بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Assessment of renal funtion tests among patients with rheumatoid arthritis in Khartoum state.

تقييم اختبارات وظائف الكلى بين المرضى الذين يعانون من التهابات المفاصل الروماتويدي في ولاية الخرطوم

A Dissertation submitted in Partial Fulfillment for the requirement of M.Sc Degree in Medical Laboratory Science (Clinical Chemistry)

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قال تعالي :

(قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيم)

صدق الله العظيم

سورة البقرة(32)

Dedication

To my sister pure soul Israa……. The strongest, bravest person I have ever known

We missed you beyond measure, may Allah have mercy on you and makeup for your youth in paradis

To my mother …….. I owe you everything

Acknowledgements

First of all, I thank God for all the beneficent and most merciful.

I am very thankful to my supervisor

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Abstract

Background:

Rheumatoid arthritis patients had higher risk of developing chronic kidney disease and glomerulonephritis which increase mortality with a hazard ratio (HR) of 2.77-4.45.

Objective:

The aim of this study is to assess urea ,creatinine , sodium and potassium levels in rheumatoid arthritis patients .

Materials of Methods: This comperative study was conducted at Military Hospital in Khartoum State, the study carried out on a total sample of 100 individual s including 50 with Rheumatoid arthritis patients as case and 50 healthy individuals as control group. Serum urea and creatinine levels were estimated using spectrophotometer biosystem-310, analyze. Sodium and potassium were estimated by easy light. Data analyzed were using SPSS software program version 20.

Results: This study showed that, the levels of urea and creatinine were significantly increased in rheumatoid arthritis patients with (43.64± 15.78 versus 26.86±7.65 mg/dl, P value = 0.000) for urea and (1.31±0.795 versus 0.736±0.179 mg/dl , P value = 0.000) for creatinine, while there was no difference in level of sodium (136.9±18.31 versus 140.1±4.36 mmol/l with P value 0.214) and potassium(3.96±0.556versus 3.95±0.42mmol\l with p.value0.785).

There was modrate positive correlation between urea levels with age (R 0.644, P. value 0.000). While there were no correlation between creatinine, Na and K with age (R -0.042, P value= 0.773. R 0.034, P value= 0.813.R -0.069, P value =0.632 respectively). Also, there were no correlation between urea , creatinine Na and K with duration (R 0.003, P value= 0.986.R 0.094,

P value= 0.515.R 0.147, P value= 0.310 .R 0.072, P value= 0.620 respectively).

In Conclusion:

From the results and finding of this study, it is concluded that:

The serum levels of urea and creatinine are higher in Rheumatoid arthritis patients, and there are no observed difference in Na and K concentratin in Rheumatoid arthritis patients and the increase of urea levels is correlated directly with age.

المستخلص

الخلفية : مرضى التهاب المفاصل الرماتويدي لدي خطورة عالية للاصابة بمرض الكلى المزمن والتهاب كبيبات الكلي مما يزيد معدل الوفيات بنسبة خطر من 2.77-4.

الاهداف : الغاية من هذه الدراسه تقييم مستويات اليوريا و الكرياتينين والصوديوم والبوتاسيوم لدى مرضى التهاب المفاصل الروماتويدي في ولاية الخرطوم.

الطريقه : اجريت هذه الدراسه في مستشفى السلاح الطبي في ولاية الخرطوم. تم تنفيذها علي عينه اجماليه من 100 شخص تشتمل علي 50 شخص مصابون بداء التهاب المفاصل الرماتويدي و 50 شخص معافى كمجموعة تحكم معدلات اليوريا والكرياتينين والصوديوم والبوتاسيوم تم قياسها بإستخدام جهاز سبكتروفوتوميتر. تم تحليل البيانات بإستخدام برنامج التحليل الاحصائي SPSS نسخة 20 .

النتائج : هذه الدراسة اظهرت ان معدلات اليوريا والكرياتينين لدى مرضى التهاب المفاصل الروماتويدي متزايده بشكل كبير مقارنه مع الاشخاص المعافيين بينما لا يوجد اختلاف في معدلات الصوديوم والبوتاسيوم
(43.64±مقابل 15.86 ± 7.65(مليغرام/ديسليتر)قيمة P=(0.000) بالنسبه لليوريا و (1.31±0.795 مقابل 7.36 ±0.179ميكرومول/ ديسليتر)قيمة P =(0.000)بالنسبه لليكرياتينين و(140.1 40.36± مقابل 136.9 ±18.31ميمول/ليتر) قيمة P =(0.214)بالنسبه للصوديوم و (3.96 +/- 0.556 مقابل 3.95 ± 0.42 مليمول / ليتر) قيمة P= (0.785) بالنسبة للبوتاسيوم . كان يوجد ترابط ايجابي معتدل بين معدلات اليوريا والعمر(R 0.644 قيمه P= 0.000). بينما لا يوجد ترابط ايجابي بين معدلات الكرياتينين والصوديوم والبوتاسيوم مع العمر (R 0.042 قيمة P=0.773 , R 0.034 قيمة P=0.813, R 0.069 قيمة P =0.632) علي التوالي..

ايضا لا يوجد ترابط ايجابي بين معدلات اليوريا والكرياتينين و الصوديوم والبوتاسيوم مع مدة المرض (0.003 R قيمة P =0.986 ,0.094 R قيمة P =0.515 , 0.0147 R قيمة P =0.0310 , 0.072 R قيمة P =0.620) علي التوالي.

الخلاصة:

من نتائج هذه الدراسة استخلصت ان معدلات اليوريا والكرياتينين مرتفعة لدى مرضى التهاب المفاصل الروماتويدي ولا يوجد اختلاف في معدلات الصوديوم والبوتاسيوم ويوجد ارتباط ايجابي بين ارتفاع معدلات اليوريا والعمر.

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List of Abbreviations

Abbreviations	Meaning
ACPA	Anti-citrullinated protein antibodies
ANCA	Anti-neutrophil cytoplasmic antibody
ADH	Antidiuretic hormone
CKD	Chronic kidney disease
ECF	Extracellular Fluid
GI	Gastrointestinal
GFR	Glomerular Filtration Rate
HR	Hazard Ratio
HLA-DR4	Human Leukocyte Antigen
IgM	Class of Immunoglobulins
IgG	Class of Immunoglobulins
ISE	Ion Selective Electrod
MHC	Major Histocompatibility Complex
MSPGN	Membranoproliferative Glomerulonephritis
PSS	Progressive Systemic Sclerosis
RTA	Renal Tubular Acidosis
RPGN	Rapidly Progressive Glomerulonepheitis
RA	Rheumatiod Arthritis
SRC SSC	Scleroderma Renal Crisis Systemic Sclerosis
TIN	Tubulointerstitial Nephritis

Chapter one

Introduction

Rationale

Objective

Chapter One

1.1 Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder, which causes chronic inflammation of the joints. Autoimmune diseases are illnesses that occur when the body's tissues are mistakenly attacked by their own immune system. The immune system contains a complex organization of cells and antibodies designed normally to "seek and destroy" invaders of the body, particularly infections. Patients with autoimmune diseases have antibodies and immune cells in their blood that target their own body tissues, where they can be associated with inflammation. While inflammation of the tissue around the joints and inflammatory arthritis are characteristic features of rheumatoid arthritis, the disease can also cause symptoms of RA. (Hill *etal*.,2009)

The cause of rheumatoid arthritis is believed to be a combination of genetic and environmental factors. Family history of rheumatoid arthritis increases the risk around three to five times.Smoking,Caucasian population,increasing risk of rheumatoid arthritis.Periodontal disease has been associated with rheumatoid arthritis though infectious agents such as viruses, bacteria, and fungi have long been suspected. The disease progresses as phase1,non – specific inflammation to phase 2 ,amplification in the synovium to phase 3 or chronic inflammation *(Murtaza etal ., 2018)*. Rheumatic disease and kidney disease are both common in the general population. Rheumatologist are thus frequently exposed to patient with concomitant renal disease. In fact 18% of rheumatology clinic patients were reported to have a glomerular filtration rate (GFR) of 60 ml/minute or less as compared with the 5% reported within the general population. When patients present with both,

arthritis and kidney disease, the following question have to be addressed. Is kidney disease a complication of rhematic disease or its management, or are they both manifestation of asingle systemic auto-immune disease. The present review addresses these question and may help attending specialists, either rheumatologists or nephrologists,to manage patients with concomitant rheumatic disease and kidney disease (Hill *etal 2009).*

There is study done by (Anders *etal*.,2011),they found renal disease is common in patients with rheumatoid arthritis based on regular assessment of serum and urine parameters of renal function and concluded that, patients with rheumatoid arthritis must be routinely monitored by blood and urinary parameters for concomitant chronic kidney disease(CKD).

1.2 Rationale:

Rheumatoid arthritis is a chronic inflammatory autoimmune disease that affects many body tissues and lead to joint destruction and other morbidity and mortality .In particular considerable incidence of renal disease. Renal disease in rheumatoid arthritis is clinically important because it not only restricts the management of primary disease, but also increase mortality. With a hazard ratio(HR) of 2.77-4.45. This study will lead to open new gates for studying the effect of rheumatoid arthritis in kidney function to avoid complication of kidney disease and decrease mortality.

1.3 Objectives

1.3.1 General Objective:

To assess the level of urea, creatinine , Na and K in rheumatoid arthritis patients.

1.3.2 Specific objectives:

1-To measure the levels of urea, creatinine , Na and k in rheumatoid arthritis patients compare to control qroup.

2-To correlate between biochemical parameters and study variables(Age, duration).

Chapter two

Literature review

Chapter Two

2. Literature review

2.1 Rheumatoid arthritis (RA):

is an autoimmune disease that results in a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated. (Hill e*tal.*,2009).

The process involves an inflammatory response of the capsule around the joints (synovium) secondary to swelling (turgescence) of synovial cells, excess synovial fluid, and the development of fibrous tissue (pannus) in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis (fusion) of the joints. RA can also produce diffuse inflammation in the lungs, the membrane around the heart (pericardium), the membranes of the lung (pleura), and white of the eye (sclera), and also nodular lesions, most common in subcutaneous tissue. Although the cause of RA is unknown, autoimmunity plays a big part, and RA is a systemic autoimmune disease. It is a clinical diagnosis made on the basis of symptoms, physical exam, radiographs and labs. (Salesi *etal*.,2009).

About 0.6% of the United States adult population has RA, women two to three times as often as men (Salesi *etal*.,2009).

The name is based on the term "rheumatic fever", an illness which includes joint pain and is derived from the Greek word -rheuma (nom.), rheumatos (gen.) ("flow, current"),The first recognized description of RA was made in 1800 by Dr. Augustin Jacob Landré-Beauvais (1772–1840) of Paris. (Salesi *etal*.,2009)

2.1.1 Signs and symptoms of rheumatoid arthritis: RA primarily affects joints, however it also affects other organs in 15–25% of individuals Arthritis of joints involves inflammation of the synovial membrane. Joints become swollen, tender and warm, and stiffness limits their movement. With time, multiple joints are affected (it is a polyarthritis). Most commonly involved are the small joints of the hands, feet and cervical spine, but larger joints like the shoulder and knee can also be involved. Synovitis can lead to tethering of tissue with loss of movement and erosion of the joint surface causing deformity and loss of function. (leung *etal.*,2007).

RA typically manifests with signs of inflammation, with the affected joints being swollen, warm, painful and stiff, particularly early in the morning on waking or following prolonged inactivity. Increased stiffness early in the morning is often a prominent feature of the disease and typically lasts for more than an hour. Gentle movements may relieve symptoms in early stages of the disease. These signs help distinguish rheumatoid from non-inflammatory problems of the joints, often referred to as osteoarthritis or "wear-and-tear" arthritis. In arthritis of non- inflammatory causes, signs of inflammation and early morning stiffness are less prominent with stiffness typically less than one hour, and movements induce pain caused by mechanical arthritis. The pain associated with RA is induced at the site of inflammation and classified as nociceptive as opposed to neuropathic. (Koseki *etal.*,2015)

The joints are often affected in a fairly symmetrical fashion, although this is not specific, and the initial presentation may be asymmetrical, the pathology progresses the inflammatory activity leads to tendon tethering and erosion and destruction of the joint surface, which impairs range of

movement and leads to deformity. The fingers may suffer from almost any deformity depending on which joints are most involved. Specific deformities, which also occur in osteoarthritis, include ulnar deviation. (Karie *etal.*,2008)

Fibrosis of the lungs is a recognized response to rheumatoid disease. It is also a rare but well recognized consequence of therapy (for example with methotrexate and leflunomide). Caplan's syndrome describes lung nodules in individuals with

RA and additional exposure to coal dust. Pleural effusions are also associated with RA. Another complication of RA is Rheumatoid Lung Disease. It is estimated that about one quarter of Americans with RA develop Rheumatoid Lung. (Helin *etal.*,2005).

