROL

Table 4-8 summarizes the various factors that influence quality control in the clinical laboratories. In order to assess the effect of different parameters (problems) that hinders quality control in the laboratories, responses to series of questions asked are captured on the above table. Responders were asked to choose which of these factors pose a major problem in their quest to adhere to good quality control practices. For cost of control materials 7 responders representing 87.5% of the private laboratories sampled and 35% of the total sample deemed it as a problem. However no participant from a government facility said it was a problem. For cost of reagent, cost of assessing QC results and cost of investigating and subsequent removal of the problem, all (100%) private laboratories assessed considered it as a major problem. However 1%, 3% and 5% of government responders in the order above consider it as a problem. In all 40%, 45% and 55% of the total responders sees it as a major problem. Handling of control charts was a major problem with most of the laboratories. A significant 93.33% of all laboratories assessed had a problem with handling control charts. Even though 25% said they use Westgard-multirule chart and 25% said they use CUSUM charts, a significant 45 percent use no chart at all. With the number of personnel with QC training, 4 (50%) of the private laboratories and 45% of the government laboratories responded that they had limited number of personnel with QC training. Ease of handling QC was also a major problem faced by the laboratories.

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Chapter 5

DISCUSSION

5.1 TOTAL CHOLESTEROL (TC)

Cholesterol level measurement is influenced by long-term or clinically significant biologic factors, transient or insignificant biologic factors, and measurement error (de Jonge *et al.*, 2004). The autoanalyzers used by the various laboratories are stable considering a within-day CV less than 3% and the between-day CV generally less than 9% for all three laboratories. A validation study of another auto analyser (Cobas 6000 analyzer series module c501) which uses the same principle as the one used by the laboratories for the measurement of cholesterol by Smolcic et al (2001), recorded a within run and between-day CVs for the cobas 6000 as 7.4% and 1.5% respectively. Cholesterol measurement were found to be acceptable when compared to quality specification. However even though the within-day CVs for all laboratories accessed were acceptable and in line with their work, the between-day CVs with the exception of the manual methods did not meet quality specification. Both the within-day run and the between-day CVs from the laboratories were consistently higher than those obtained by the WHO recommended manual methods. However the existence of method dependent differences in precision and accuracy of various cholesterol assays has been amply demonstrated (Demacker *et al.*, 1983; Boerma *et al.*, 1986). Variation in reported cholesterol levels is partially the consequence of the varied methods used to test cholesterol, but substantial variation occurs even among laboratories using the same method (D'Orazio *et al.*, 2000). In a research conducted by The College of American Pathologists, a sample specimen whose cholesterol concentration was determined by CDC to be 262.6 mg/dl was sent to 5,000 clinical laboratories. The cholesterol values reported by the surveyed laboratories

ranged from 101 to 524 mg/dl (Sawa *et al.*, 2011). This was found to be in line with the results obtained with respect to the differences in the estimated CVs. For example comparing CVs for total cholesterol for human serum (Table 4-2) Medilab recorded a CV which was 0.22% higher than KATH whiles SDA was 4.09% higher than KATH.

The stability of the analytical processes for cholesterol from the various laboratories was assessed using the short and long term capability indices. The medical allowable tolerances defined by the CLIA-88 proficiency testing criteria for acceptable performance for cholesterol is 10 %(Westgard 2008). A good analytical process should have a capability index greater or equal to 1(Jenny, 1994). In a study conducted Jonge et all, (2004) the process capability for the selected tests including total cholesterol range from 3 to 15, which means that all selected analytes were within good analytical control. Even though the cholesterol capabilities assessed by Jonge and others were higher than what was obtained by the laboratories, the within-day capability indices were all above the minimum value of 1. This shows that the analytical process were highly stable for cholesterol in the short term. In contrast the between-day capability index shows instability in the analytical systems since most of the laboratories recorded capability indices less than 1. Cholesterol measurement from SDA was by far the most unstable process among the assessed laboratories this may be attributed the constant breakdowns and repairs of the autoanlyzer being used.

Inaccuracy in the measurement of total cholesterol was assessed using bias, from Bland-Altman analysis. Both within-day and between-day measurement produced results that were generally accurate per the quality specification. Exception was results from SDA which mostly did not meet the quality specification for bias. Accuracy was generally good for the measurements of cholesterol from all three laboratories this influenced most of the acceptable

total error. However for SDA estimated total error for the between-day measurement did not meet recommended standards and this might have resulted from the presence large inaccuracies and precision inherent in the analytical procedure.

