Diagnosis of Kidney Conditions Using Low-Cost Paper Diagnostics

by

Md. Nazibul Islam

Student ID: 1015022024

A thesis submitted to the Department of Chemical Engineering in partial fulfillment of the requirements for the degree of

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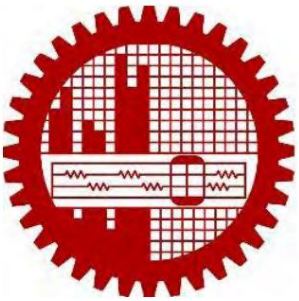
(CHEMICAL)



Department of Chemical Engineering

Bangladesh University of Engineering and Technology, Dhaka

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CERTIFICATION OF THE DISSERTATION

We, the undersigned, are pleased to certify that **Md. Nazibul Islam**, a candidate for the Degree of Master of Science in Engineering (Chemical) has presented this dissertation on the subject **'DIAGNOSIS OF KIDNEY CONDITIONS USING LOW-COST PAPER DIAGNOSTICS'**. The thesis is acceptable in form and content. The student demonstrated a satisfactory knowledge of the field covered by this thesis in an oral examination held on **January 21, 2018**.

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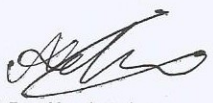

Md. Nazibul Islam

Student #P1015022024

January 21, 2017

Abstract

Kidneys are important body organ, which prevent waste buildup in human body, maintain stable electrolyte levels and regulate blood pressure and blood pH. In recent years, complications related to decreased kidney function and kidney failure have become a worldwide public health problem. A gradual reduction in kidney function can lead to Hyperuricemia (high uric acid), formation of kidney stones, kidney tumor and other chronic kidney diseases and can cause kidney failure. However, some of these adverse effects can be prevented or delayed by early detection and subsequent treatment. Paper diagnostic devices (PADs) can play a vital role in low-cost and rapid diagnosis of kidney condition, resulting in early detection of kidney complications. Paper diagnostics are paper and cellulose based analytical devices capable of qualitative or quantitative detection of biomarkers (molecules, genes, antibodies, antigens etc.) from biofluids (blood, urine etc.). In this study, different biomarkers for kidney diagnosis and detection techniques for specified biomarkers were analyzed, and low-cost paper diagnostic devices for qualitative and quantitative detection of important kidney biomarkers (for example: uric acid and creatinine) from biofluids (for example: urine) were developed. To detect, uric acid and creatinine concentration in human urine samples, colorimetric reactions were used. The reactions produce color signals on paper surface, and these signals can be analyzed and quantified using image analysis software. The temperature effect on color signal formation was measured, and use of urinary uric acid to creatinine ratio for diagnosis of other diseases was explored. Laboratory trials on three adult male samples were performed to assess the validity of the proposed techniques. Finally, codes were developed for image analysis using MATLAB application which could be potentially used in telemedicine using cell phone and computers.

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Chapter 1: Introduction

1. Introduction

Kidneys are important body organ, which act as filter to remove wastes from blood. Kidneys also regulate blood pressure, blood pH, excrete hormones and maintain stable electrolyte level [1-8]. Any disorder affecting these functions or kidney structure is known as kidney disease [9] which in recent years have become a global health problem [10]. Common kidney diseases include, Hypertensive Nephrosclerosis, Kidney Stones, Glomerulosclerosis, Urinary Tract Infection (UTI), Diabetic Kidney Disease (DKD) and Analgesic Nephropathy [11]. Prolonged occurrence (> 3 months) of any of these diseases can lead to decreased kidney function, kidney damage and eventually to chronic kidney disease (CKD) [9]. Furthermore, CKD can lead to kidney failure, cardiovascular disease (CVD) and premature death [10]. According to world health organization (WHO), in 2012, an estimated 864,226 deaths (1.5% of deaths worldwide) were attributed to CKD [12] and it is the 14^{th} leading cause of global deaths [12]. Kidney diseases may also develop into end-stage renal disease (ESRD), requiring costly renal replacement therapy in the form of dialysis or transplantation [13, 14]. However, early detection of kidney disease can reduce the risk of kidney failure progression and cardiovascular disease by up to 50% [15]. Therefore, a simple and rapid kidney diagnostic technique would be invaluable to doctors and patients.

In general, blood and urine tests are performed to diagnose kidney condition [16-18]. Common biomarkers for kidney analysis include, serum creatinine, urea nitrogen (BUN), glucose (fasting), parathyroid hormone, cystatin C, and urinary uric acid, creatinine and albumin [13, 15, 17-21].

