Dedication

To may dear parents, who have played an indispensable role and conserved no effort to teach me .And keep encouraging me and show happiness .for my success

To my brothers and sister who shared the task of carrying out this study with me, really without their .assistance this work couldn’t have been as it is

Acknowledgement

On completion of this study, I would like to appreciate the supervision of professor A.H.Khattab.Who paid a considerable effort to guide and direct me to conduct this study .Without his assistance, really, this work couldn't have appeared also the contribution and the assistance of others who have played a remarkable role which made this work possible. Those include:

-

-Director of the Directorate for Quality Assurance in Clinical Laboratory

-Colleagues in the regulatory laboratory for quality in the National Health Laboratory, Federal Ministry of Health.

- Colleagues in the department of clinical chemistry in the National Health Laboratory.

-Professor Mahjoub Awad Yusuf .The Owner of Hipocrate Company.

-Mr. Awad Mustafa, the Owner of Rokn El-Mazaya.

Abstract

This study was conducted in the National Health Laboratory intending the comparison of glucose, urea and creatinine using reagents produced by BioSystems and Linear S.L. on the comparison covers base of their linearity and sensitivity to assure their suitability to be used by health laboratories for diagnostic purposes.

One kit from each source was used to analyze specimens containing several concentrations of the three analytes. Normal and high concentrations were examined to study the linearity of the reagents, while lower concentrations were examined to study the sensitivity of these reagents. All reagents from both companies showed acceptable linearity and sensitivity. No significant difference between the results obtained by the two kits.

الخلاصة

هذة الدراسة تم إجراؤها في المعمل القومي بغرض مقارنة نتائج سكر الجلوكوز والبولينا والكرياتينين المتحصل عليها باستخدام كواشف منتجة بواسطة شركتي بايوسسيستم ولينيرعلى أساس خطيتها وحساسيتها لتأكيد أهليتها للاستعمال بواسطة المعامل الصحية لأغراض التشخيص .

تم استعمال كاشف واحد من كل مصدر لتحليل عينات تحتوى على تركيزات مختلفة من تلك المواد الثلاث المراد تقديرها.تم تحليل عينات ذات تراكيزات طبيعية مرتفعة لدراسة خطية تلك الكواشف .كما تم استعمال عينات ذات تركيزات منخفضة لدراسة حساسيتها.

كل الكواشف المستعملة والمنتجة بواسطة الشركتين اظهرات خطية وحساسية مقبولتان

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Abbreviations

GLP	Good Laboratory Practice
IVD	In Vitro Diagnostics
SOPs	Standard Operating Producers
IQC	Internal Quality Control
EQC	External Quality Control
EN	European Standard Series

ISO	International Standard Organization
NCCLS	National Committee For Clinical Laboratory Standards
EQA	External Quality Assessment

CHAPTER 1

ND LITRITURE INTRODUCTION A REVIEW

Introduction and Literature review

1.1 Laboratory reagents:

In many countries, a major problem for those responsible for providing good laboratory services is the quality and consistency of supply of laboratory reagents. The contribution that medical laboratory makes to the health programmers of a country is dependent upon the clinical usefulness of the

service provided to the health care structure, whether it is at the intermediate or peripheral level. Good laboratory practice (GLP) in all disciplines of medical laboratory is the cornerstone of such service but good laboratory practice is impossible without a constant supply of quality reagents.

Due to the importance of this subject many similar studies on the same subject have been conducted in Sudan, but they did not study products from the companies targeted by this studies (Linear S.L. and BioSystems).

1.1.1 Linear chemicals, S.L.:

Linear chemical S.L. is Spanish company established in early 1994 with activities in the developing manufacturing of in vitro diagnostics (IVD) products including different instruments and reagents covering clinical chemistry, serology, urinalysis, Hematology and Immunology and marketing them under the brand name of cromatest ®. Linear, today is represented around the world through a distributor's network that has contributed to its globalization, supplying products with the highest quality at competitive prices.

1.1.2 BioSystems:

BioSystems is a Spanish company it is based in Barcelona. It manufactures and markets diagnostic test kits and reagents to customers worldwide and also supplies a range of analytical instruments. BioSystem has a comprehensive range of products covering the areas of serology clinical biochemistry immunochemistry, rapid (one step) tests, blood grouping coagulation electrophoresis, instruments analyzers.

1.2 ERRORS

Many kits are been marketed in sudan.so it is important to choose the appropriate kits to use in our laboratory .there may be significant difference in the results of different reference results so to compare different kits using .quality control sera to help in the choice is important

The kits show different types of errors that should be recognized .The errors -:that are likely to occur can be classified as following

1.2.1 Crude Errors:

Crude errors are caused by serious mix-ups, e.g. of samples, pipettes, reagents, measurement wavelength etc.

They often result in implausible measured values , making it easy to detect these types of errors.

Crude errors can basically be avoided by ensuring good laboratory organization and following the “SOPs”.

1.2.2 Random errors:

If you measure the concentration of a substance repeatedly many times in the same sample under identical condition, you do not obtain identical results, but rather a scattering of values .this scattering can never be completely avoided و but it can be minimized by using careful working procedures and high-quality reagents and analyzer systems. Large random errors mean high imprecision.

1.2.3 Systematic errors:

Systematic errors are usually caused by defective pipetters or dosing devices, slight deviations in temperature control (especially in determinations of enzyme activity), defective reagents and analyzer systems, or erroneous data interpretation. The measured values often indicate a trend, i.e., they are systematically located above or below the expected value.

Large systematic errors mean high inaccuracy (1).