It is highly important that across laboratory methods are standardized to establish homogeneity across an array of laboratories (Myers *et al.,* 2000). The main objective for standardization is to ensure that reported results for analytes agree across measurement systems and laboratories and over time (Myers *et al.*, 2000). With the exception of the brands of the automated instrument being used by Kath, Medilab and SDA there are basically no differences(Between the autoanalyzers and the manual methods) in the chemistry underlying the reaction used in the determination of total cholesterol (Cholesterol esterase – peroxidase). Any difference will be associated to the sensitivity of the autoanalyzers to detect accurately the concentration of cholesterol in the control sera used. It is therefore not surprising that, for all control material used (with the exception of bovine from KATH), total cholesterol from both Medilab and KATH showed complete concordance with the WHO manual methods. However total cholesterol from SDA was consistent with both constant and proportional error (Table 4-5). This is also expected as there were problems with both accuracy and precision in the measurements from SDA. When the results obtained from the autoanalyzers from the laboratories were compared among themselves, there was complete agreement for cholesterol among all the laboratories.

5.2 SODIUM AND POTASSIUM

The routine analysis of sodium and potassium in almost every laboratory is very important in the diagnosis of diseases (Fogh-Andersen and Siggaard-Andersen, 1994). Generally good precision and accuracy have been associated with the use of ISE for the analysis of sodium and potassium by several researchers (Fogh-Andersen and Siggaard-Andersen, 1994; D'Orazio *et al.*, 2000; Albert *et al.*, 2011). However other authors have also reported imprecision higher than recommended in the measurement of sodium using ISE based autoanalyzers (Jasna et al., 2010). In the analytical evaluation study conducted by Jasna and co, 2011 on the Olympus AU2700 plus (Olympus CO Ltd., Tokyo, Japan). Between-day and within-day sodium were 0.7% and 1.0% respectively, which are above the recommended CV of 0.4%. Even though some drawbacks such as low throughput, requirement of manual operation, as well as time consuming procedure has been attributed to the flame photometry (Albert *et al.*, 2011). The manual method used for the analysis consistently produced results that are accurate and precise per the quality specification (Table 4-1 and Table 4-2). Within-day and between-day imprecision for sodium for both Medilab and KATH did not meet quality specification. Also result for potassium from both KATH and Medilab were imprecise per the quality specification. However, slow response time, instability, or drift requiring frequent calibration, nonlinear response in the clinically useful range, non-specificity, lack of sensitivity, and protein build-up on the electrodes have been cited as major draw backs of the ion selective electrode. (Owiredu *et al.*, 2007a and (Albert *et al.*, 2011). For both between-day and within-day measurements, sodium (KATH and Medilab) did not meet the recommendation for total error. This was due to the fact that both imprecision and inaccuracy are high in the measurements. Also the rule for the acceptance of total error is very strict (0.9%). This is due to the low analytical variation

expected in the measurement of sodium ((Albert *et al.*, 2011). However with the exception of bovine serum from KATH the quality specification was met for potassium (both bovine and human serum).

Agreement between the ISE and the flame photometry has been established by several authors(Fogh-Andersen and Siggaard-Andersen, 1994). A comparison study between an ISE based autoanalyzer(Beckman Coulter Synchron CX9 PRO) and the flame photometer established 95% limit of agreement for sodium in serum (-7.8±17.3 (-42.2 to 26.6)) and in urine (-22±41 (-104 to 60)). Similarly, the mean difference between the two methods was found to be -0.25±0.75 (-1.75 to 1.25) and in urine was -5.3±38.9 (-83.1 to 72.5). With 95% confidence interval, the value of sodium and potassium as determined by both the methods fell between the upper and lower limit thus showing 95% limits of agreement. They concluded that good degree of agreement was achieved when the two methods were compared for measuring the electrolytes and hence the use of Synchron CX9 in place of Flame photometer for electrolyte analysis in serum and urine is justified and the two can be used interchangeably (Diaz Moreno *et al.*, 2011) This is similar to what was observed in this study. A passing and bablock regression (Table 4-5) comparing the flame photometric methods and the ISE being used by the laboratories showed complete agreement for both bovine and human sera and thus these two methods can be used interchangeably. A comparison of potassium measurement between KATH and Medilab showed complete agreement between the two ISE based methods and there were no deviations from linearity between results obtained from the two laboratories.