RA may affect the kidney glomerulus directly through a vasculopathy or a mesangial infiltrate but this is less well documented (though this is not surprising, considering immune complex-mediated hypersensitivities are known for pathogenic deposition of immune complexes in organs where blood is filtered at high pressure to form other fluids, such as urine and synovial fluid. (Nakano *etal.*,2008).

People with RA are more prone to atherosclerosis, and risk of myocardial infarction (heart attack) and stroke is markedly increased. Other possible complications that may arise include: pericarditis, endocarditis, left ventricular failure, valvulitis and fibrosis. (Immonen *etal.*,2009).

2.1.2 Causes of rheumatoid arthritis:

RA is a form of autoimmunity, the causes of which are still not completely known. It is a systemic (whole body) disorder principally affecting synovial tissues. There is no evidence that physical and emotional effects or stress

could be a trigger for the disease. The many negative findings suggest that either the trigger varies, or that it might in fact be a chance event inherent with the immune response, Half of the risk for RA is believed to be genetic, It is strongly associated with the inherited tissue type major histocompatibility complex (MHC) antigen HLA-DR4.

Smoking is the most significant non-genetic risk. the RA being up to three times more common in smokers than non-smokers, particularly in men, heavy smokers, and those who are rheumatoid factor positive. (Scott *etal.*,2002)

Epidemiological studies have confirmed a potential association between RA and two herpes virus infections, Epstein-Barr virus and Human Herpes Virus 6 . (Scott *etal.*,2004).

Vitamin D deficiency is common in those with RA and may be causally associated, Some trials have found a decreased risk for RA with vitamin D supplementation while others have not. (Sihvonen *etal.*,2004).

2.1.3 Pathogenesis of rheumatoid arthritis:

Once the abnormal immune response has become established (which may take several years before any symptoms occur), plasma cells derived from B lymphocytes produce rheumatoid factors and ACPA of the IgG and IgM classes in large quantities. These are not deposited in the way that they are in systemic lupus. Rather, they activate macrophages through Fc receptor and complement binding, which seems to play an important role in the intense inflammatory response present in RA. (Sihvonen *etal.*,2004).

2.2Renal Anatomy:

The kidneys are pair of bean-shaped retroperitoneal structures that are

normally located along the posterior wall of the abdominal cavity between the transverse processes of T12-L3 vertebrae, with the left kidney typically somewhat more superior in position than the right. The upper poles are normally oriented more medially and posteriorly than the lower poles. (Hill e*tal.*,2009) . The kidneys purify toxic metabolic waste products from the blood in several hundred thousand functionally independent units called nephrons. A nephron consists of one glomerulus and one double hairpin-shaped tubule that drains the filtrate into the renal pelvis. The glomeruli located in the kidney cortex are bordered by the Bowman's capsule. They are lined with parietal epithelial cells and contain the mesangial with many capillaries to filter the blood. The glomerular filtration barrier consists of endothelial cells, the glomerular basement membrane and visceral epithelial cells (also known as podocytes).

All molecules below the molecular size of albumin pass the filter and enter the tubule, which consists of the proximal convoluted tubule, the loop of Henle and the distal convoluted tubule. An intricate countercurrent system forms a high osmotic gradient in the renal medulla that concentrates the filtrate. The tubular epithelial cells reabsorb water, small proteins, amino acids, carbohydrates and electrolytes, thereby regulating plasma osmolality, extracellular volume, blood pressure and acid–base and electrolyte balance. Non-reabsorbed compounds pass from the tubular system into the collecting ducts to form urine. The space between the tubules is called the interstitium and contains most of the intrarenal immune system, which mainly consists of dendritic cells, but also of macrophages and fibroblasts. (Andres *etal*.,2011).

2.3 Renal physiology:

The kidneys serve important functions, including filtration and excretion of metabolic waste products (urea and ammonium); regulation of necessary electrolytes, fluid, and acid-base balance; and stimulation of red blood cell production. They also serve to regulate blood pressure via the renin-angiotensin. aldosterone system, controlling reabsorption of water and maintaining intravascular volume. The kidneys also reabsorb glucose and amino acids and have hormonal functions via erythropoietin, calcitriol, and vitamin D activation. (Andres *etal*.,2012).

2.4 Maintenance of Homeostasis:

The kidneys maintain the homeostasis of several important internal conditions by controlling the excretion of substances out of the body.

2.2 Urea: Urea is a nitrogen- containing compound formed in the liver as the end product of protein metabolism.About 85% of urea is eliminated via kidneys; the rest is excreted via gastrointestinal tract.Serum urea is increased in conditions where renal clearance decreased(in acute and chronic renal failure /impairment)(Weening *etal.*, 2014). Uremia,a clinical condition associated with worsening renal function, is characterized by fluid, electrolyte and hormone imbalance in addition to metabolic abnormalities.The literal meaning of uremia is “Urine in the blood” and the condition develops most commonly in the setting of chronic and end-stage renal disease,but may occur as aresult of acute kidney injury(Alenius *etal*., 2019).Urea may also increase in other conditions not related to renal disease such as upper GI bleeding, dehydration, catabolic states and high protein diets.Urea may decreased in starvation, low protein diets and severe liver disease(Christopher *etal*., 2017).

2.3: Creatinine: Creatinine is the by- product of creatine phosphate in muscle, and it is produced at a constant rate by the body.For the most part, creatinine is cleared from the blood entirely by the kidney.Decreased clearance by kidney results in an increased blood creatinine.The amount of creatinine produced per day depends on muscle bulk,and thus, there is a difference in creatinine ranges between male and female with lower creatinine values n children and those with decreased muscle bulk. Diet also influences creatinine values.Creatinine can change as much as 30% after ingestion of red meat.As GFR increases in pregnancy lower creatinine values are found in pregnancy.Additionally, serum creatinine is a later indicator of renal impairment-renal function is decreased by 50% before a rise in serum creatinine is observed.(Ginsberg *etal.,* 2013).

2.4 : Sodium: Sodium is the major cation of extracellular fluid.The mean body content of sodium in the adult male is 92 g , half of which (46g) is located in the ECF at a concentration of 135-145 mmol/l,~11g is found in the intracellular fluid at the concentration of ~10mmol\l, and ~35g is found in the skeleton.(Weening *etal*., 2014).Sodium is necessary for the body to maintain fluid balance and is critical for normal body function.It is also help to regulate nerve function and muscle contraction. Hpernatremia is serum sodium concentration more than 145mEq/l. It implies a deficit of total body water relative to total body sodium caused by water intake being less than water loss (Tebbe *etal*., 2017).Hyponatremia is decrease in serum sodium concentration less than 136mEq/l caused by an excess of water relative to solute.Commen cause include diuretic use,diarrhea,heart failure, liver disease renal disease and syndrome of inappropriate ADH secretion.(Kasitanon *etal.,* 2017).

2.5: Potassium: Potassium (K) is the most abundant intracellular cation with more than 98% of total body K located intracelluarly and less than 2% extracellulary.The steep trans-cellular Kgradient,generated in an energy-dependent (Na-K-ATPase) manner, is vital to the maintenance of cell membrane potential and multiple cellular functions. Kidneys, in response to increased serum K, aldosterone, distal renal tubular sodium(Na)delivery and tubular fluid flow,excrete 98%of dialy K intake and are the organs that play a major role in the maintenance of K homeostasis.Kidney disease inevitably lead to K derangements and increased risk of adverse cardiovascular events and mortality(Siedner *etal*.,2017). Hyperkalemia is one of the most common and life threatening electrolyte disorder in chronic kidney disease and end stage renal disease ,it becomes increasingly prevalent as CKD advances.(Siedner *etal.*,2017). Hypokalemia although equally dangerous, hypokalemia is less common in CKD patients,as impaired renal K excretion usually leads to hyperkalemia.CKD patients can, however, develop hypokalemia due to gasterointestinal K loss from diarrhea or vomiting or renal K loss from non- K- sparing diuretics (Siedner *etal*., 2018).

2.6 Relationship between kidney diseases and rheumatoid arthritis:

Kidney diseases and rheumatoid arthritis demonstrates a close relationship. They can as causative factors of each other. In particular, renal manifestation or renal involvement of rheumatoid arthritis is clinically significant because of the increase in mortality and morbidity in rheumatoid arthritis patients with renal dysfunction .

Thus, early diagnosis and proper management of renal involvement in rheumatoid arthritis may improve overall or renal prognosis of rheumatoid arthritis patients.

The clinical and histologic manifestations of renal involvement in were investigated throughout this review.

Renal involvement can be caused by anti-rheumatic drugs and rheumatic diseases. RA mainly induces glomerulonephritis, such as membranous nephropathy, mspGN,

and amyloidosis. pSS induces tubular dysfunction including TIN and RTA. In addition, ANCA-associated vasculitis mainly induces RPGN accompanied by acute renal dysfunction. SSc is a relatively rare disease, but renal involvement of SSc including SRC may be fatal in view of renal prognosis. In relatively frequent patients with gout, renal involvement may be related to hyperuricemia. In conclusion, a more effective approach for a definite diagnosis and proper care of rheumatoid arthritis can be achieved by accurately grasping the clinical characteristics of renal involvement in rheumatoid arthritis. (Seon *etal.,* 2017).

ChapterThree

Materials and metho

Chapter Three

3.Materials and methods

3.1. Materials:

3. 1.1. Study design:

Cross sectional -case control , hospital-based study.

3. 1.2. Study area:

The study was conducted at Military Hospital in Khartoum State.

3. 1.3. Study period:

The study period from April to October 2019 .

3.1.4. Study Population:

The study population intended 50 patients with Rheumatoid arthritis and 50 apparently health subjects serves as control.

Inclusion criteria: Patient with Rheumatoid arthritis and healthy subject as control were included **.**

Exclusion criteria: Infectious diseases, usage of particular medications, metabolic disease, family history of some endocrinopathies, renal diseases , liver and otherchronic disease.

3.1.5. Sampling technique:

samples was collected in the morning the samples was separated by centrifugation at 3000 rpm for 10 minutes.

3.1.6. Ethical considerations:

Procedure of blood sampling was explained to the participants. All participants were informed about the research objectives and procedures during the interview period. A verbal consent was obtained from all participants.

3.1.7. Data collection:

Personal and clinical data from all participants were collected using special form of questionnaire.

3.2. Laboratory Experiments:

During specimens collection the patients and the normal health individuals are relaxed and after taking the samples and centrifugation all sample was freezer and preserve and until the completion of the total number and then analysed.

3.3. Methods:

3.3.1. Estimation of urea concentration using the enzymatic (urease)

3.3.1.1.Principle of method:

Urease enzyme catalyses the conversion of urea in to carbon dioxide and ammonia, which react with salisylate and alkaline hypochlorite in presence of nitroprusside (catalyst increase rate of reaction) to give indophenol which

dissociated with alkaline solution to give (indophenol) blue to green color, which can be measured calorimetrically at 600nm the color directly proportional to the concentration of urea in the sample.

3.3.1.2 Procedure of urea: (appendix II)

3.3.2 Estimation of creatinine concentration using kinetic method: (appendix

III).

3.3.2.1.Principle of method:

Creatinine was thought to be react with alkaline picrate with in one minute, after additional of sample to working reagent, while pseudocreatinine (ketone bodies, Aceto acetate) react during first 30 second and other pseudocreatinine react with working reagent after 90 minutes of additional of sample(after reaction finished).

3.3.2.2 Procedure of creatinine (appendix 11).

3.3.3. Estimation of Na+ and K+ levels:

3.3.3.1. Principle of method (ISE):

An Ion selective electrode consists of a detector electrode and an

Electrically conductive membrane which separates the sample solution of Unknown activity from a solution of fixed ion activity which fills the Electrode. A difference in ionic composition of the two solutions causes an Electrical potential difference to develop across the membrane, change in Potential across the selective membrane are measured with respect to a Reference electrode. The potential of which is constant. The change in Potential difference between the reference electrode and the ion selective Electrode for the sample is proportional with the potential difference for a Calibration solution of known composition (Tietz, 1987).

3.3.3.2. Procedure of Na+ and K+ measurement. (Appendix II).

3.4 Data analysis:

Data was analyzed to obtain means standard and correlation of the sampling using statistical package for social (SPSS) computer programmed version 20, t test and persons correlation were used for mean and correlation.

3.5 Quality control:

The precision and accuracy of all methods used in this study were checked at least once per day with commercially available control, was done at least two levels of control (normal and abnormal).

ChapterFour

Results

Chapter Four

4.Result:

4-1 Base line characteristics of patients:

Table (4-1) illustrate the age ,gender and duration of disease. 34(68%) of patient at age less than 40 years and 16(23%) of patient at age range between 40-80 years. 37(74%) of patients were female while 13(26%) of patients were male.

The duration of disease in 35(70%) of patient less than 5 years and 35(70%) of patient at duration range between 5-12 years..