For human, uric acid is the final bi-product of purine metabolism [22, 23]. Human beings have a mutation in the gene for uricase, the enzyme responsible for uric acid degradation. This mutation has affected the ability to regulate uric acid levels. So, a change in dietary

behavior such as an increase in consumption of purine rich foods such as meat and seafood, sugar-sweetened beverages, and alcohol can result in an increase in uric acid levels in urine and blood [22-26].

An increase in uric acid may indicate defects of uric acid transport in the nephron and renal under-excretion of uric acid [23, 27]. A higher level of serum uric acid (>7 mg/dl for men and >6.5 mg/dl for women) or urinary uric acid (>700 mg/day) is known as hyperuricemia [22-24, 27-30]. Hyperuricemia may disrupt renal function by causing renal arteriolar changes and glomerular damage which may lead to *de novo* renal disease such as glomerular hypertrophy, tubulointerstitial fibrosis, glomerulo-sclerosis etc. as well as accelerate existing renal diseases [23, 27-30]. In addition, hyperuricemia is a precursor of gout, and is also associated with the development of type 2 diabetes, obesity, Lesch-Nyhan Syndrome and arteriolar hypertension which may lead to cardiovascular disease [22-24, 27-32]. Therefore, it is important to have a reliable, low cost and user friendly technique to detect uric acid from human biofluids.

Urinary creatinine is also an important indicator of kidney condition. The formation of creatinine has a direct relationship with total muscle mass and body weight and is reasonably constant throughout the day [33]. An abnormal value in urine creatinine level may indicate renal dysfunction such as necrosis tubulus, glomerulonephritis and low glomerular filtration rate (GFR) [19, 20, 34, 35]. The rate of change of urinary creatinine excretion has been correlated with patients with chronic kidney disease (CKD) stages 3 and 4 and can be used to correlate between CKD and cardiovascular disease (CVD) [19]. The urinary albumin-creatinine ratio can be used to diagnose Proteinuria (excess amount of protein in the urine) [18, 36], metanephrine-creatinine ratio for Pheochromocytoma and calcium-creatinine ratio for pregnancy induced hypertension [37, 38]. In addition, on-spot urinary creatinine concentration is widely used as an indicator of sample abuse for urine samples used in drug tests [39-42].

Urinary uric acid to creatinine ratio can be used to diagnose diseases such as Perinatal Asphyxia (impairment of exchange of the respiratory gases in newborns) [43-46] and sleep hypoxemia (lack of oxygen in blood during sleep) [47, 48].

Several analytical methods for detecting uric acid and creatinine from human biofluids have been reported, which include sophisticated and expensive equipment such as, spectroscopy, chromatography, electrochemistry, membrane and capillary electrophoresis [22, 49-66]. These techniques often require out sourcing/routing of samples to specialized laboratories, which makes these detection techniques expensive and time consuming. Hence, there is a need for instantaneous and low cost point-of-care detection technique capable for qualitative and quantitative detection of uric acid and creatinine in human biofluids. Paper based diagnostics, often used for health and environmental purposes [67-71]; offer an attractive option for detecting biomarkers such as uric acid and creatinine from biofluids such as urine, serum, sweat and/or saliva [68-71].

This study reports simple, low-cost and user friendly paper based diagnostic technique for detecting uric acid and creatinine from human urine and use these values to diagnose kidney condition. For the proposed technique, ferric chloride and potassium ferricyanide solutions for uric acid detection and sodium-picrate for creatinine detection were used as reagents. Potassium ferricyanide reacts with uric acid present in human urine to form potassium ferrocyanide. Potassium ferrocyanide than reacts with ferric chloride to form prussian blue on the paper surface [72]. Sodium hydroxide reacts with picric acid to form sodium-picrate, which reacts with creatinine present in human urine to form picrate-creatinine complex (orange color) on paper surface [33, 73, 74]. Reaction rate of these reaction mechanisms were studied. The rate of reaction on paper was slightly higher than that in liquid solution, indicating a faster reaction. This is in accordance with other published results [68]. Color intensities with respect to uric acid and creatinine concentration were analyzed. The color

intensity of the resultant prussian blue and picrate-creatinine complex is linearly proportional with the concentration of uric acid and creatinine respectively. Calibration curves were developed based on the color intensity. Effect of temperature on color intensity was also studied. Using the ranges of urinary uric acid and creatinine concentration, a diagram was created which can be used by doctors and patients to diagnose kidney condition on-spot. Laboratory trials on human samples were performed to assess the validity of the proposed detection techniques. Finally, MATLAB codes were generated as possible baseline for the development mobile based applications. Uric acid and creatinine concentration in human urine can be quantified using a simple scanner or mobile application or with a gradient paper.