5.3 GLUCOSE

Within-day run glucose measurement from KATH and Medilab were very precise per the quality specification, a comparison of the CVs and standard deviation with those of the WHO recommended manual methods shows no significant difference especially for bovine serum(Table 4-1). SDA however recorded an imprecision in the within-day measurement of glucose. Even though the difference in deviation between SDA and the Manual methods were insignificant, the mean deviation recorded by SDA were significantly high thereby influencing a high within-day imprecision. A research conducted by Smolcic et al., (2011) on another Hexokinase base autoanalyzer (Cobas 600) recorded an acceptable between-run CV of (1.5%). However the within-run CV (1.5%) was unacceptable per the quality specification (2.9%). In contrast, the within-run CV for the autoanalyzers except (SDA) were acceptable whiles imprecision was rather high in the between-day measurements. The hexokinase method has been as giving higher values than normal(Romano, 1973). This has been attributed to the ability of the alkaline copper-neocuproine to react with non-glucose reducing material to give results that are falsely high (Fingerhut, 1968) (Powell and Djuh, 1971). High imprecision was observed in the measurements of glucose low as compared to glucose high. With the exception of the WHO recommended manual method and KATH, both Medilab and SDA recorded unacceptable variability in the measurements of glucose low even though glucose high was precise by the quality specification.

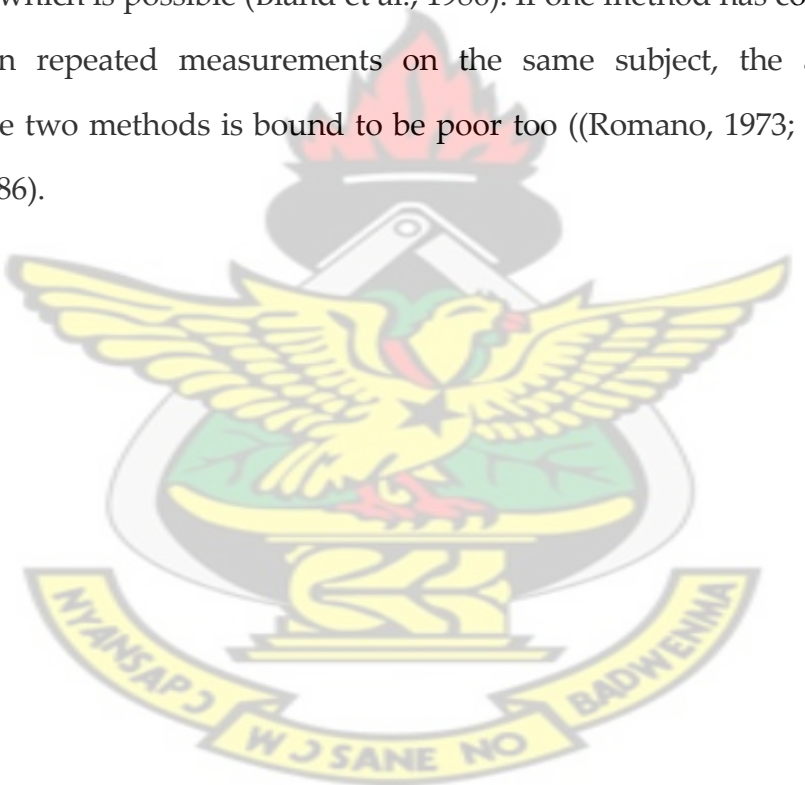
The medically allowable tolerances defined by the CLIA-88 proficiency testing criteria for acceptable performance for glucose is 10 %(Westgard, 2008). This was used to compute the capability index for glucose measurements. Within-day capability index for all laboratories were acceptable (greater than 1). This indicates the stability of the autoanlyzers in the short term. However the capability indices observed for between-day studies were below the

recommended values. When compared to the manual method they were significantly lower. Glucose high was however stable even in the long term.