Table (4-2) shows mean comparison of urea , creatinine ,Na and K levels in patients with RA compared to control group. There were significant increase in urea and creatinine levels compared to control group (43.64 ± 15.78 versus 26.86± 7.65 mg/dl,p value = 0.000) for urea and (1.31±0.795 versus 0.736±0.179 Mmol/l, p value= 0.000) . While there were no significant difference in mean concentration of Na and K in case compared to control group(136.9± 18.31 versus 140.1± 4.36 mmol/l ,p value 0.214)for Na and (3.96 ±0.556 versus 3.95 ±0.426 mmol/l , p value 0.785).

Figure 4.1 Show correlation between urea level and age(R 0.644, P =0.000),there was moderate positive correlation.

Figure(4-2) Show correlation between creatinine level and age (R= -0.042 , P = 0.773) , there was no correlation.

Figure(4-3) Show correlation between sodium level and age (R=0.034, P=0.813), there was no correlation.

Figure (4-4) Show correlation between potassium level and age (R=-0.069 , P =0.632),there was no correlation.

Figure (4-5) Show correlation between urea level and duration (R=0.003, P =0.986) , there was no correlation.

Figure(4-6) Show correlation between creatinine level and duration

(R=0.094 , P =0.515), there was no correlation.

Figure (4-7) Show correlation between sodium level and duration(R= 0.147 , P = 0.620) , there was no correlation.

Figure (4-8) Show corrrlation between potassium level and duration (R = 0.072 , P = 0.620), there was no correlation.

Table (4-1) Baseline characteristics of patients

Variables	Frequency	Percentage
Duration		
Less than 5y	35	70%
5-12	15	30%
Age group		
Less than 40	34	68%
40 – 80	16	32%
Gender		
Female	37	74%
Male	13	26%
Total	50	100%

Table (4-2) Mean comparison of urea, creatinine , Na and K levels in case versus control group

Parameters	Case (Mean± SD)	Control (Mean± SD)	*P-value*
Urea(mg/dl)	43.64±15.78	26.86±7.65	0.000
Creatinine (Mmol/l)	1.31±0.795	0.736±0.179	0.000
Na(mmol/l)	136.9±18.31	140.1±4.36	0.214
K(mmol/l)	3.96±0.556	3.95±0.426	0.785

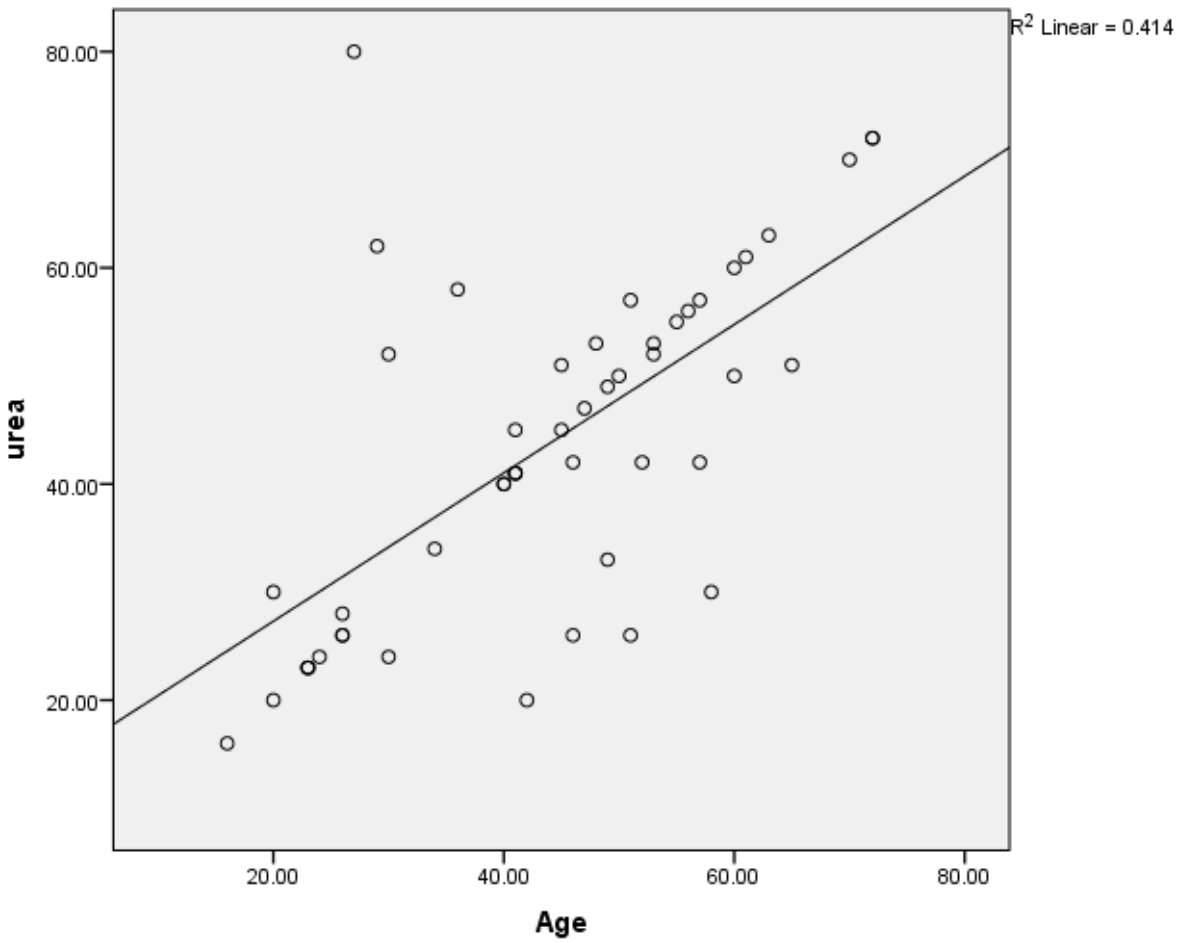


Figure 4.1(A) correlation between urea level and age(R = 0.644, P = 0.813).

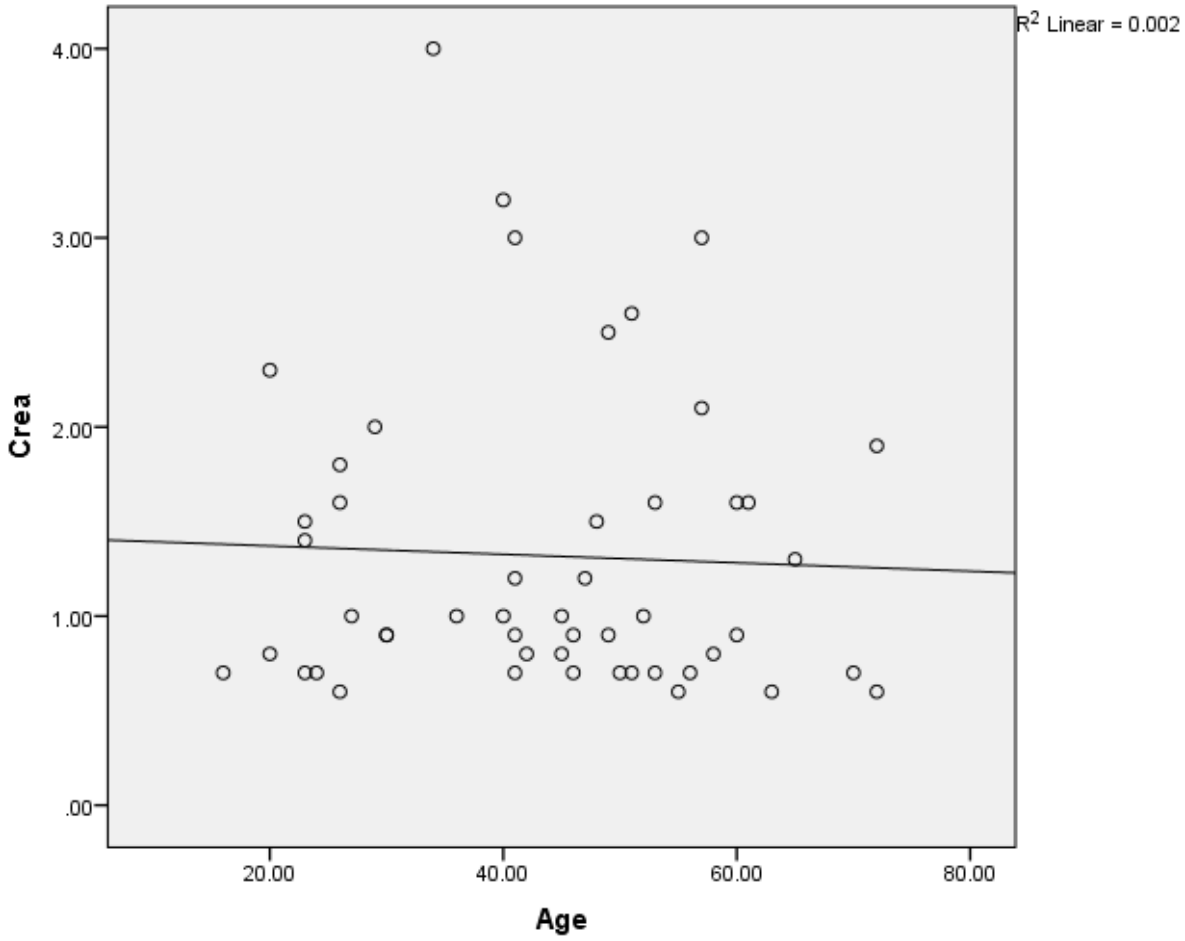


Figure 4.2(4) correlation between creatinine level and age(R = -0.042, P = 0.773).

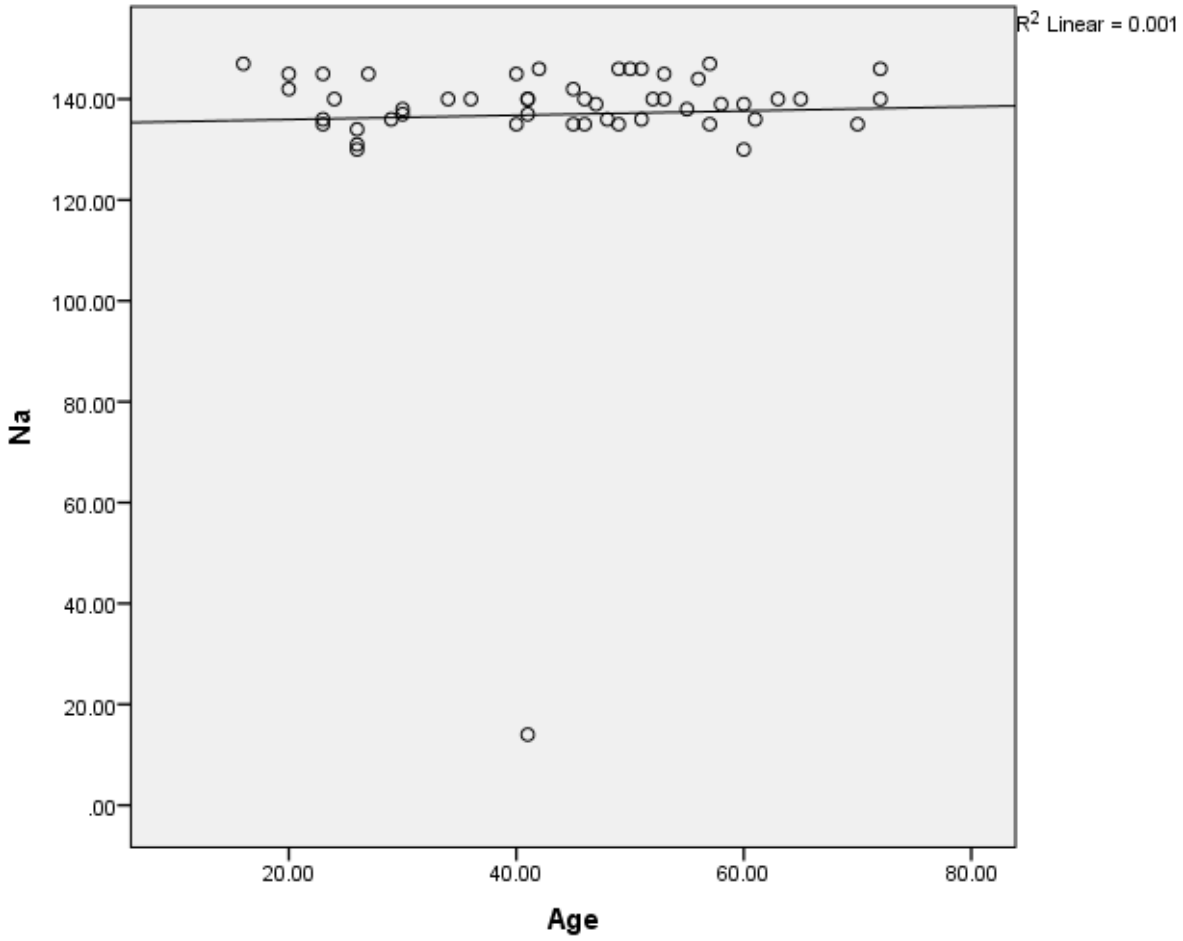


Figure 4.3(D) correlation between Sodium level and Age(R = 0.034, P = 0.813).