1.1 Objectives of the Study

The objectives of the study are:

- To find out different biomarkers for kidney diagnosis and to study different detection techniques for specified biomarkers.
- To develop low-cost paper diagnostic devices for qualitative and quantitative detection of important kidney biomarkers from biofluids (for example: urine).

1.2 Thesis Organization

The first chapter of this thesis briefly introduces kidney function, diseases and biomarkers. Paper based detection techniques coupled with telemedicine to detect kidney biomarkers are also discussed in this chapter.

Chapter 2 provides detailed discussion o Chronic Kidney Diseases (CKDs), Biomarkers of CKDs, existing detection techniques for urinary uric acid and creatinine, Paper Based Diagnostics (PADs) and the importance of Urinary Uric Acid: Creatinine Ratio as a biomarker.

Chapter 3 discusses the reaction kinetics involved in colorimetric detection of urinary uric acid and creatinine.

Chapter 4 describes the experimentation part of the thesis

Chapter 5 describes the experimental determination reaction rate constants for urinary uric acid and creatinine detection.

Chapter 5 reviews the qualitative and quantitative detection of urinary uric acid and creatinine.

Chapter 6 explores the effects of temperature on paper-based diagnostics.

Chapter 8 analyzes pathological trials

Chapter 9 introduces to the concept of Telemedicine and the application of proposed detection techniques in telemedicine.

The thesis ends with chapter 10 which provides the conclusion and recommendations for future work on the topic.

Chapter 2: Literature Review

2.1 Chronic Kidney Disease

Each kidney contains around 1 million nephrons. Nephrons are the renal functional unit which removes waste and excess water from the body [9, 11, 75]. Each nephron contains a filtering body, the glomerulus and a long tube made of differentiated segments [9]. The final parts of these tubules are interconnected to form the collecting ducts, which open into the renal pelvis (a funnel-shaped structure in each kidney through which urine is discharged into the ureter) [9, 76]. Apart from waste removal, kidney is also involved in immune function and regulation of several hormones [9, 11].

Kidney disease is defined as a heterogeneous group of disorders affecting kidney structure and function [9]. Common kidney diseases include, Hypertensive Nephrosclerosis, Kidney Stones, Glomerulosclerosis, Urinary Tract Infection (UTI), Diabetic Kidney Disease (DKD) and Analgesic nephropathy [11]. Prolonged occurrence (> 3 months) of any of these diseases can lead to decreased kidney function, kidney damage and eventually to chronic kidney disease (CKD) [9]. CKD is a global public health problem, which can lead to kidney failure, cardiovascular disease (CVD) and premature death (Appendix, Figure: A) [10]. Figure 1 illustrates a conceptual model of the association of kidney disease with adverse outcome [9, 10, 13, 77].