1.3 Quality Control

This describes the steps taken by the laboratory to ensure that tests are performed correctly also describes all steps that can be taken in (IQC) and out (EQC) side the laboratory to recognize and minimize analytical errors. (2)

1.3.1 Accreditation and Certification:

Accreditation is a procedure by which an authoritative body gives formal recognition that a body or person is complement to carry out specific tasks. On the contrary, certification is a procedure by which a third party gives written assurance that a product, process or service conforms to a specific requirement. (3)

. Certification according to EN ISO 9000 ff is the only internationally recognized from of external certification available to date. It is a point of differentiation in the marketplace for individual laboratory and manufacture of invitro diagnostic products. (1)

1.3.2 Quality control sera:

A control sample is necessary to check for bias (in accuracy) in tests. It is available in two types:

Analyzed pooled sera:-

This can be prepared and calibrated in the laboratory.

Commercially prepared sera:

It is available in two forms:-

- Freeze dried “lyophilized” sera
- Liquid synthetically manufactured sera (2)

The needs for quality assurance in chemical measurements are vital to a variety of human interests.

Two types of quality assessment measurements are encountered .The first involves the evaluation of the accuracy and precision of the methods of measurement. The second deals with the quality of a manufactured product that is being sold to the public (4)

1.3.3 Reference materials and reference methods:-

ISO defines the term reference material: material or substance one or more of whose property values are sufficiently homogenous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials. It is characteristic for primary reference materials that they are highly purified chemicals and that they can be directly weighed or measured to produce a solution whose concentration is exactly known (5,6).

The term reference method is defined as: thoroughly investigation measurement procedure, clearly and exactly describing the necessary conditions and procedures, for the measurement of one or more property values that has been shown to have trueness of measurement and precision of measurements in accordance with its intended use and that can therefore be used to access accuracy of other measurement procedure for the same properties, particularly in permitting the characterization of a reference

1.3.4 Requirements set for manufacturers and products:-

The devices must be designated and manufactured in such away that they are suitable for the purpose, as specific by the manufacturer. They must achieve the performance; in particular, where appropriate in terms of analytical sensitivity, diagnostic sensitivity, analytical specificity, diagnostic specificity, accuracy, repeatability, reproducibility, including control of known relevant interference and limits of detection stated by the manufacturer (7) .The objective of IVDs design is to produce medically useful results.

Manufacturers conforming to ISO quality system standards must follow a disciplined design control when developing a new IVD system (8, 9, 10).

Design control has five general steps (11):-

Define user requirements

Translate user requirements into design specifications.

Design and develop the product to satisfy the requirements and design specifications.

Verify the product meets the specifications.

Validate the product by demonstrating that the user requirements are met.

The prime objective of design control in the development of medical devices is to deliver the product to market economically and have to perform safety and effectively for its intended use (12, 13).

1.4 Evaluation and expression of measurement quality:-

1.4.1 The assessment of analytical methods:

For routine use, a method with adequate performance should be selected, as a poor method is unlikely to give satisfactory results even with elaborate quality control procedures. The reliability and practicability characteristics are important and knowledge at these comes from individual

assessment and collective experience over a period, preferably using standardized procedures after a period of initial familiarization.

1.4.2 Analytical performance:-

From the medical laboratory perspective, information on the reliability of results is necessary for several reasons. First, a laboratory professional has to evaluate the fulfillment of quality goals in method validation, establishing of IQC or in running daily quality control (14,15,16) .Secondly, it is important that the result of a measurement is accompanied with information of the error or uncertainty (17,18,19) . Thirdly, the competence of the laboratory may be, and is often judged against the analytical performance in EQA (20) .Or third party assessment according available international standards (21, 22).

1.4.3 Method description:

The aim when selecting a method is to choose the method that has the best chance of achieving the laboratory service requirement. The process of selection consists of defining those requirement, searching the technical literature to survey information about available methods, then selecting the method information about available methods, then selecting the methods whose characteristics best satisfy the laboratory's service requirements.

In general the characteristics of method can be divided into:

- **Application characteristics**: Are factors that determine whether a method can be implemented in a particular laboratory situation .They consist of cost per test, type of specimens that can be analyzed, sample volume, turn around time, work load, equipment and personnel requirements, space, portability and safety consideration.

- **Methodology characteristics**: Are factors which, in principle, should contribute to best performance in general, these are concerned with the analytical sensitivity and analytical specificity of the method of analysis. They consider the choice of chemical reaction, optimization of reaction conditions, principle of standardization and calibration, and the rigor of the analytical procedure.
- **Performance characteristics**: Are factors which, in practice, demonstrate how well a method performs. These include the working range, precision, recovery, interference, accuracy and sometimes detection limit.

1.4.4 Method validation:-

The evaluation process is confirmation by examination and provision of objective evidence that the particular requirement for a specific intended use are fulfilled (23).

A process very close to validation i.e. “verification” is performed when a laboratory wishes to confirm that specified requirements have been fulfilled. In laboratory medicine, validation can be understood as an adequate examination of laboratory or POCT method of measurement intended for a clinical investigation i.e. monitoring or diagnosis. Clinical laboratory professionals meet the need for selection and evaluation of either new or modified methods recurring. At the time, standardized and non standardized methods shall be covered (24, 12).

The outlines of validation (and verification) shall consist of :(25.26)

-Planning, timing, and follow-up.

-Performance according to reasonable schemes.

-Documentation.

-Reporting.

-Acceptance.

1.4.4.1 The linearity or reportable range experiment:

-Purpose:

It's important to assess the useful analytical range of laboratory methods i.e. the lowest and highest test results that are reliable and can be reported. Manufacturers make claims for the reportable range of their method by make claims by stating the upper and lower limits of the range (23).