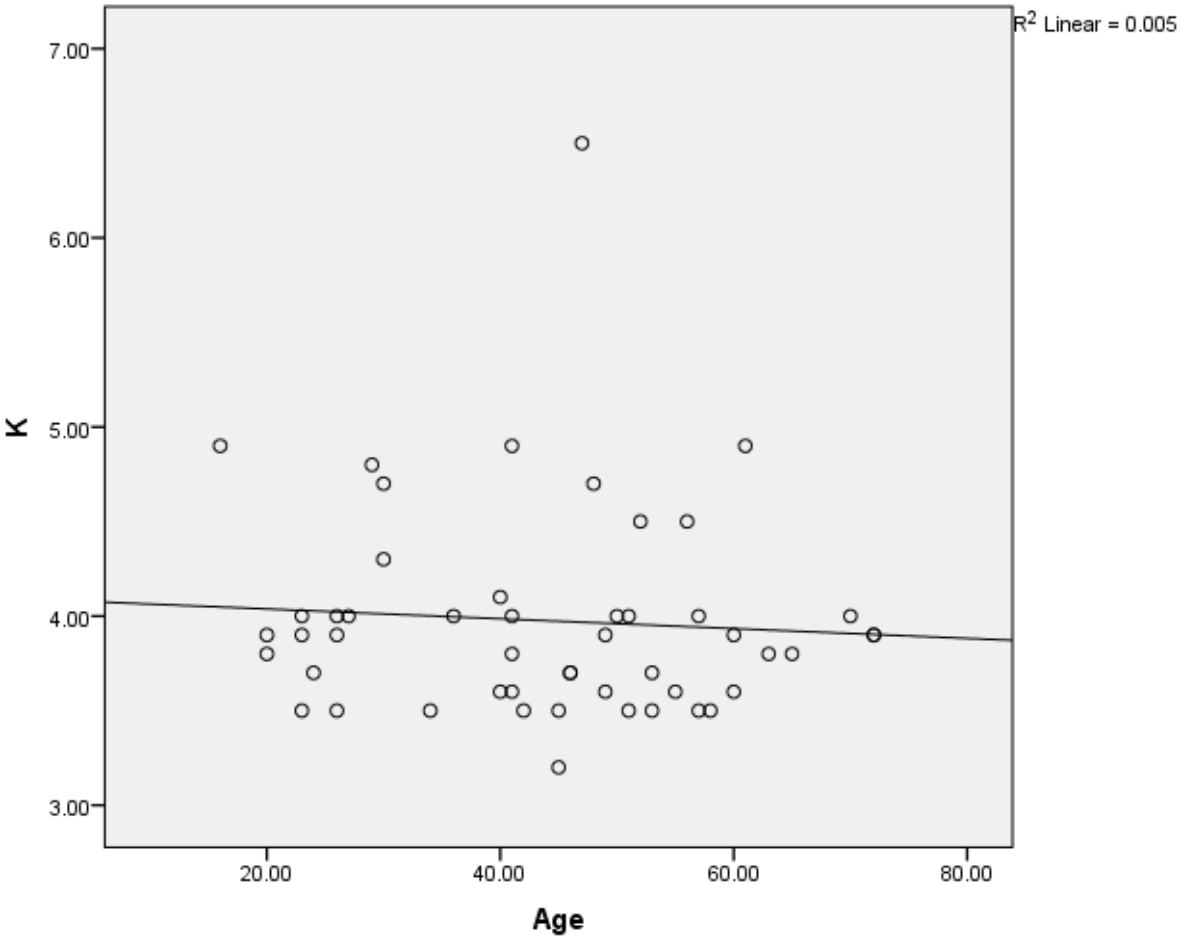


Figure 4.4(E) correlation between potassium level and age(R =- 0.069, P= 0.632).

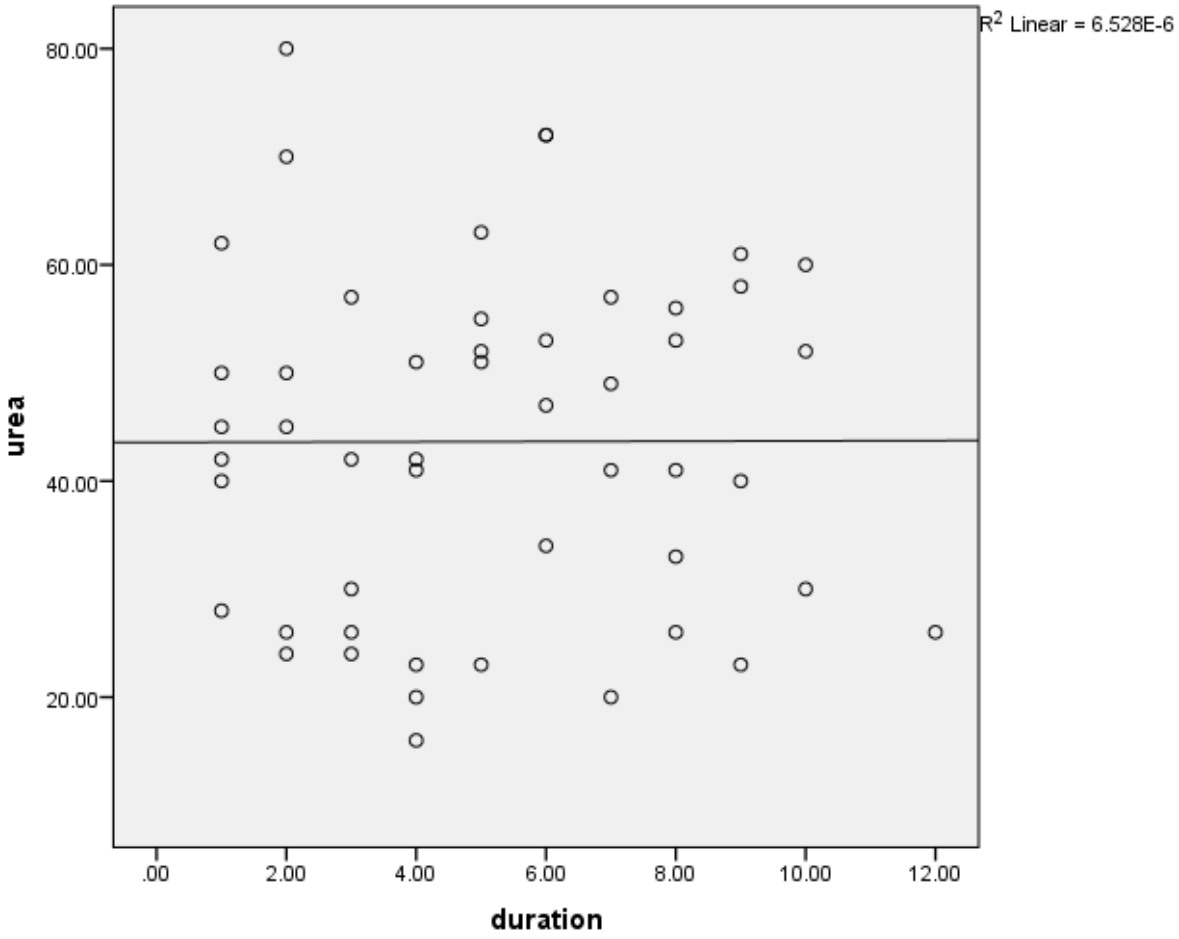


Figure 4.5(F) correlation between urea level and duration(R = 0.003, P = 0.986).

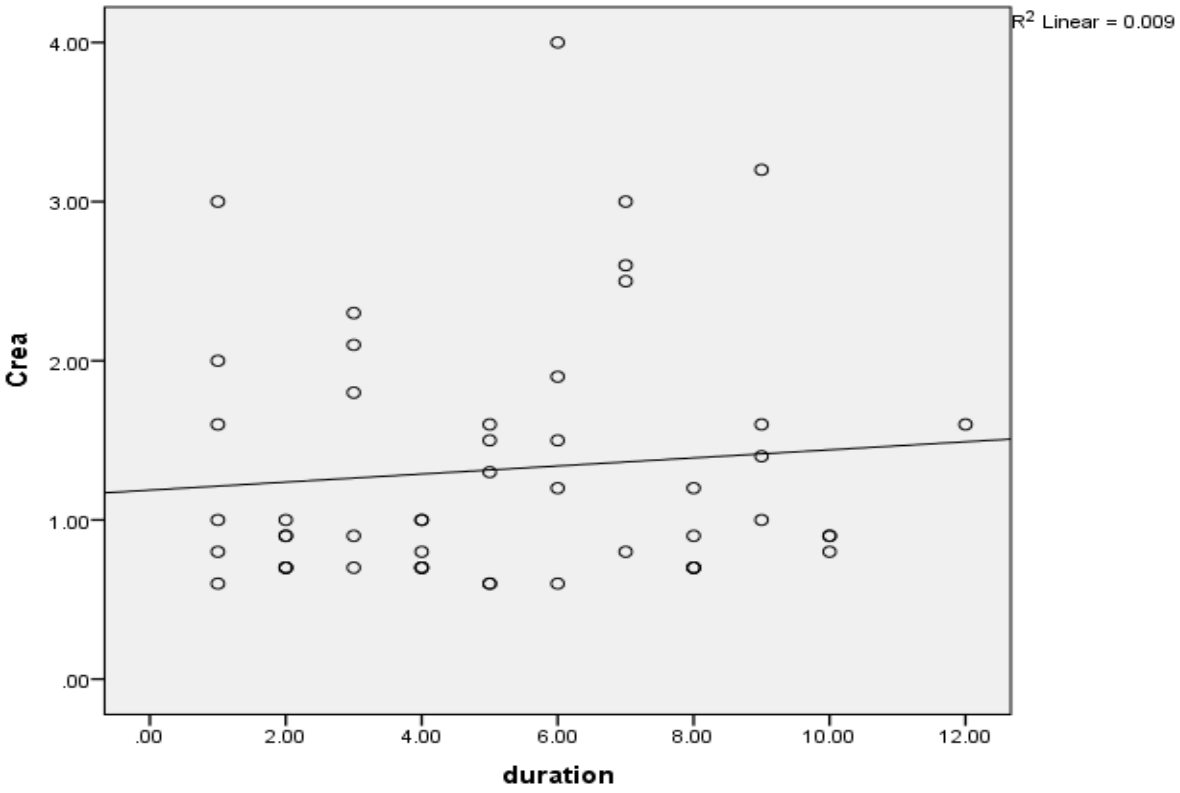
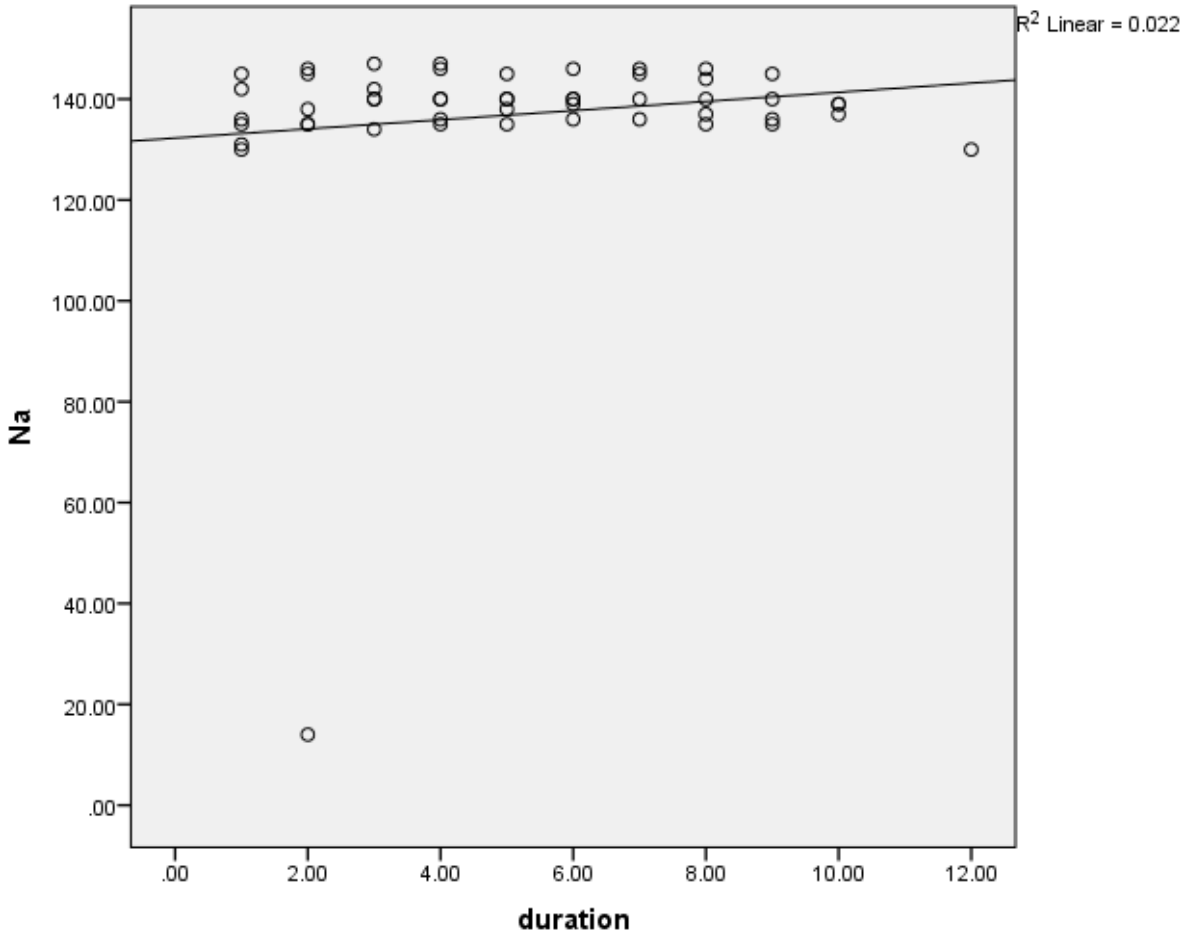


Figure 4.6(F) correlation between creatinine level and duration (R=0.094 , R=0.515).



i

Figure4.7(G) Correlation between sodium level and duration (R=0.147,P=0.310).