Assessment of glucose analytical process in terms of total error for both within-day and between-day studies revealed an acceptable within-day total error for both KATH and Medilab. A closer look at the results shows an acceptable within-day inaccuracy (Table 4-4) as well as imprecision (Table 4-1) for both KATH and Medilab. On the other hand SDA recorded an acceptable total error for human serum but the error produced in the bovine serum measurements was larger than recommended.

Differences between the hexokinase and the glucose oxidase methods have been reported (Gochman *et al.*, 1975). An interlaboratory comparison of serum glucose methods applied to instrumental adaptations for hexokinase based method used with the Du Pont "aca," (Abbot Bichromatic Analyzer) and a glucose-oxidase based method (Beckman Glucose Analyzer) analysed both normal samples and samples that contained potential interfering substances or were otherwise abnormal. The researchers observed that all methods were satisfactorily precise, however they also noted several cases especially in the abnormal specimens where the hexokinase – based method was generally more variable. They also noted the consistency in the data obtained from the glucose oxidase method. (Gochman *et al.*, 1975)). In line with the current study, a comparison of the hexokinase and the glucose oxidase method (Manual) using the passing and babblock regression show both constant and proportional difference for both three laboratories (except glucose for bovine serum which showed a proportional difference). However glucose high was in agreement with results obtained by the manual methods. It is important that a based line reference is set for each procedure and necessary adjustment made if the two methods are to be used interchangeably (Gochman *et al.*, 1975). Especially when measuring low concentrations of glucose. Comparison of glucose measurement

between KATH and Medilab showed complete agreement for all control materials except for (Bovine serum). However a comparison of results between KATH and SDA recorded proportional differences for all control materials. It might be surprising that even though KATH, SDA and Medilab basically uses the same method, complete agreement was not achieved for KATH-SDA (Proportional difference) and Medilab-SDA (constant difference). This may be due to the high imprecision recorded by the auto analyser used by SDA. Repeatability is relevant to the study of method comparison because the repeatabilities of the two methods of measurements, limit the amount of agreement which is possible (Bland et al., 1986). If one method has considerable variation in repeated measurements on the same subject, the agreement between the two methods is bound to be poor too ((Romano, 1973; Bland and Altman, 1986).



5.4 UREA

Urea measurement was one of the most consistent and precise analytes accessed from the laboratories. Generally within-day CV was less than 4% whilst between-day CV was less than 6.5%. When compared to the quality specification, all the laboratories produced within-day imprecisions that were acceptable. Observation of urea results in terms of total error and comparing them with European quality specification for allowable total error revealed an acceptable within–day total error (Table 4-6). This is as a result of within-day imprecision and inaccuracy that were both acceptable per the quality specification. This confirms the results obtained by Smolcic and others which reported a within-day imprecision of 4.0% and a between-day 2.1% on the cobas integra 6000. Also between-day assessment of total error from KATH, Medilab and SDA (except human serum for SDA) were within the quality specification for all control materials. This is also in line with what was reported by Smolcic and co.

Basically the main principle behind the manual method and the one used by the automated analysers for the measurement of urea are the same and thus any differences could be attributed to occurrences in the analytical systems. Agreement between the manual methods and the autoanalyzers were assessed using the passing and bablock regression. Complete concordance was achieved for all control materials in all the laboratories. Also a CUSUM test for linearity showed linear relationship between the manual method and the autoanalyzers. Thus it is possible for the two methods to be used interchangeably in the analysis of samples. Comparison of urea measurement between KATH and Medilab showed complete agreement for all control materials used but results for KATH–SDA were consistent with proportional difference whiles constant differences were founds in the comparison of Medilab and SDA.

5.5 FACTORS WHICH INFLUENCE QUALITY CONTROL

Clinical laboratories have a major influence on clinical decisions and the past years have seen an evolution brought about by advances in information technology and automation. The impact of the ever-changing technology in regard to responsibilities and training therefore needs continual appraisal (George *et al.*, 2008).