-Factors to consider:

Linearity experiment use to check the reportable range for a test in this experiment a laboratory will analyze a series of samples with known concentrations or a series of diluted samples.

-Number of levels:-

The national committee for clinical laboratory standards (NCCLS) recommends a minimum of at least 4 and preferably 5 different levels or concentration (27).

1.4.4.2 The detection limits experiment:

-Purpose:

The detection limit experiment is intended to estimate the lowest concentration of an analyte that can be measured.

-Factors to consider:

Two different kinds of sample are generally prepared- one sample is a "blank" that has a zero concentration of the analyte of the interest the second is a spiked sample that has a low concentration of the analyte

concentration of the analyte of interest. Both the blank and the spiked samples are measured repeatedly in a replication type of experiment, then the means and SDS are usually calculated from the values observed for the samples- different estimates of detection limit may be calculated from the data on blank and spiked samples.

-Blank solution:

One aliquot of the blank solution is typically used for the blank and another aliquot is used to prepare the spiked sample, ideally the blank solution should have the same matrix as the regular patient samples. However it's common to use the, zero standard, from a series of calibrators as the blank and the lowest standard as the spiked sample.

-Spiked sample:

In validating the performance of a method, the amount of analyte added to the blank solution should represent the detection concentration claimed by the manufacturer; prepare several spiked samples whose concentrations are in the analytical range of the expected detection limits.

-Number of replicate measurements:

20 replicates measurements are usually recommended in the manufactures often recommend 10 measurements in their verification protocols to minimize cos

2.1 Rationale

Many diagnostic Reagent Kits are produced worldwide by different companies, using different technologies, and variation in the test results are possibly detected when the investigators use more than one reagent kit, or results may vary from a laboratory to another. So, it is very important to check the best stable, linear, sensitive, specific and efficient diagnostic reagent kits.

The reagent kits from BioSystem and linear have been chosen for this study because to my knowledge they are predominantly used by the Federal Hospital Laboratories according to a survey of external quality assessment conducted by laboratory of quality assurance relative to the general directories of laboratory and researches, relative to federal Ministry of health.

And the reagent for glucose, urea and creatinine have been targeted because they are among the most basic and routine parameters investigated by most laboratories and that many laboratories are still facing problems in achieving reliable results when they are estimating them.

2.2 Objectives

2.2.1 General objective:

To identify which of the two companies, BioSystems and linear S. L., has products of the best quality. So, that they will have the priority for use to estimate glucose, urea and Creatininen in Sudan.

2.2.2 Specific objectives:

1- To evaluate the linearity and sensitivity of the reagent kits.

2- To compare the results obtained by the two kits in relation to quality control sera results.

Chapter two

Materials and Methods

3.1. Study approach:

Quentitative approach to choose which of the two companies, BioSystem and Linear; produce the best reagents kits for determination of glucose, urea and creatinine.

3.2. Study design:

Descriptive cross sectional.

3.3. Study population

Glucose, urea and creatinine measurement using reagents produced by linear and BioSysem companies.

3.4. Data analysis:

Data analysis by SPSS.

3.5. Study variables:

3.5.1. Linearity:

Linearity of the reagent is the highest level of concentration of an analyte at which that reagent can provide an accurate result.

3.5.2. Sensitivity:

Sensitivity is defined as the ability of diagnostic kits to detect small quantities of the measured component.

Also it is a measure of the incidence of positive results in patients known to have a condition is true positive (TP).

Linear

Glucose:-

Enzymatic colorimetric methods:

Endpoint:

Principle:

In the tinder reaction the glucose is oxidized to D-gluconate by the glucose oxidase (GOD) with the formation of hydrogen peroxide. In the presence of peroxidase (POP), a mixture of phenol and 4- aminoantipyrine (4.AA) is oxidized by hydrogen peroxide to from a red quaineimine dye proprofional to the concentration of glucose in the sample.

B-D glucose +H2 +O2 GOD D.Gluconate +H2O2

4- AA+phenol H2O2/POD quainoneimine + H2O

Reagent composition:

R1:

Monoreagent: phosphate buffer 100mmol/L PH 7.5 glucose oxidase 716 KUL, peroxidase 72KU/L, 4. aminoantipyrine 0.5mmol/L, phenol 5 mmol/L.

CAL glucose standard:

Glucose 100mg/dl (5.5mmol/l) organic matrix based primary standard traceable to SRM 914a and 909.

Reagent preparation:

The mono reagent and the standard are ready to use.

Samples:

Serum or heparin plasma free of hemolysis.

Storage and stability:

Store of 2-8℃.

The monoreagent and standard are stable until the expiry date stated on the label.

Interferences:

- Highly icteric or lipemic samples require blank correction.

Procedure:

1) Bring reagents and samples to room temperature.

2) Pipette into labeled tubes:

Tubes	*Blank*	*Sample*	*Standard*
Monoreagent	*1.0ml*	*1.0mL*	*1.0mL*
Sample	-	*10µl*	-
Standard	-	-	*10µL*

3- Mix and let stand the tubes 10minutes at room temperature or 5 minutes of 37℃.

4- Read the absorbance (A) of the samples and the standard at 500nm against the reagent blank.

The color is stable for about 2 hours protected from light.

Calculations:

$\frac{\text{Sample}}{\text{Standard}}$ X concentration of standard = mg/dl glucose.

Linearity:

up to 400mg/dl.

Sensitivity:

1mg of glucose will produce a net absorbance of approximately 0.004.

Urea:

Enzymatic colorimetric method

End point:

Principle:

Urea is hydrolyzed by urease into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield blue cromophore.