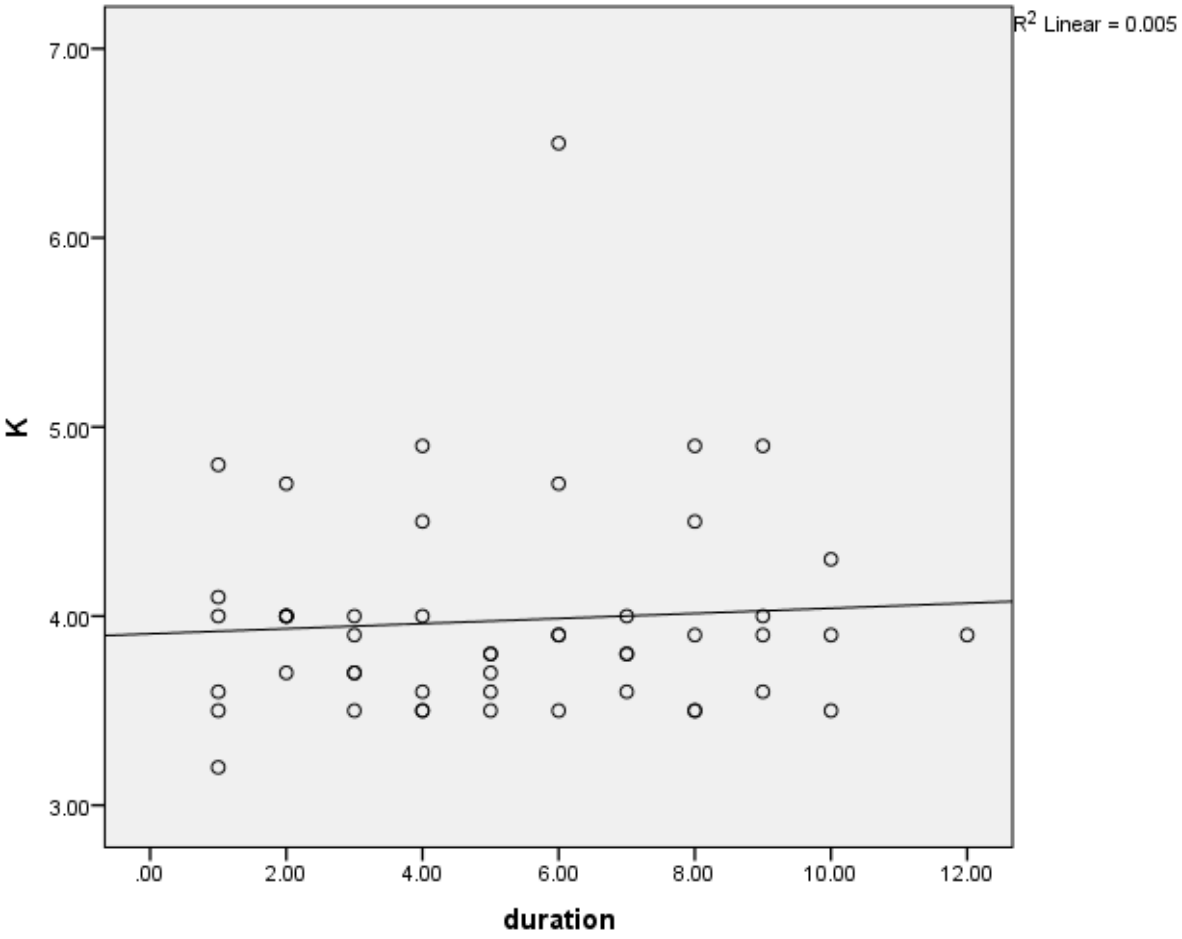


Figure 4.8(H) Correlation between potassium level and duration (R=0.072 , P =0.620).

ChapterFive

Discussion

Chapter Five

5.1. Discussion:

Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated. (Hill e*tal.*,2009) , This study conducted to measure urea , creatinine sodium and ,potasium among Rheumatoid arthritis patients.

The present study revealed significant increase in mean of urea and creatinine levels among case when compared to control group with *p-value(* 0.000),(0.000) respectively. This finding agreed with studies done by (Koseki *etal*., 2015). Also results of a study conducted by (Anders *etal* .,2011) showed significant increase in the level of urea and creatinine in Rheumatoid arthritis cases than control group.. The present study revealed insignificant difference in sodium and potassium levels with *p-value* 0.214 / p-value 0.785 respectively. This result similar to another results which found no significant difference in Na and K levels in RA patients compared to control group (Marouen *etal* .,2017;Kianifard *etal* .,2018).

There was moderate positive correlation between urea levels with age (R 0.644, P. value= 0.000) . There was no correlation between creatinine and Na and K with age (R -0.042, P value= 0.773 , R 0.034 ,P value= 0.813 ,R -0.069 P. value= 0.632) Also, there was no correlation between urea , creatinine and Na, K with duration (R 0.003, P value= 0.986. R 0.094, P value= 0.515.R 0.147, P value= 0.310 .R 0.072 , P value= 0.620).This result agreed with study done by (Seon *etal*., 2017), which found there was positive correlation between urea and age , while there were no correlation between creatinine , sodium , potassium with age and duration of disease.

5.2.Conclusion:

From the results and finding of this study, it is concluded that:

The serum levels of urea and creatinine are higher in Rheumatoid arthritis patients, and there are no observed change in Na, K concentration in Rheumatoid arthritis patients. There is positive correlation between urea levels with age.

5.3 Recommendation:

1.Renal function test should be done as routine investigation in RA patients to avoid the progression of chronic kidney disease.

2. Patients with rheumatoid arthritis and renal co-morbidity should be managed through close collaboration between a rheumatologist and a nephrologist.

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Appendixes

Sudan University

of Science and

Technology

College of

Graduate Studies

QUESTIONNAIRE No ( )

Date: / /2019

Name: …….

Age: .....Years.

Duration of disease ……

History of kidney disease : Yes …… No ……

Medications: ……

Result:

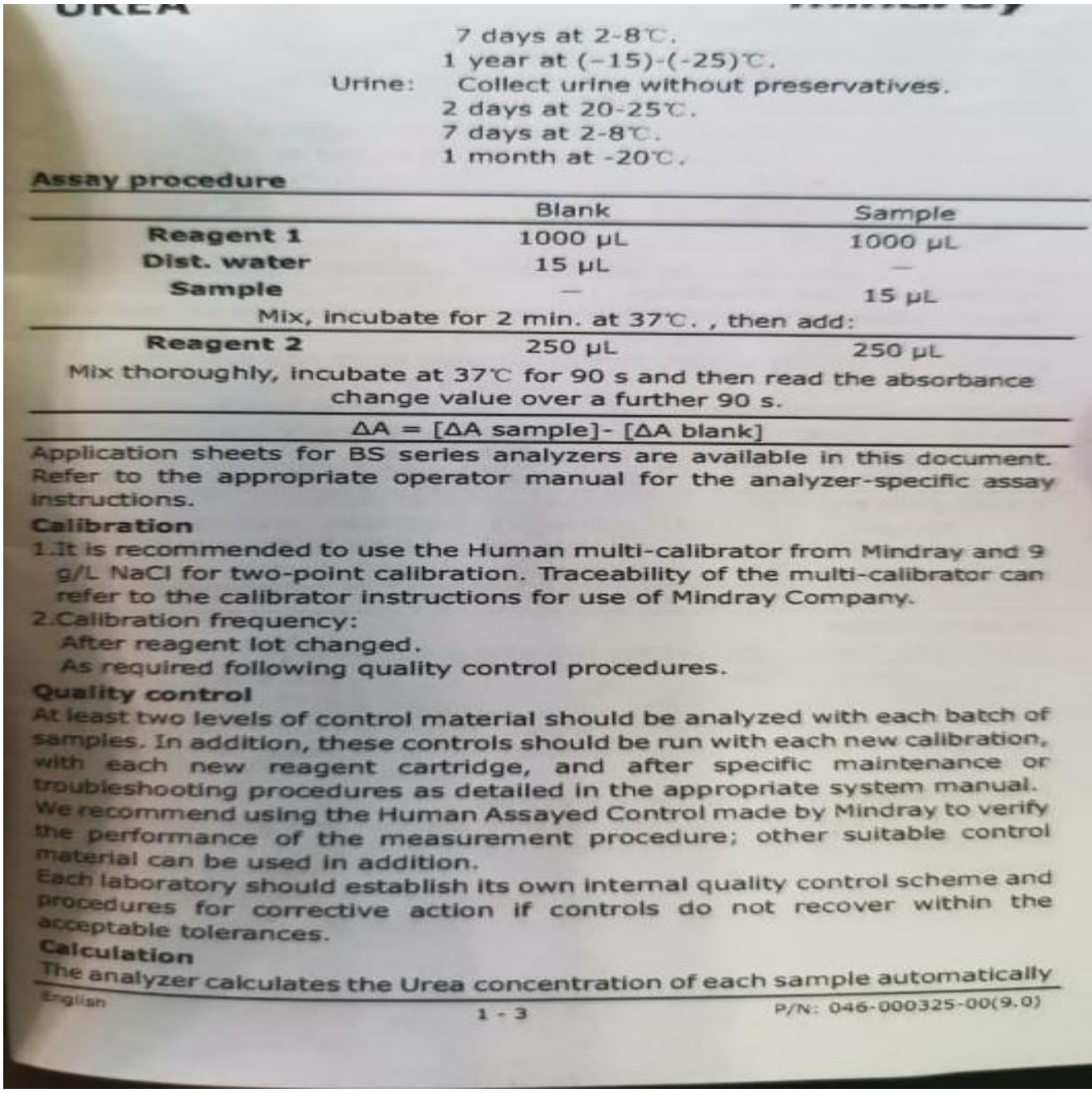
Urea……. (mg/dl)

Creatinine…… (M l)

Sodium…… (mmol/l)

Potassium……(mmol/l)

Appendixes II :



UREA

7 days at 2-8℃.
1 year at (−15)-(-25)℃.

Urine: Collect urine without preservatives.
2 days at 20-25℃.
7 days at 2-8℃.
1 month at -20℃.

Assay procedure

	Blank	Sample
Reagent 1	1000 µL	1000 µL
Dist. water	15 µL	—
Sample	—	15 µL
Mix, incubate for 2 min. at 37℃. , then add:		
Reagent 2	250 µL	250 µL

Mix thoroughly, incubate at 37℃ for 90 s and then read the absorbance change value over a further 90 s.

ΔA = [ΔA sample]- [ΔA blank]

Application sheets for BS series analyzers are available in this document. Refer to the appropriate operator manual for the analyzer-specific assay instructions.

Calibration

1.It is recommended to use the Human multi-calibrator from Mindray and 9 g/L NaCl for two-point calibration. Traceability of the multi-calibrator can refer to the calibrator instructions for use of Mindray Company.

2.Calibration frequency:
After reagent lot changed.
As required following quality control procedures.