Structured self-administered questionnaires were provided for laboratory managers in and around the Kumasi metropolis. This sought information on various quality control practices in their laboratories. All laboratories assessed indicated that, daily calibration of their analytical instrument is done before commencement of the daily activities. However there was divided response on the practice of internal quality control, 40%, 35% and 25% respectively maintain they practice IQC daily, weekly and monthly. An observation of the results reveal that, a higher percentage of the sampled laboratories use commercially prepared QC material mostly and only one concentration level is assessed. As much as 45% of the laboratories did not use any control charts, whiles others seem to have some knowledge about Westgard-multirule control chart and the CUSUM chart. Method comparison seems to be a major problem for these laboratories. Most of the responders (75%) admitted they occasionally undertake method comparison studies. From the results it was evident that most laboratories face a lot of challenges in quality control assessment. Some of these factors were assessed with series of response in Table 4-7. It is clear that government and private laboratories face some common problems; however there were others that were mostly associated with either private establishments or government institution. For example cost (financial) was a major problem for the private institutions as the cost of control materials, reagents, error investigations and subsequent removal remained a major problem. This may be due to the fact that government agencies are supported

by public funds unlike the private institutions. On the other hand time cost was however problematic for the government agency. A possible explanation to this may be due to the number of patients that flood the governments hospitals hence making analysts over burdened with work. Again the use of control charts seems to be a major problem for both government and private laboratories. About 93.33% of all responders had a problem with the use of control charts.

Chapter 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Within-day imprecision for majority of the tested analytes from all the laboratories as well as the manual methods were acceptable when compared with quality specification, however this was in contrast to the 20-day replication study. It is evident that for most of the analytes which did not meet quality specification for total error, the outcome was heavily influenced by imprecision rather than inaccuracies. Total cholesterol and urea analytical process from KATH and Medilab are stable. However glucose measurements especially low concentrations of glucose seem to be a problem for all the three laboratories. Most of the analytical processes assessed from the laboratories had good process capability and are reliable in the short term. With the exception of the WHO recommended manual methods, the capability index of most of the analytical processes assessed from KATH, Medilab and SDA in the long term was low.

All the WHO recommended manual methods examined provided reasonable estimates for all the analytes for routine clinical decision making and patients care. One important revelation was the fact that none of the analytical processes assessed from the laboratories recorded a significant deviation from linearity when assessed with the Cusum test for linearity. Complete agreement between the WHO recommended manual methods and autoanalyzers (KATH and Medilab) was achieved for urea, total cholesterol, sodium and potassium. Urea results from SDA achieved full concordance with the WHO recommended manual methods. Most of the private laboratories assessed responded in the affirmative that financial cost provided an

impediment in the adherence to good quality control. As much as the above mentioned factor does not actually pose a problem for most of the government hospitals, time cost was however a problem. Knowledge about the use of quality control charts is a major problem for both the private and government institutions.

6.2 RECOMMENDATION FOR FURTHER STUDIES

Analytical methods that did not fulfil quality specification criteria require more frequent quality control protocol which includes several runs of control material in series. Also, there is need for continuous monitoring of bias and CV in routine laboratory work and stricter rules for allowable CV and bias. Further studies should be conducted in this area and should include the estimation of other analytical processes which has not been included in this study. Further studies which employ all the three control levels (Normal, low and High) for each analyte should be conducted since the performance of the two different glucose levels by the autoanalyzers were not the same

There is need for policies that will encourage the training of laboratory managers and analyst in the area of quality control. It also recommended that private laboratories are supported by the government with additional funds that will help them in the practise of stricter quality control. It is also essential that an oversight body is established that will regulate the continuous practice of method comparison studies in other to ensure between laboratory methods agreements.