Urea + H2O urease 2NH3 +CO2

NH4 + salycilate +Naco nitroprusside indophenol +Nacl

OH

Reagent composition:

R1: Enzyme reagent:

Urease 7500u/ml stabilizers.

R2 Buffered chromogen:

Phosphate buffer 20mol/L PH 6.9.

EDTA 2mmol/L, sodium salycilate 60mmol/L sodium nitroprusside 3.4 mmol/L.

R3 Alkaline hypochlorite:

Sodium hypochlorite 10mmol/L, NaOH 150mmol/L

CAL Urea Standard:

Urea 50 mg/dl.

- ***Storage and stability:***
    - Store of 2-8C.

- ***Reagent preparation***

  Working reagent mix 1volume of R1 +24 volumes of R2 stable for 4 weeks at 2-8C and for 7 days at 15-25C.

Samples:

- Serum or heparinized plasma free of hemolysis and urine.
- interference:
- Bilirubin < 20mg/dl and triglycerides <109/L.

Procedure:

1- Pipette:

Tubes	*Blank*	*Sample*	*Standard*
Working reagent	*1.0 ml*	*1.0 ml*	*1.0 ml*
Sample	-	*1.0 µl*	
Standard	-	-	*10µL*

Mix and incubate for 5minutes of 37C or for 10minutes at room temperature (16-25C).

Pipette:

R3 1.0 ml 1.0 ml 1.0 ml.

Mix and incubate for 5 minutes at 37C or for 10mintes at room temperature (10-25C).

Linearity:

up to 300mg/dl.

Sensitivity:

1mg of urea will produce absorbance of 0.002.

Creatinine:

Kinetic colorimetric method.

Fixed time.

Principle:

This procedure is based upon a modification of the original picrate reaction (Jaffe) creatinine under alkaline conditions reacts with picrate 10ns forming a reddish complex.

Creatinine + picric acid PH>12 Red addition complex

37C

Reagent composition:

R1 picric acid.

R2 Alkaline buffer.

CAL creatinine standard.

- ***Storage:***

  Store at 15-30C.

- ***Reagent preparation:***

  Working reagent : mix volume of R1 +1 volume of R2 stable for I week at room temperature.

Samples:

Serum or heparinized plasma and urine.

Procedure:

1- pipette into acuvette.

Working reagent 1.0 ml.

Sample or standard 100µL.

2- Mix – insert curette into the instrument.

3- Record absorbance at 500nm after 30 seconds (A1) and after 90 seconds (A2).

Calculation:

$$\frac{A2 - A1 \text{ sample}}{A2 - A1 \text{ standard}} \times \text{concentration of standard}$$

Linearity:

up to 20mg/dl.

Sensitivity :

1mg of creatinine read ΔA/min 0.020.

BioSystems

Glucose:

Principle:

Glucose in sample originals by means of the coupled reactions described below.

Glucose oxidase:

Glucose + ½ O2 +H2O Glucose +H2O2.

Peroxidase.

2H2O2 + 4 aminoantipyrine +phenol quinoneimine +4H2O.

Reagent Composition:

A Reagent:

phosphate 100mmol/L , Phenol 6mmol/L, glucose oxidase 710U/ml , peroxidase 7/U/ml, 4- aminoantipyrine 0.4mol/L , PH 7.5

- S Glucose standard 100mg/dl.

Storage:

Store at 2-8C.

Reagent preparation:

ready to use.

Samples :

Serum or plasma.

Procedure:

Tubes	*Blank*	*Sample*	*Standard*
Reagent A	*1.0 ml*	*1.0 ml*	*1.0 ml*
Standard	-	-	*10µL*

Sample	-	*10µL*	-

Mix incubate for 10minutes at room temperature (16-25C) of for 5mintes at 37C.

Measure the absorbance (H) of sample and standard at 500nm.

Linearity:

500mg/dl = 27.7mmol/l.

Detection limit:

0.23mg/dl = 0.0126mmol/L.

Interference:

Hemoglobin (>3g/L) ,lipemia (triglycerides > 1.25g/L) and bilirubin (10mg/dl).

Urea/Bun Color:

Urease / salicylate.

Principle:

Urea in the sample consumes, by means of the coupled reactions coloured complex that can be measured by spectrophotometery.

Urea + H2O Urease 2NH4+CO2.

NH4 + salicylate +Nacio nitroprusside indphenol.

Reagent Composition:

A1 reagent:

sodium salicylate 62 mmol/L sodium nitroprusside 3.4mmol/L, phosphate buffer 20mmol/L PH60.

A2 reagent:

urease 7500 U/ML.

B Reagent hypochloride 7mmol/L sodium hydroxide 150mmol/L.

S Urea standard 50mg/dl

- ***Storage:***

Store at 2-8C.

- ***reagent preparation.***

Reagent B and standard (S) ready to use.

Reagent A: transfer the contents of one A2 vial into A1 bottle.

Sample:

Serum plasma or urine.

Procedure:

	Blank	***Standard***	***Sample***
Urea standard	-	***10µL***	-
Sample	-	-	***10µL***
Reagent A	***1.0 ml***	***1.0 ml***	***1.0 ml***

Mix incubator for 10minutes at room temperature (16-25C) or for 5minutes at 37C read the absorbance at 600nm

Linearity limit:

300mg/dl.

Detection limit:

1.3mg/dl.

Interference:

Lipemia (Triglycerides

10g/L). hemolysis

Ceatinine:

Alkaline picrate:

Principle:

Creatinine in the sample reacts with picrate in alkaline medium forming acoloured complex.

Reagent Composition.

A. reagent sodium hydroxide 0.4 mol/L.

B. reagent Picric acid 25mmol/L.

S.creatinine standard 2mg/dl.