Quality control

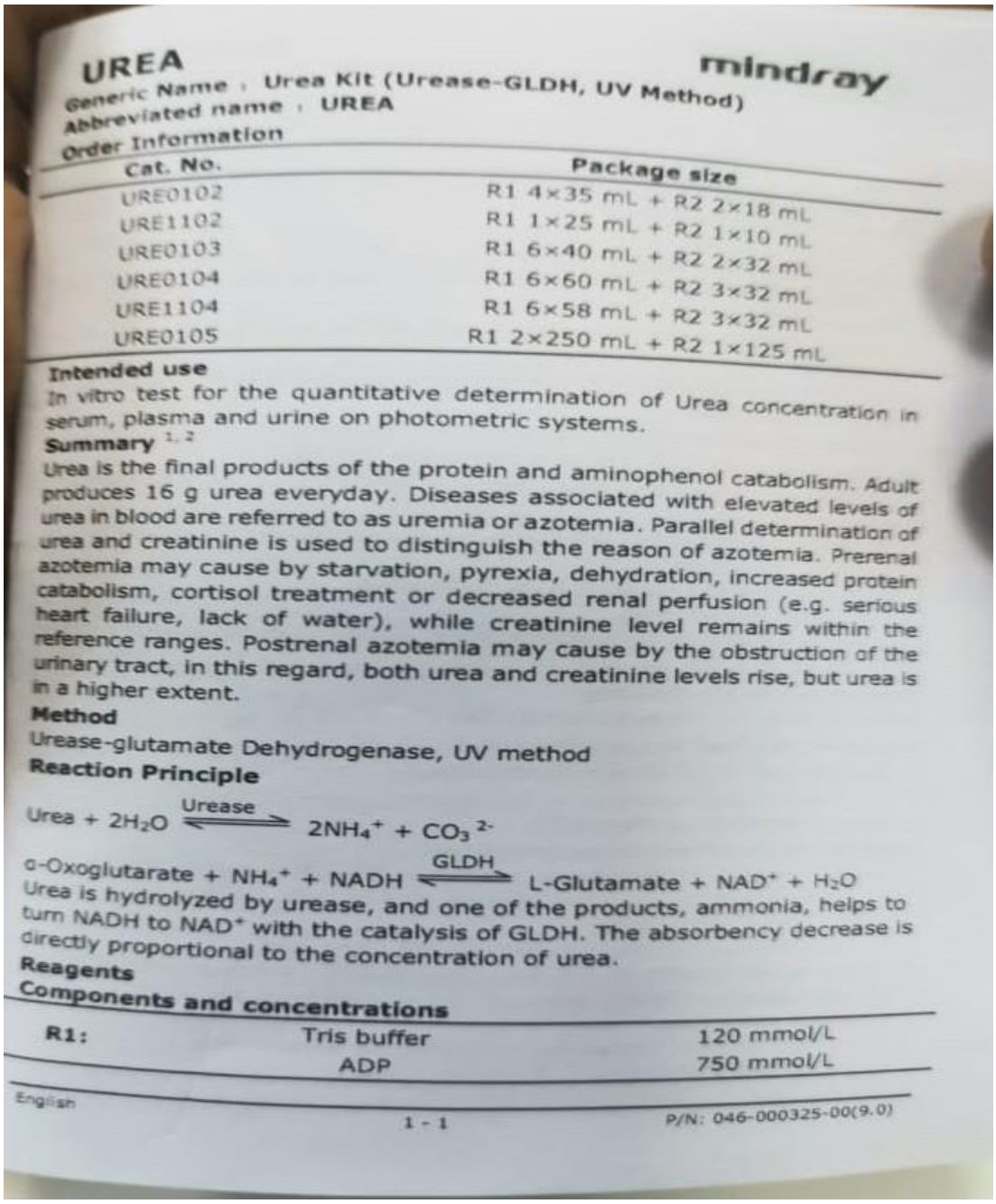
At least two levels of control material should be analyzed with each batch of samples. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual.
We recommend using the Human Assayed Control made by Mindray to verify the performance of the measurement procedure; other suitable control material can be used in addition.
Each laboratory should establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Calculation

The analyzer calculates the Urea concentration of each sample automatically

English 1 - 3 P/N: 046-000325-00(9.0)

AppendixesII:



UREA mindray

Generic Name : Urea Kit (Urease-GLDH, UV Method)
Abbreviated name : UREA

Order Information

Cat. No.	Package size
URE0102	R1 4×35 mL + R2 2×18 mL
URE1102	R1 1×25 mL + R2 1×10 mL
URE0103	R1 6×40 mL + R2 2×32 mL
URE0104	R1 6×60 mL + R2 3×32 mL
URE1104	R1 6×58 mL + R2 3×32 mL
URE0105	R1 2×250 mL + R2 1×125 mL

Intended use

In vitro test for the quantitative determination of Urea concentration in serum, plasma and urine on photometric systems.

Summary [1, 2]

Urea is the final products of the protein and aminophenol catabolism. Adult produces 16 g urea everyday. Diseases associated with elevated levels of urea in blood are referred to as uremia or azotemia. Parallel determination of urea and creatinine is used to distinguish the reason of azotemia. Prerenal azotemia may cause by starvation, pyrexia, dehydration, increased protein catabolism, cortisol treatment or decreased renal perfusion (e.g. serious heart failure, lack of water), while creatinine level remains within the reference ranges. Postrenal azotemia may cause by the obstruction of the urinary tract, in this regard, both urea and creatinine levels rise, but urea is in a higher extent.

Method

Urease-glutamate Dehydrogenase, UV method

Reaction Principle

$$\text{Urea} + 2H_2O \xrightleftharpoons{\text{Urease}} 2NH_4^+ + CO_3^{2-}$$

$$\alpha\text{-Oxoglutarate} + NH_4^+ + \text{NADH} \xrightleftharpoons{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

Urea is hydrolyzed by urease, and one of the products, ammonia, helps to turn NADH to NAD^+ with the catalysis of GLDH. The absorbency decrease is directly proportional to the concentration of urea.

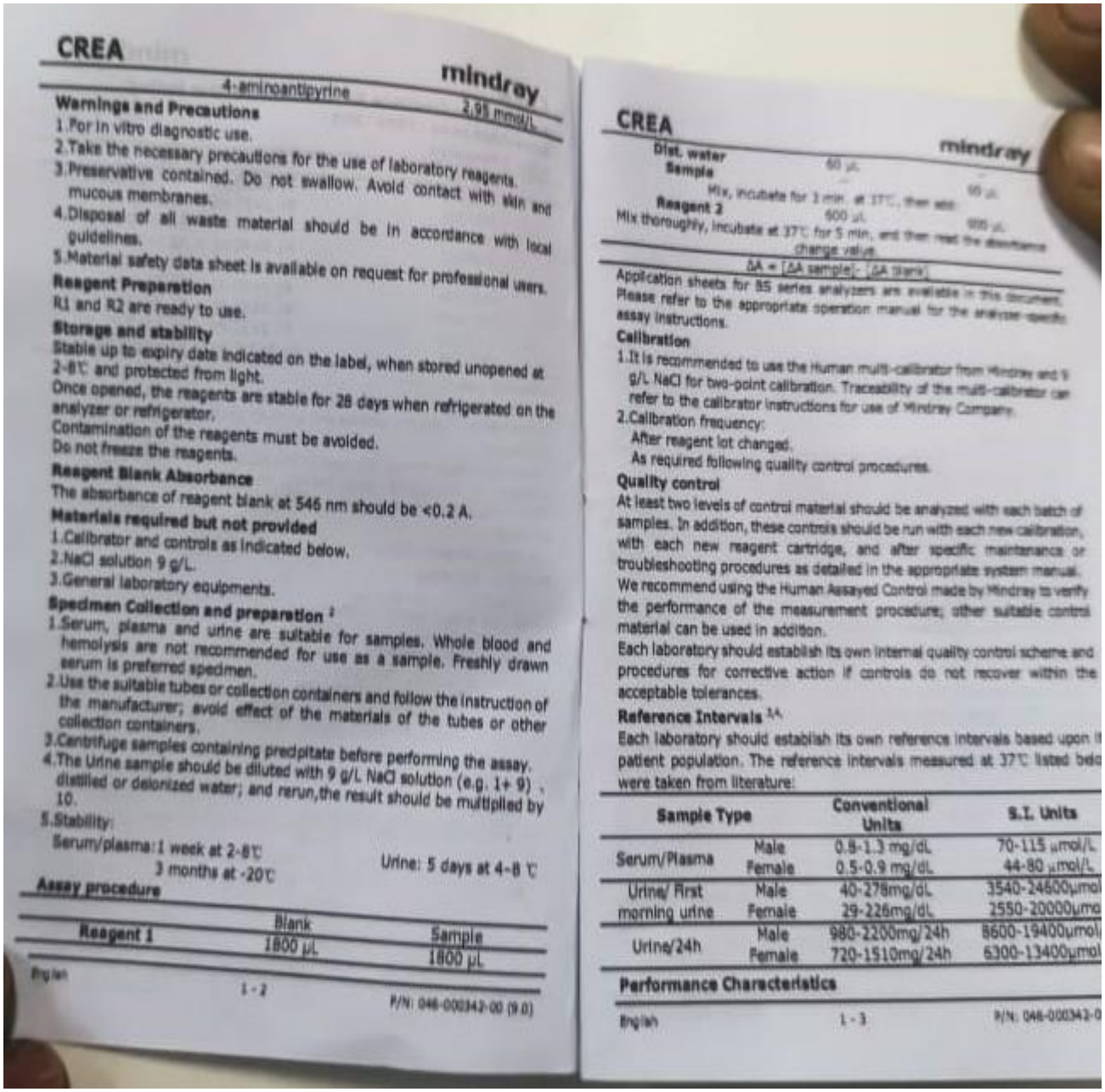
Reagents

Components and concentrations

R1:	Tris buffer	120 mmol/L
	ADP	750 mmol/L

English 1 - 1 P/N: 046-000325-00(9.0)

Appendixes III :



CREA

mindray

4-aminoantipyrine 2.98 mmol/L

Warnings and Precautions

1.For in vitro diagnostic use.
2.Take the necessary precautions for the use of laboratory reagents.
3.Preservative contained. Do not swallow. Avoid contact with skin and mucous membranes.
4.Disposal of all waste material should be in accordance with local guidelines.
5.Material safety data sheet is available on request for professional users.

Reagent Preparation

R1 and R2 are ready to use.

Storage and stability

Stable up to expiry date indicated on the label, when stored unopened at 2-8℃ and protected from light.
Once opened, the reagents are stable for 28 days when refrigerated on the analyzer or refrigerator.
Contamination of the reagents must be avoided.
Do not freeze the reagents.

Reagent Blank Absorbance

The absorbance of reagent blank at 546 nm should be <0.2 A.

Materials required but not provided

1.Calibrator and controls as indicated below.
2.NaCl solution 9 g/L.
3.General laboratory equipments.

Specimen Collection and preparation [2]

1.Serum, plasma and urine are suitable for samples. Whole blood and hemolysis are not recommended for use as a sample. Freshly drawn serum is preferred specimen.
2.Use the suitable tubes or collection containers and follow the instruction of the manufacturer; avoid effect of the materials of the tubes or other collection containers.
3.Centrifuge samples containing predpitate before performing the assay.
4.The urine sample should be diluted with 9 g/L NaCl solution (e.g. 1+ 9) , distilled or deionized water; and rerun,the result should be multiplied by 10.
5.Stability:
Serum/plasma:1 week at 2-8℃ Urine: 5 days at 4-8 ℃
3 months at -20℃

Assay procedure

	Blank	Sample
Reagent 1	1800 µL	1800 µL

English 1 - 2 P/N: 046-000342-00 (9.0)

CREA

mindray

Dist. water	60 µL	-
Sample	-	60 µL
Mix, incubate for 3 min at 37℃, then add:		
Reagent 2	600 µL	600 µL

Mix thoroughly, incubate at 37℃ for 5 min, and then read the absorbance change value.

ΔA = [ΔA sample] - [ΔA blank]

Application sheets for BS series analyzers are available in this document. Please refer to the appropriate operation manual for the analyzer-specific assay instructions.

Calibration

1.It is recommended to use the Human multi-calibrator from Mindray and 9 g/L NaCl for two-point calibration. Traceability of the multi-calibrator can refer to the calibrator instructions for use of Mindray Company.
2.Calibration frequency:
After reagent lot changed.
As required following quality control procedures.