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APPENDIX

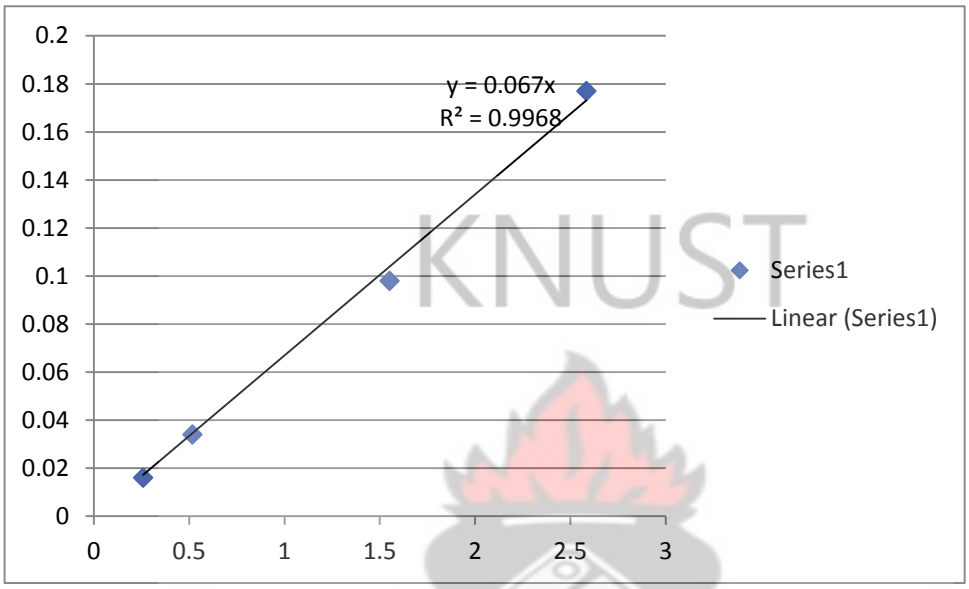

Figure 3Standard Curve for Cholesterol

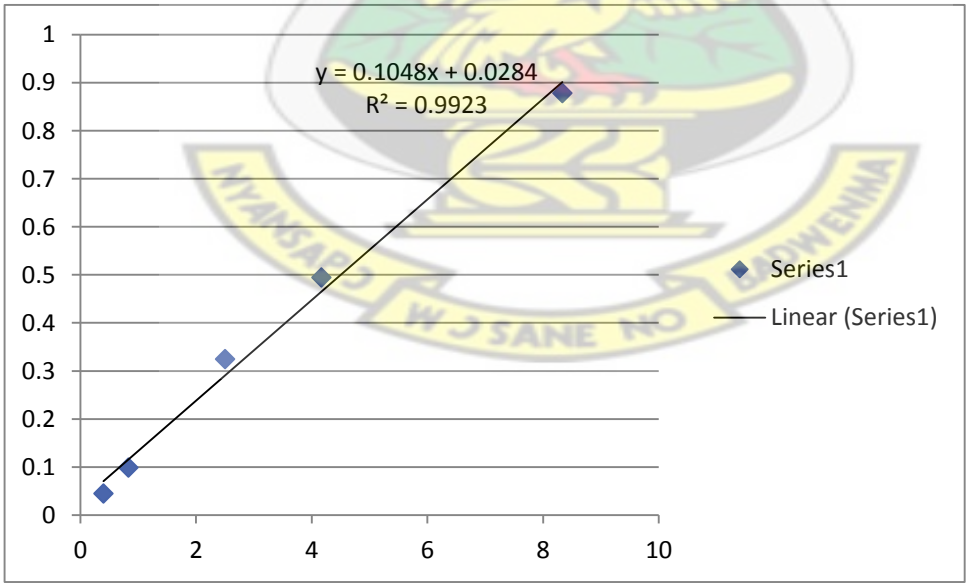

Figure 4 Standard Curve for Urea