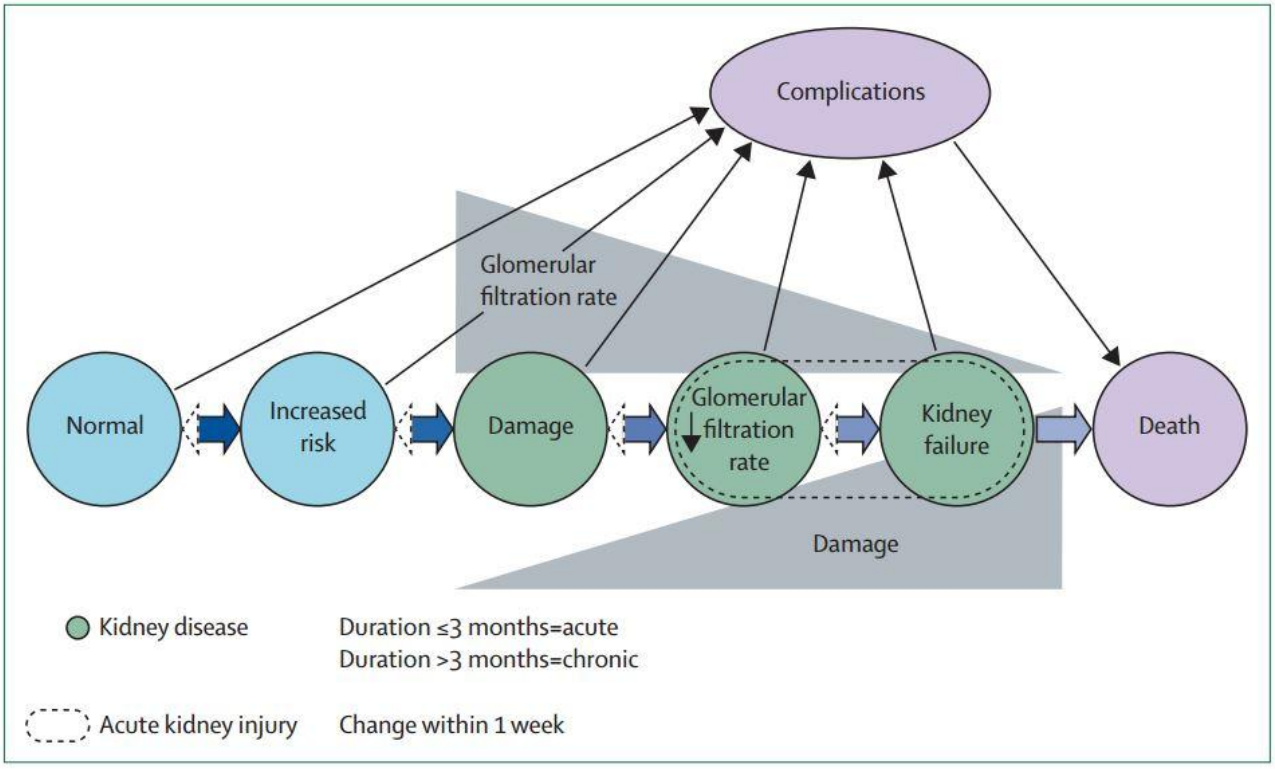


Figure 1: Factors associated with increased risk of kidney disease (blue), stages of disease (green), and complications (including death; purple) [9, 10, 13, 77]

Chronic kidney disease (CKD) is defined by a sustained reduction in glomerular filtration rate (GFR) or evidence of structural or functional abnormalities of the kidneys on urinalysis, biopsy, or imaging [78]. The prevalence of CKD is estimated between 8-16% worldwide [77, 79]. CKD was ranked 27th in the list of causes of total number of global deaths in 1990 (annual death rate 15.7 per 100000), but rose to 18th in 2010 (16.3 per 100000) [79]. According to world health organization (WHO), in 2012, an estimated 864226 deaths (1.5% of deaths worldwide) were attributed to CKD [12]. This death rate is expected to increase over the next few years [12]. Moreover, in 2012, CKD accounted for 1.1% of disability-adjusted life-years and 1.3% of life years lost globally [79]. Figure 2 represents the proportion of total mortality attributed to kidney disease in 2012 [12].

Another important outcome of CKD is end-stage renal disease (ESRD), requiring costly renal replacement therapy in the form of dialysis or transplantation. ESRD is a major cost driver for health-care systems [13, 14]. Over the past few decades, the annual growth of dialysis programs ranged between 6% to 12% and is continuing to grow, especially in the developing countries [14].