Quality control

At least two levels of control material should be analyzed with each batch of samples. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual.
We recommend using the Human Assayed Control made by Mindray to verify the performance of the measurement procedure; other suitable control material can be used in addition.
Each laboratory should establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Reference Intervals [3,4]

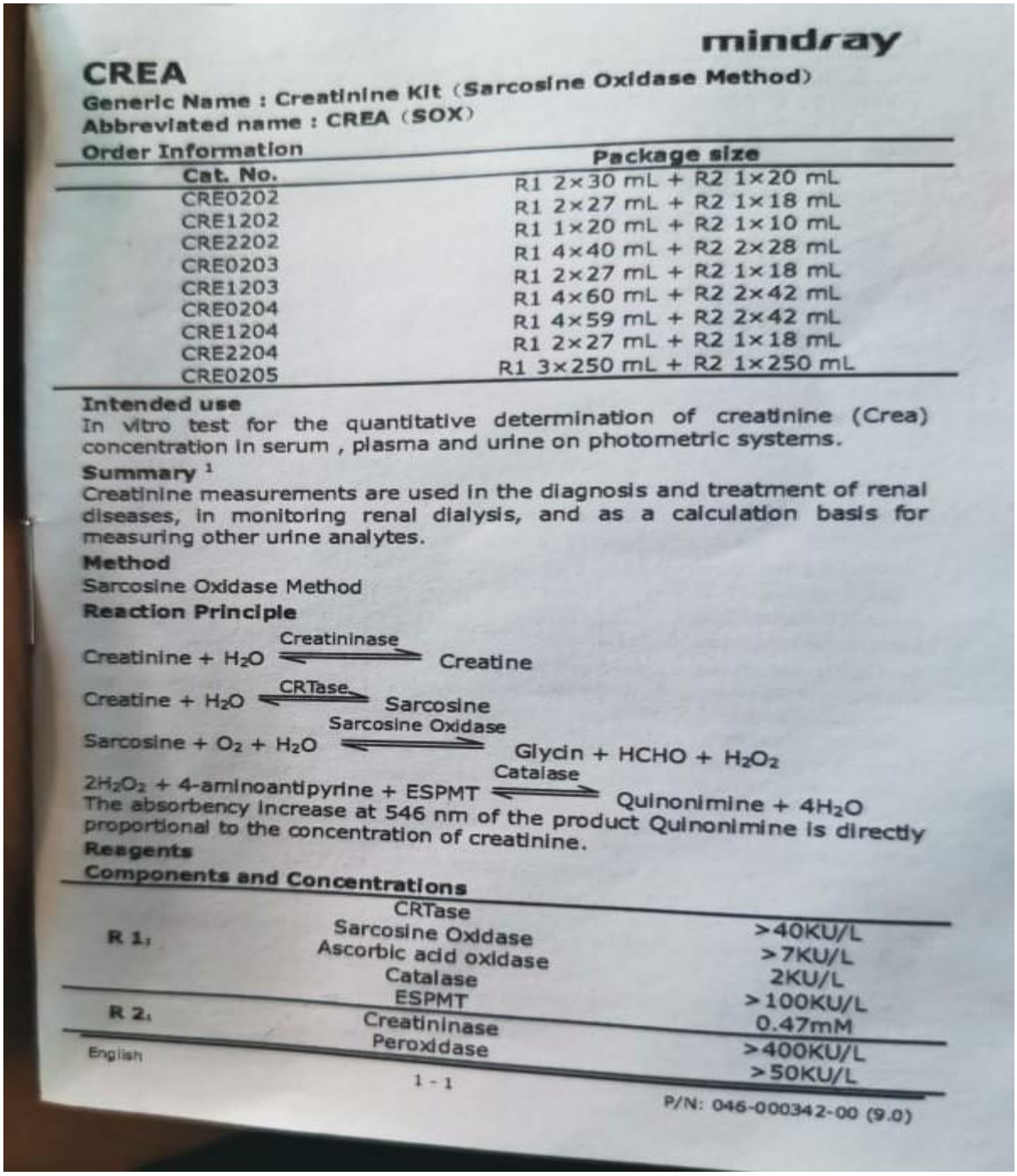
Each laboratory should establish its own reference intervals based upon its patient population. The reference intervals measured at 37℃ listed below were taken from literature:

Sample Type		Conventional Units	S.I. Units
Serum/Plasma	Male	0.8-1.3 mg/dL	70-115 µmol/L
	Female	0.5-0.9 mg/dL	44-80 µmol/L
Urine/ First morning urine	Male	40-278mg/dL	3540-24600µmol
	Female	29-226mg/dL	2550-20000µmo
Urine/24h	Male	980-2200mg/24h	8600-19400µmol
	Female	720-1510mg/24h	6300-13400µmol

Performance Characteristics

English 1 - 3 P/N: 046-000342-0

Appendixes III :



mindray

CREA

Generic Name : Creatinine Kit (Sarcosine Oxidase Method)
Abbreviated name : CREA (SOX)

Order Information

Cat. No.	Package size
CRE0202	R1 2×30 mL + R2 1×20 mL
CRE1202	R1 2×27 mL + R2 1×18 mL
CRE2202	R1 1×20 mL + R2 1×10 mL
CRE0203	R1 4×40 mL + R2 2×28 mL
CRE1203	R1 2×27 mL + R2 1×18 mL
CRE0204	R1 4×60 mL + R2 2×42 mL
CRE1204	R1 4×59 mL + R2 2×42 mL
CRE2204	R1 2×27 mL + R2 1×18 mL
CRE0205	R1 3×250 mL + R2 1×250 mL

Intended use
In vitro test for the quantitative determination of creatinine (Crea) concentration in serum , plasma and urine on photometric systems.

Summary [1]
Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for measuring other urine analytes.

Method
Sarcosine Oxidase Method

Reaction Principle

$$\text{Creatinine} + H_2O \xrightleftharpoons{\text{Creatininase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightleftharpoons{\text{CRTase}} \text{Sarcosine}$$

$$\text{Sarcosine} + O_2 + H_2O \xrightleftharpoons{\text{Sarcosine Oxidase}} \text{Glycin} + HCHO + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{ESPMT} \xrightleftharpoons{\text{Catalase}} \text{Quinonimine} + 4H_2O$$

The absorbency increase at 546 nm of the product Quinonimine is directly proportional to the concentration of creatinine.

Reagents

Components and Concentrations

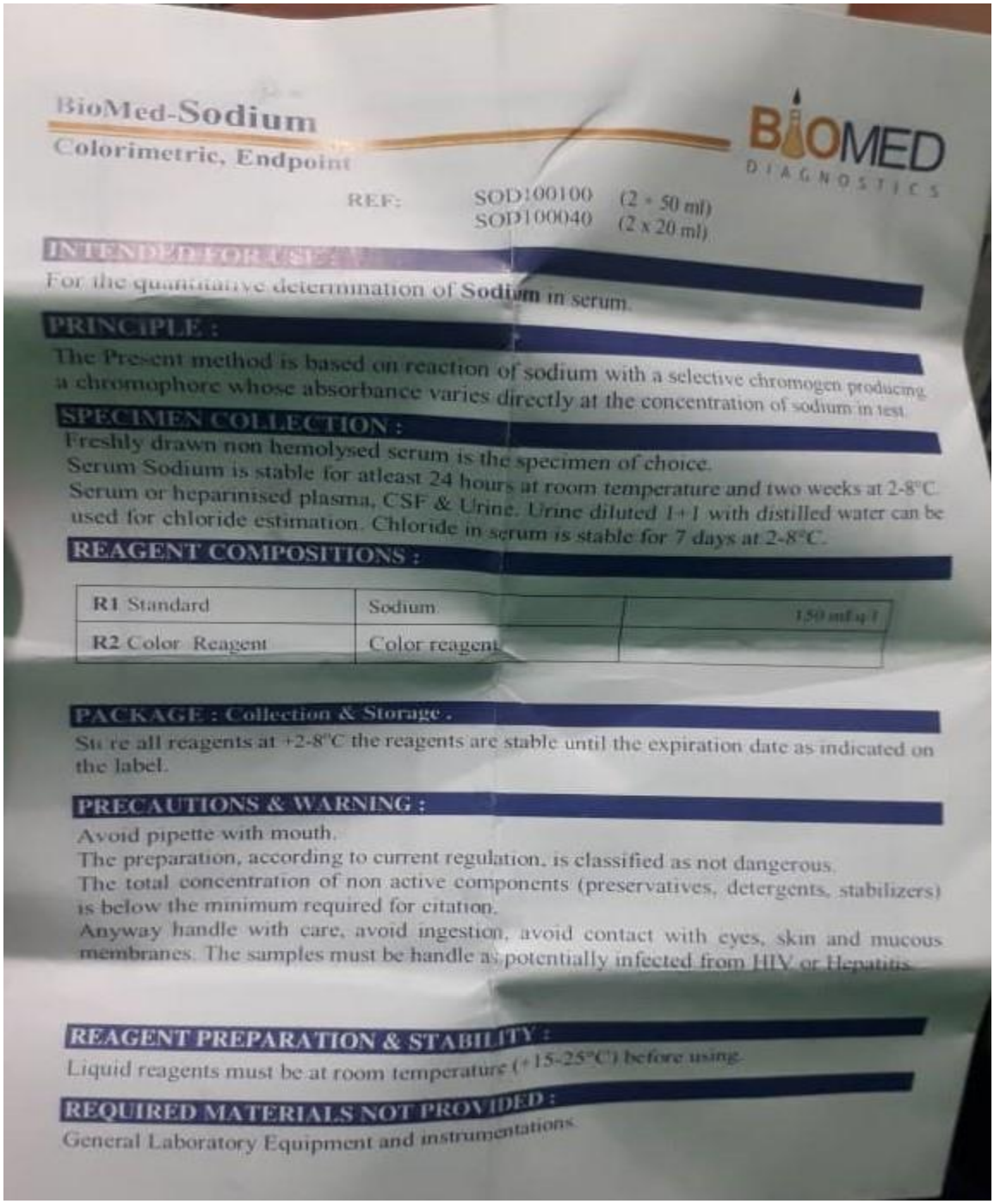
R 1:	CRTase	>40KU/L
	Sarcosine Oxidase	>7KU/L
	Ascorbic acid oxidase	2KU/L
	Catalase	>100KU/L
	ESPMT	0.47mM
R 2:	Creatininase	>400KU/L
	Peroxidase	>50KU/L

English

1 - 1

P/N: 046-000342-00 (9.0)

Appendixes IV :



BioMed-Sodium

Colorimetric, Endpoint

BIOMED DIAGNOSTICS

REF: SOD100100 (2 × 50 ml)
SOD100040 (2 x 20 ml)

INTENDED FOR USE :

For the quantitative determination of **Sodium** in serum.

PRINCIPLE :

The Present method is based on reaction of sodium with a selective chromogen producing a chromophore whose absorbance varies directly at the concentration of sodium in test.

SPECIMEN COLLECTION :

Freshly drawn non hemolysed serum is the specimen of choice.
Serum Sodium is stable for atleast 24 hours at room temperature and two weeks at 2-8°C.
Serum or heparinised plasma, CSF & Urine. Urine diluted 1+1 with distilled water can be used for chloride estimation. Chloride in serum is stable for 7 days at 2-8°C.

REAGENT COMPOSITIONS :

R1 Standard	Sodium	150 mEq/l
R2 Color Reagent	Color reagent	

PACKAGE : Collection & Storage .

Store all reagents at +2-8°C the reagents are stable until the expiration date as indicated on the label.

PRECAUTIONS & WARNING :

Avoid pipette with mouth.
The preparation, according to current regulation, is classified as not dangerous.
The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.
Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle as potentially infected from HIV or Hepatitis.

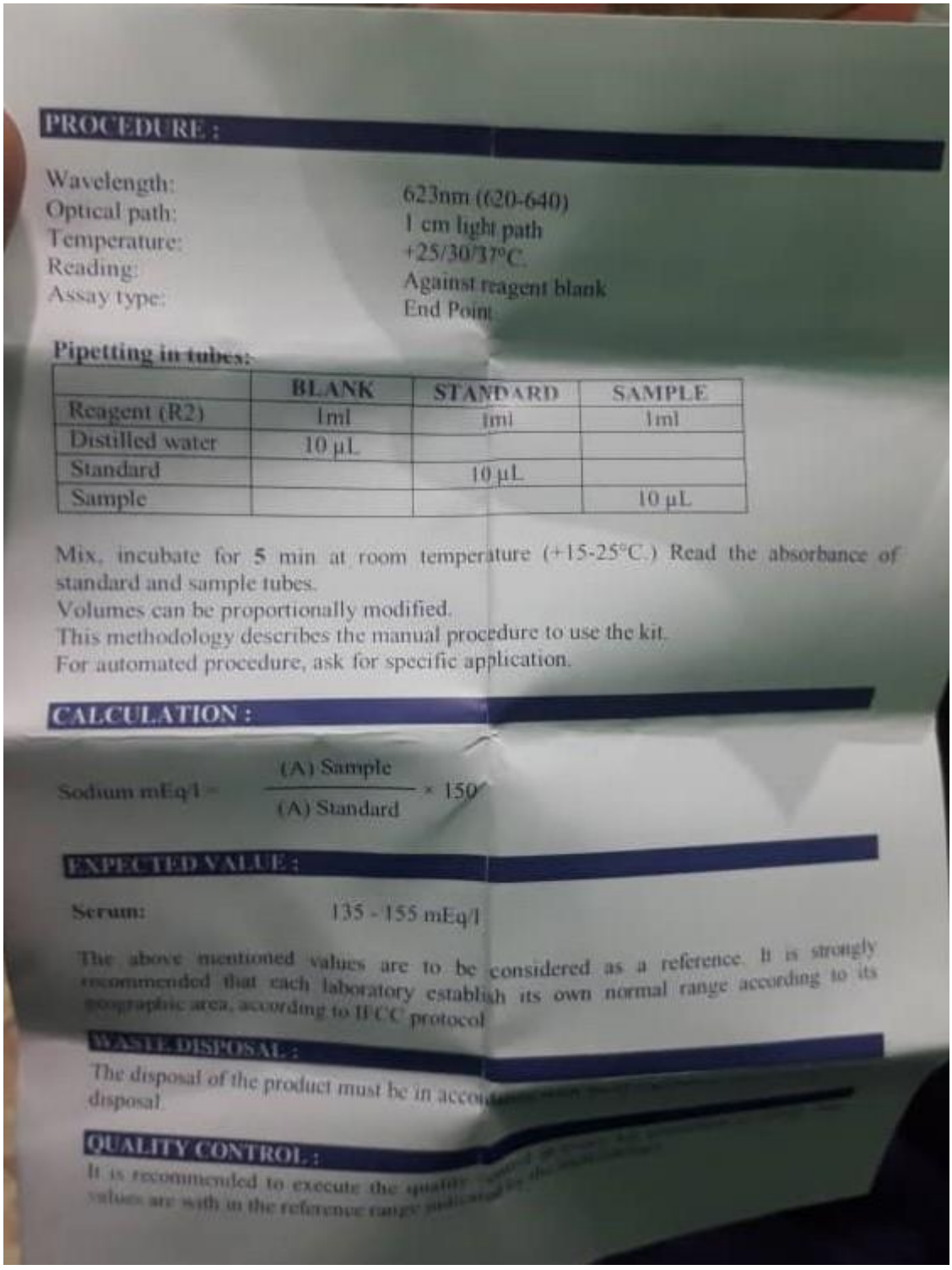
REAGENT PREPARATION & STABILITY :

Liquid reagents must be at room temperature (+15-25°C) before using.