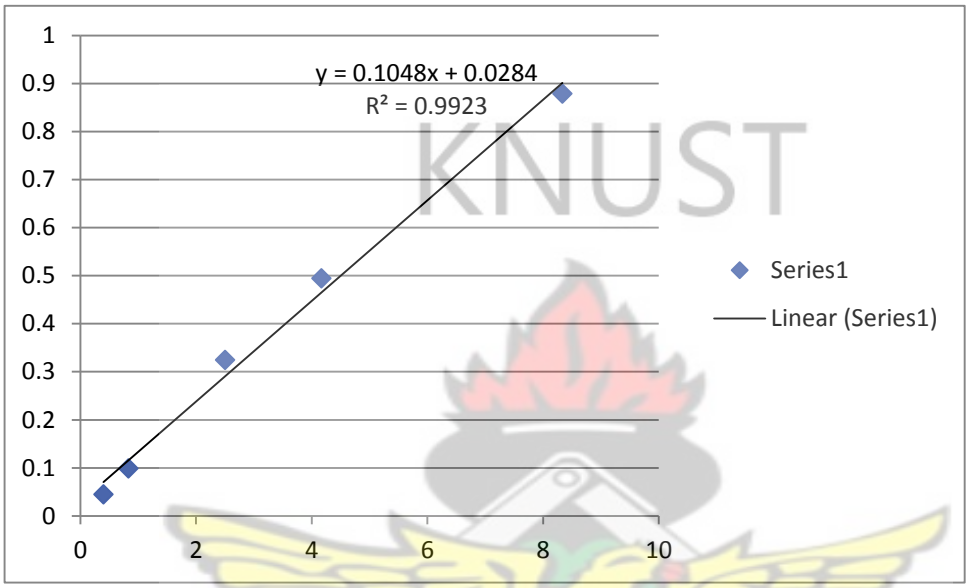

Figure 5 Standard Curve for Glucose

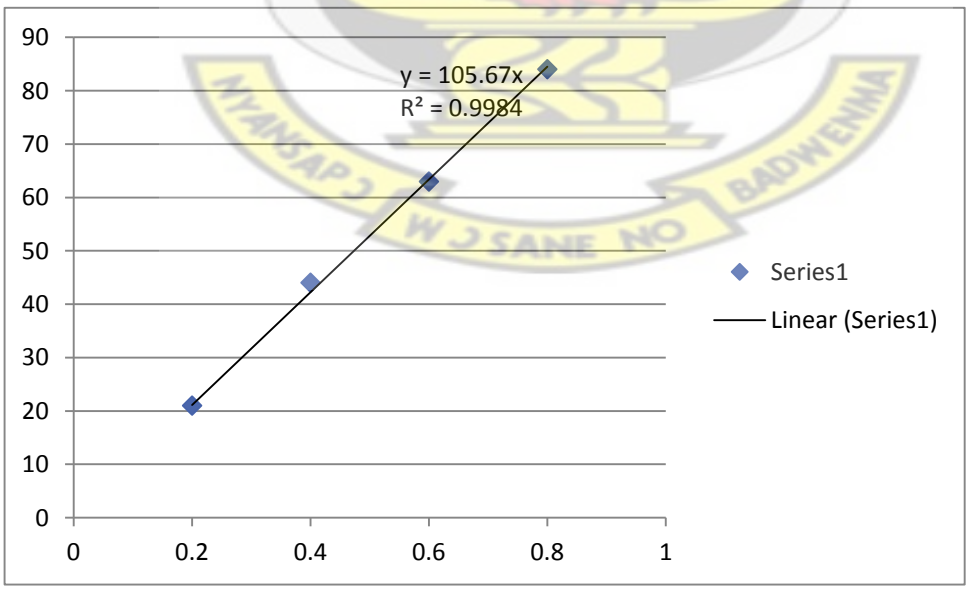

Figure 6 Standard Curve for Sodium

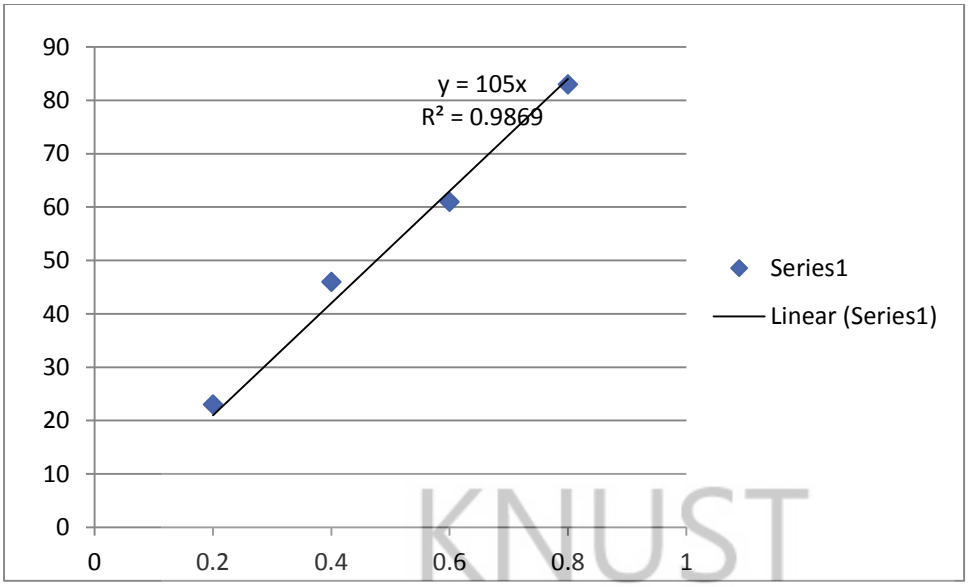

Figure 7 Standard Curve for Potassium