Storage:

store at 15-30 C

Reagent preparation:

Working reagent mix equal volumes of A and B stable for 1 month at 2-8C.

Samples:

Serum , plasma or urine

Procedure:

Pipette into acuvette:

Working reagent = 1.0ml.

Standard or Sample 0.1ml.

Mix, read absorbance at500ml after 30 seconds and after 90 seconds .

Linearity limit:

20mg/dl.

Detection limit:

0.03mg/dl.

Interference:

Hemoglobin (10g/L), bilirubin (10mg/dl), and Lipemia (triglycerides >2g/L).

METHODOLOGY

Specimens:

Human plasma was pooled, and its glucose, urea and creatinine contents estimated using a fully automated chemistry analyzer (***Selectra***) and a half-automated spectrophotometer (***Microlab***). The concentrations of the three analytes were as follows:

- *Glucose: 480 mg/dl.*
- *Urea: 19mg/dl.*
- *Creatinine: 1.2mg/dl.*

Using the formular wt/gm=mw *x* concentration, dry forms of the three chemicals were, then, added to the pooled plasma to increase the concentrations to 700 mg/dl for glucose , 380mg/dl for urea and 60.4mg/dl for creatinine. The target concentrations of the three analytes were .confirmed by retesting the plasma three time and the means were calculated

Linearity:

To study the linearity of the test reagents, serial dilutions of the specimens were prepared using the following formula:

Z=A+ (N-1) D was applied to determine the differences between the concentrations, where:

Z was the highest concentration (700mg/dl)

A was the lowest concentration (0mg/dl)

D was the difference between the concentrations.

Serial concentrations of glucose were prepared as follows:

700=0+ (5-1) D.

700 = (4) D D= 700/4

D=175mg/dl.

Pool 1 = Zero.

Pool 2 is then = 175mg/dl.

Pool 3 = 175+175 = 350mg/dl.

Pool 4 = 350+175 = 525mg/dl.

Pool 5 = 525 +175 = 700mg/dl.

To prepare the glucose pools, the formula RV/O was applied, where:

R was the required concentration,

V was the required volume.

O was the original concentration.

175 = 175x0.4 / 700 = 0.1ml.

0.1ml was taken from pool 5 and added to 0.3ml from pool 1.

350 = 350x0.4/ 700 = 0.2ml.

0.2ml was taken from pool 5 and added to 0.2 ml from pool 1.

500 = 500x0.4/ 700 = 0.285ml.

0.285ml was taken from pool 5 and added to 0.114 ml from pool 1.

525 = 325x0.4/ 700 = 0.3ml.

0.3ml was taken from pool 5 and added to 0.1ml of pool1.

700 = 700x0.4 / 700 = 0.4

0.4ml is than taken from pool 5 without an addition to pool 1. Glucose concentrations were estimated to ten times for each in these pooled specimens using BioSystem and linearity glucose kits as mentioned in pages (33) & (25) the formula Z = A+ (N-1)D was applied to determine the difference between the concentrations of urea in the specimens:

380= 0 + (5-1) D, 380 = 4D, D = 380/4 = 95.

So the pools were:

0, 95, 190, 285 an 380 mg/dl.

To prepare the urea pools, the formula RV /O was applied as follows:

$$95 = \frac{95 \times 0.4}{380} = 0.1.$$

0.1 was taken from pool 5 and added to 0.3 from pool I

$$190 = \frac{190 \times 0.4}{380} = 0.2$$

0.2ml was taken from pool 5 and added to 0.2ml of pool 1

$$285 = \frac{285 \times 0.4}{380} = 0.3$$

0.3ml of pool 5 was taken and added to 0.1 of pool 1

$$300 = \frac{300 \times 0.4}{380} = 0.316$$

0.316ml of pool 5 was taken and added to 0.084 of pool 1

$$380 = \frac{380 \times 0.4}{380} = 0.4$$

0.4ml of pool 5 was taken without an addition to pool 1

Urea concentrations in these pooled specimens were estimated ten times for each using BioSystem and linear urea kits as mentioned in pages (35) , (28).

The formula Z = a + (N-1) D applied to determine the difference between the concentrations of the creatinine in the specimens:

$$60.6 = 0 + (4-1)\,d,\ 60.6 = 3d,\ D = \frac{60.6}{3} = 20.2$$

So, the pools were:
O, 20.2, 40.4, and 60.6mg/dl
To prepare the creatinine pools, the formula RV/O was applied as follows:

20.0 x2 / 60.6 = 0.660

0.660ml was taken from pool 5 and added to 1.339m of pool1

20.2 x2 / 60.6 = 0.667

0.667ml was taken from pool 5 and added to 1.333m of pool1

40.4 x2/ 60.6 =1.333

1.333 ml of was taken from pool5 and added to 0.667ml of pool 1

60.6 x2/ 60.6 =2

So, 2ml of pool 5 is taken without an addition to pool 1

Creatinine concentration in these pooled specimen were estimated ten times for each using BioSystem and linear creatinine kits as mentioned in pages (36-37) (31).

Sensitivity:

Reagents from linear SL:

a) Glucose:

It was stated by the manufacturer that 1mg glucose produces a net absorbance of proximately 0.004. A specimen of human plasma containing 50mg/dl glucose was used to prepare a 1mg/dl specimen using the formula of RV/O

$1 = \frac{1x0.5}{50}$ 0.01ml

0.01ml was taken from the specimen and added to 0.04 ml normal saline. This specimen was, then, tested 3 times for glucose concentration and it proved to contain 1mg/dl of glucose. These tests were done using glucose reagents from Randox co, Control serum was included and it proved a valid assay. This specimen was then tested ten times using glucose reagent kit from linear SL. Mean and standard deviation was calculated from the results.

b) Urea:

It is stated by the manufacturer that 1mg urea produces net absorbance of 0.002.