REQUIRED MATERIALS NOT PROVIDED :

General Laboratory Equipment and instrumentations.

Appendixes IV :



PROCEDURE :

Wavelength:	623nm (620-640)
Optical path:	1 cm light path
Temperature:	+25/30/37°C.
Reading:	Against reagent blank
Assay type:	End Point

Pipetting in tubes:

	BLANK	STANDARD	SAMPLE
Reagent (R2)	1ml	1ml	1ml
Distilled water	10 µL		
Standard		10 µL	
Sample			10 µL

Mix, incubate for **5** min at room temperature (+15-25°C.) Read the absorbance of standard and sample tubes.
Volumes can be proportionally modified.
This methodology describes the manual procedure to use the kit.
For automated procedure, ask for specific application.

CALCULATION :

$$\text{Sodium mEq/l} = \frac{\text{(A) Sample}}{\text{(A) Standard}} \times 150$$

EXPECTED VALUE :

Serum: 135 - 155 mEq/l

The above mentioned values are to be considered as a reference. It is strongly recommended that each laboratory establish its own normal range according to its geographic area, according to IFCC protocol

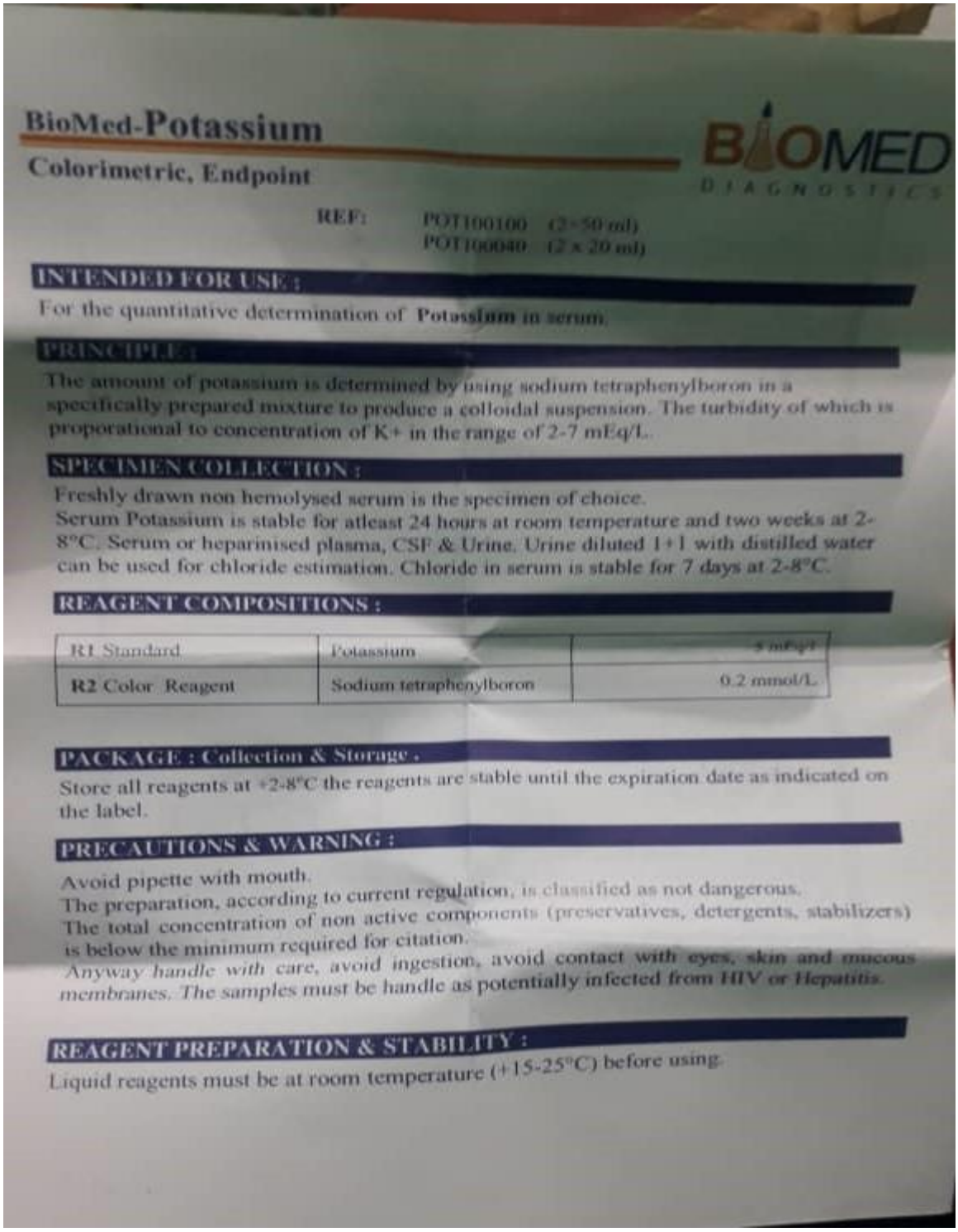
WASTE DISPOSAL :

The disposal of the product must be in acco
disposal.

QUALITY CONTROL :

It is recommended to execute the quality
values are with in the reference range

Appendixes V :



BioMed-Potassium

Colorimetric, Endpoint

BIOMED DIAGNOSTICS

REF: POT100100 (2×50 ml)
POT100040 (2 x 20 ml)

INTENDED FOR USE :

For the quantitative determination of **Potassium** in serum.

PRINCIPLE :

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proporational to concentration of K+ in the range of 2-7 mEq/L.

SPECIMEN COLLECTION :

Freshly drawn non hemolysed serum is the specimen of choice.
Serum Potassium is stable for atleast 24 hours at room temperature and two weeks at 2-8°C. Serum or heparinised plasma, CSF & Urine. Urine diluted 1+1 with distilled water can be used for chloride estimation. Chloride in serum is stable for 7 days at 2-8°C.

REAGENT COMPOSITIONS :

R1 Standard	Potassium	5 mEq/l
R2 Color Reagent	Sodium tetraphenylboron	0.2 mmol/L

PACKAGE : Collection & Storage .

Store all reagents at +2-8°C the reagents are stable until the expiration date as indicated on the label.

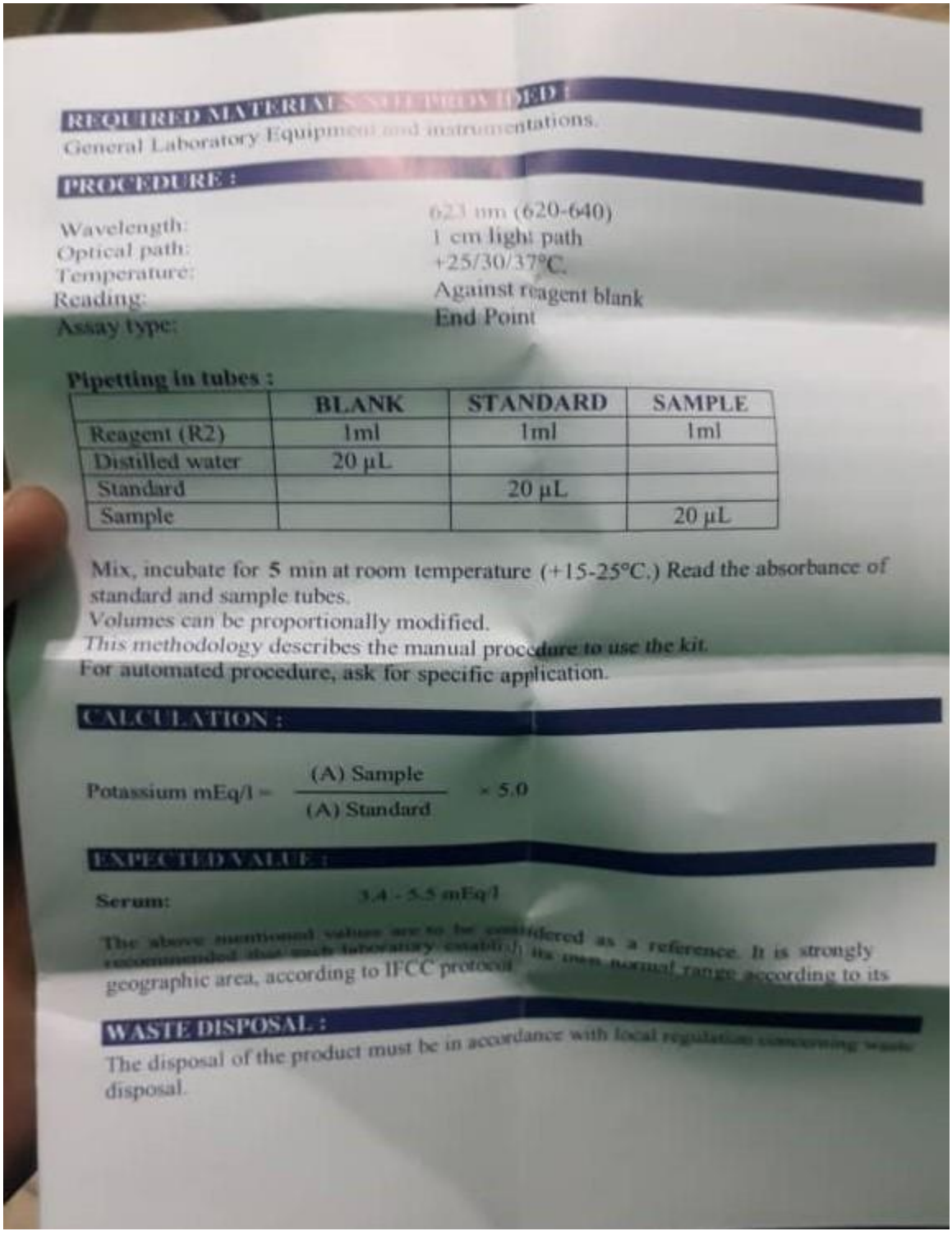
PRECAUTIONS & WARNING :

Avoid pipette with mouth.
The preparation, according to current regulation, is classified as not dangerous.
The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.
Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle as potentially infected from HIV or Hepatitis.

REAGENT PREPARATION & STABILITY :

Liquid reagents must be at room temperature (+15-25°C) before using.

Appendixes V :



REQUIRED MATERIAL [illegible] PROVIDED :

General Laboratory Equipment and instrumentations.

PROCEDURE :

Wavelength:	623 nm (620-640)
Optical path:	1 cm light path
Temperature:	+25/30/37°C.
Reading:	Against reagent blank
Assay type:	End Point

Pipetting in tubes :

	BLANK	STANDARD	SAMPLE
Reagent (R2)	1ml	1ml	1ml
Distilled water	20 µL		
Standard		20 µL	
Sample			20 µL

Mix, incubate for 5 min at room temperature (+15-25°C.) Read the absorbance of standard and sample tubes.
Volumes can be proportionally modified.
This methodology describes the manual procedure to use the kit.
For automated procedure, ask for specific application.

CALCULATION :

$$\text{Potassium mEq/l} = \frac{\text{(A) Sample}}{\text{(A) Standard}} \times 5.0$$

EXPECTED VALUE :

Serum: 3.4 - 5.5 mEq/l

The above mentioned values are to be considered as a reference. It is strongly recommended that each laboratory establish its own normal range according to its geographic area, according to IFCC protocol

WASTE DISPOSAL :

The disposal of the product must be in accordance with local regulation concerning waste disposal.

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