A specimen containing 20 mg/dl urea was used to prepare a 1mg/dl specimen using the formula RV/0

1x0.4/20 = 0.02.

0.02ml of the specimen was added to 0.02ml normal saline. This specimen was, then, tested 3 times for urea concentration and it proved to contain 1mg/dl of urea. These tests were done using urea reagents from Randox co, Control serum was included and it proved a valid assay. This specimen was then tested ten times using urea reagent kit from linear SL. Mean and standard deviation was calculated from the results.

c) Creatinine:

It is stated by the manufacturer that 1mg creatinine produces net absorbance of 0.020

A specimen containing 2 mg/dl creatinine was used to prepare a 1mg/dl specimen using the formula RV/0

1X1/2 =0.5.

0.5ml of the specimen was added to 0.5ml normal saline. This specimen was, then, tested 3 times for creatinine concentration and it proved to contain 1mg/dl of creatinine. These tests were done using creatinine reagents from Randox co, Control serum was included and it proved a valid assay. This specimen was then tested ten times using creatinine reagent kit from linear SL. Mean and standard deviation was calculated from the results.

Reagents from BioSystem:

a)glucose:

It was stated by the manufacturer that the minimum glucose concentration readable by its glucose reagent kit is 0.23mg/dl.

Specimen containg 100mg/dl glucose was used to prepare 0.23m/dl specimen using the formula RV/O.

0.23 = 0.23 x10/100 = 0.023.

0.023ml of the specimen was added to 9.977ml normal saline. This specimen was, then, tested 3 times for glucose concentration and it proved to contain 0.23mg/dl of glucose. These tests were done using glucose reagents from Randox co, Control serum was included and it proved a valid assay. This specimen was then tested ten times using glucose reagent kit from BioSystem. Mean and standard deviation was calculated from the results.

b)Urea:

It was stated by the manufacturer that the minimum urea concentration readable by its urea reagent kit is 1.3 mg/dl.

Specimen contain 20mg/dl urea was used to prepare 1.3 m/dl specimen using the formula RV/O.

1.3= 1.3 x2/20 = 0.13.

0.13ml of the specimen was added to 1.87ml normal saline .This specimen was, then, tested 3 times for urea concentration and it proved to contain 1.3mg/dl of urea. These tests were done using urea reagents from Randox co, Control serum was included and it proved a valid assay. This specimen was then tested ten times using urea reagent kit from BioSystem. Mean and standard deviation was calculated from the results.

c)Creatinine:

It was stated by the manufacturer that the minimum creatinine concentration readable by its creatinine reagent kit is 0.03mg/dl.

Specimen contain 2mg/dl creatinine was used to prepare 0.03m/dl specimen using the formula RV/O.

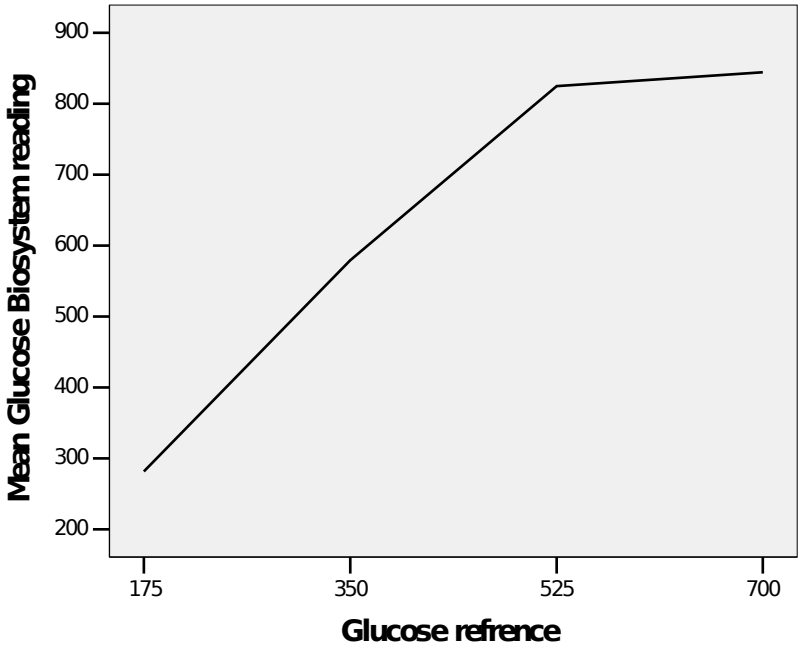
0.03 = 0.03 x10/2 = 0.15.

0.15ml of the specimen was added to 1.85ml normal saline. This specimen was, then, tested 3 times for creatinine concentration and it proved to contain 0.03mg/dl of creatinine. These tests were done using creatinine reagents from Randox co, Control serum was included and it proved a valid assay. This specimen was then tested ten times using creatinine reagent kit from BioSystem. Mean and standard deviation was calculated from the results.

Chapter three

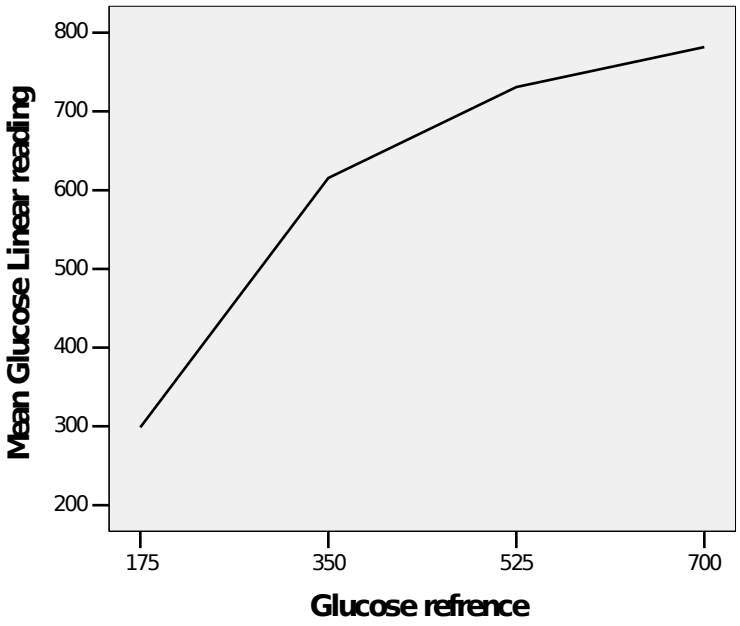
II

Result analysis



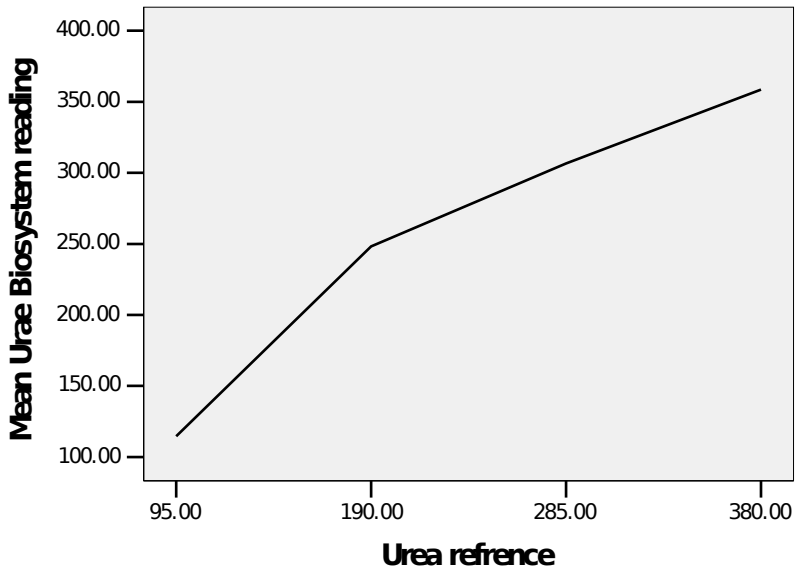
Curve 1

The curve shows the linearity of glucose by BioSystems



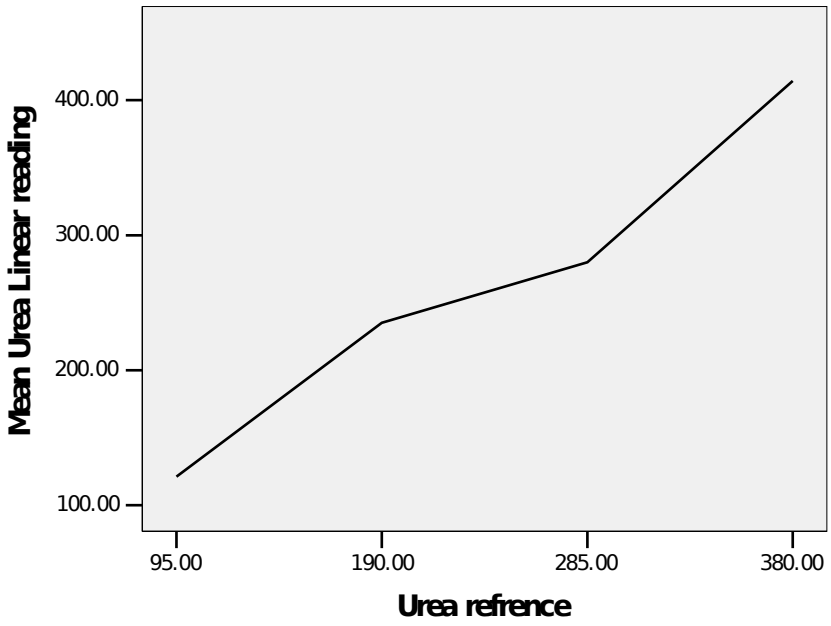
Curve 2

The curve show the linearity of glucose by linear



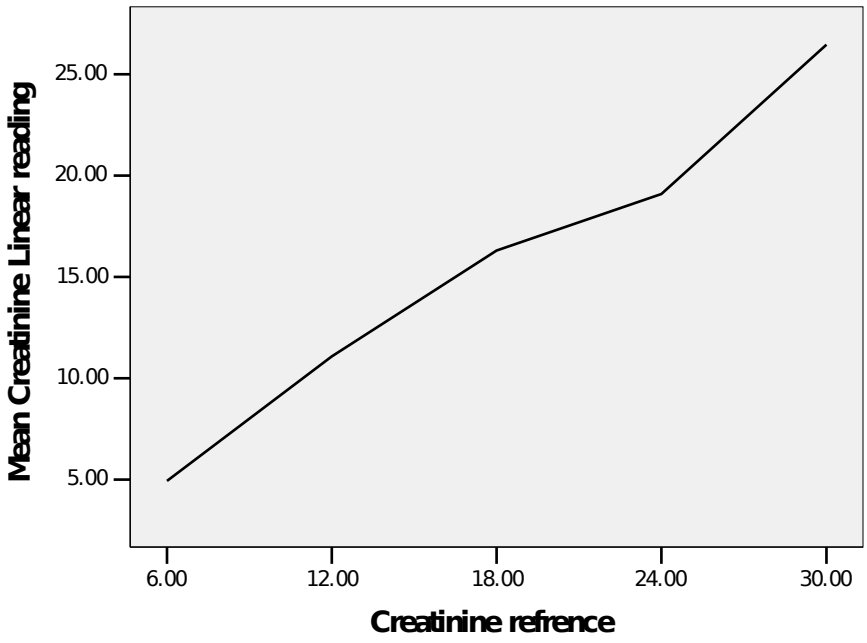
Curve 3

The curve show the linearity of urea by BioSystems



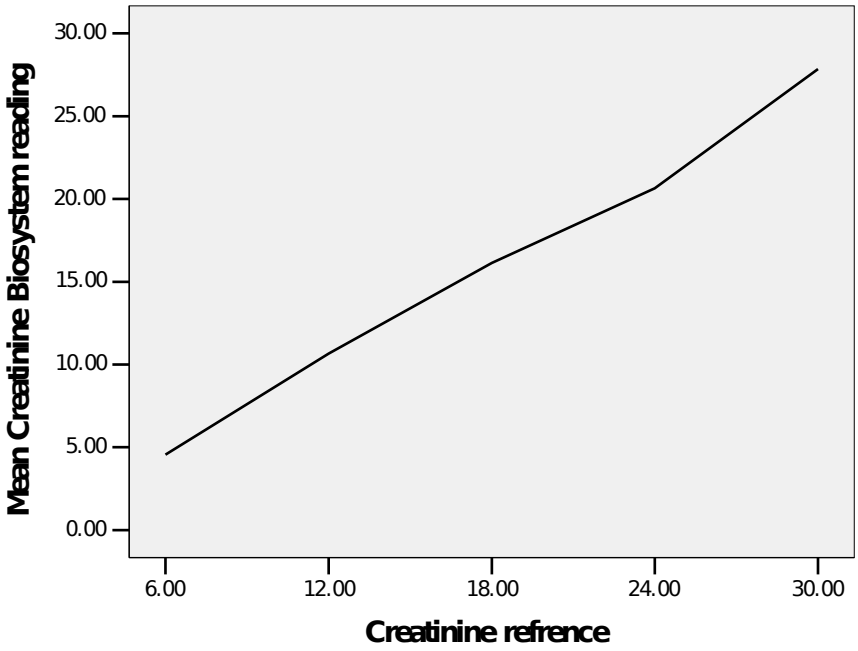
Curve 4

The curve show the linearity of urea by linear



Curve 5

The curve show the linearity of creatinine by linear



Curve 6

The curve show the linearity of creatinine by BioSystems

Chapter four

Discussion of the Results

Discussion

Linearity:

The results showed that the linearity of glucose reagents produced by Linear SL is 620mg/dl while the linearity of glucose reagents produced by BioSystems is 550mg/dl. This indicates that the linearity of Linear reagents is better than that of BioSystems reagents.

While the urea results showed that the linearity of urea reagents produced by Linear SL is 230mg/dL and the linearity of urea reagents produced by BioSystems is 259mg/dl. This indicates that the linearity of BioSystems' reagents is better than that of Linear SL reagents.

The results of creatinine showed that the linearity of creatinine reagents produced by Linear SL is 11mg/dl while the linearity of creatinine reagents produced by BioSystems is 11mg/dl. This indicates that the linearity of creatinine reagents produced by both companies is the same.

Senstivity:

Glucose reagents produced by Linear SL was supposed to be as sensitive as 1 mg/dl which produce an absorbance of 0.004. By testing the reagents produced 0.0025, which shows higher sensitivity than that stated by the manufacturer.

Glucose reagents produced by BioSystems were supposed to have 0.24 mg/dl sensitivity. By testing the reagents, they produced a sensitivity of 0.008mg/dl which proved higher sensitivity than that stated by the manufacturer.

By comparing the glucose reagents produced by the two companies, BioSystems' reagents are of higher sensitivity than that of reagents produced by Linear SL.

Urea reagents produced by Linear SL was supposed to be as sensitive as 1mg/dl which produce an absorbance of 0.002. By testing the reagents, the absorbance was 0.0015 which proved to show higher sensitivity.

Urea reagents produced by BioSystems were supposed to have 1.3 mg/dl sensitivity. By testing the reagents, they proved to show 1.007 mg/dl which was highly sensitive than that stated by the manufacturer.

By comparing the urea reagents produced by the two companies, Linear SL reagents are of higher sensitivity than that of reagents produced by BioSystems

Creatinine reagents produced by Linear SL were supposed to be as sensitive as 1mg/dl which produce an absorbance of 0.020. By testing the reagents, the absorbance was 0.06 which proved lower sensitivity.

Creatinine reagents produced by BioSystems were supposed to have 0.03 mg/dl sensitivity. By testing the reagents, they produced 0.005 mg/dl which was highly sensitive than that stated by the manufacturer.

By comparing the creatinine reagents produced by the two companies, BioSystems reagents were of higher sensitivity than that of reagents produced by Linear SL.

Conclusion

By comparing the reagents from the two companies on base of linearity, Linear SL glucose reagents were of the highest linearity, urea reagents from BioSystems were of the highest linearity, while creatinine reagents from both companies where of equal linearity.

On base of sensitivity, BioSystems' glucose and creatinine reagents were the most sensitive, Linear SL urea reagents were of the best sensitivity.

Recommendations

This study showed the need for further studies targeted to the whole method validation through which the factors affecting the quality of the reagents used to estimate blood glucose, urea and creatinine in blood.

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