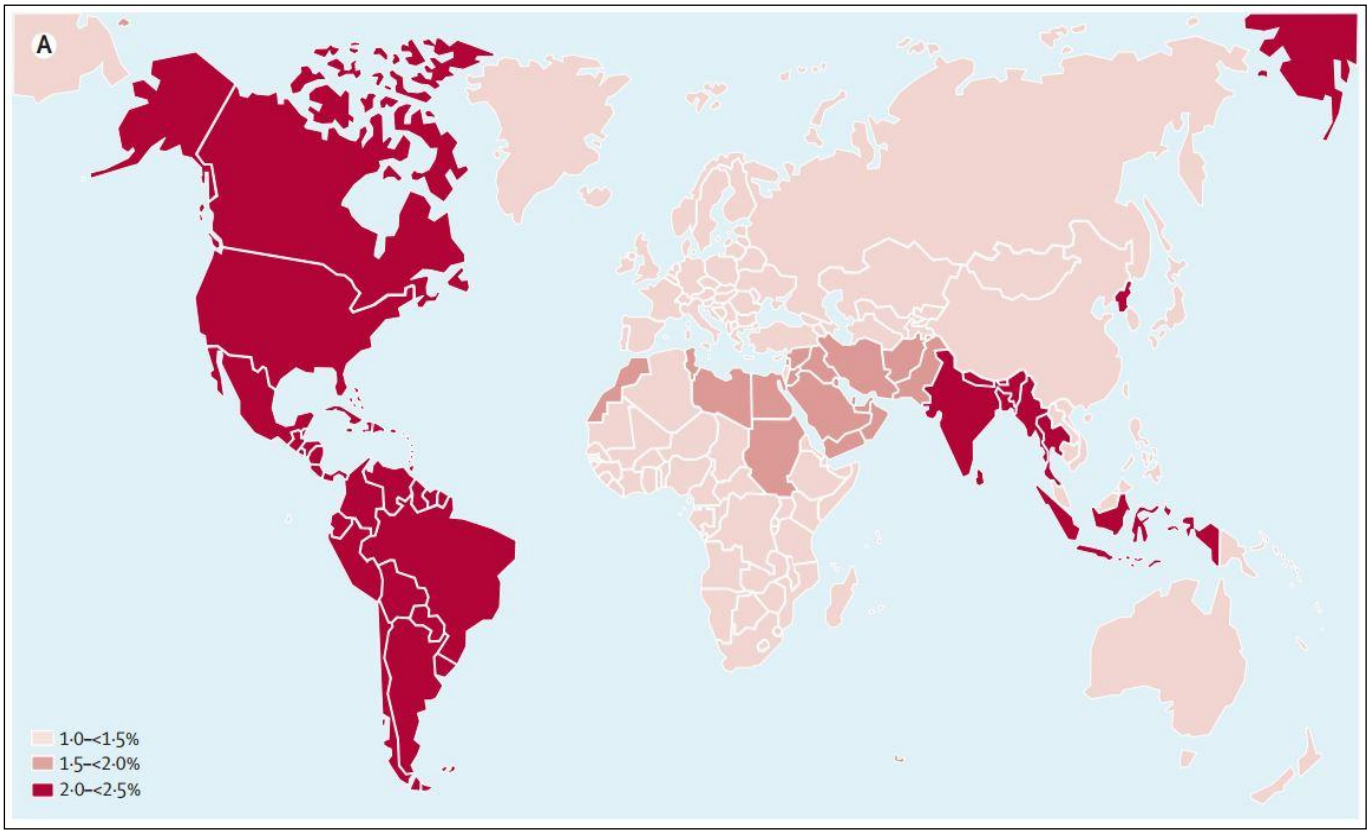


Figure 2: Proportion of total mortality attributed to kidney disease (2012) [12].

Common causes of CKD include diabetes, hypertension, inflammation and damage to glomerulus, genetic diseases (Adult Polycystic Kidney Disease), use of certain drugs, increased body-mass index (BMI), smoking, infectious diseases and exposure to chemicals such as lead, cadmium and mercury [9, 12, 78-80]. Age, hypertension, diabetes, increased BMI and smoking are the major cause of CKD in developed countries and in high income populations [9, 78, 79]. In the developing countries, diseases such as albuminurima,

glomerulonephritis and infectious diseases (tuberculosis, hepatitis B and C, malaria etc.) are major causes of CKD, in addition to the prior mentioned causes [9, 78, 79]. In Asia, Africa and Middle East, environmental and occupational exposure to lead, cadmium and mercury has also been attributed as potential cause for CKD [78].

2.2 Biomarkers for Kidney Diagnosis

The principle function of kidney is to remove waste and excess fluid from blood [9, 11, 18, 75]. To diagnose kidney condition, generally blood and urine tests are performed. Blood and urine tests can indicate kidney performance in removing body wastes, urine tests can also detect leakage of abnormal amount of proteins in urine, which is a signal of kidney injury [16-18]. In addition, imaging tests, such as Intravenous Urography, Angiography, Ultrasonography, Computed Tomography (CT scan), Magnetic Imaging Resonance (MRI) and Kidney Biopsy are performed to assess kidney condition [16, 18, 81].

Common biomarkers for kidney analysis in blood are: serum creatinine, urea nitrogen (BUN), glucose (fasting), parathyroid hormone and cystatin C [13, 15, 17, 18]. Serum creatinine is used to calculate glomerular filtration rate (GFR), an important indicator of CKD and serum Cystatin C level is closely correlated to cardiovascular disease (CVD) and CKD [13, 15, 21]. Common biomarkers for kidney analysis in urine are: urinary uric acid, creatinine and albumin [13, 15, 17, 19-21].

Urinary uric acid is an important indicator for kidney diagnosis. An increase in uric acid may indicate defects of uric acid transport in the nephron and renal under-excretion of uric acid [23, 27]. A higher level of urinary uric acid (>700 mg/day) is known as hyperuricemia [22-24, 27-30]. Hyperuricemia may lead to *de novo* renal disease as well as accelerate existing renal diseases (Appendix, Figure: C) [23, 27-30].

Urinary creatinine is also an important indicator for kidney analysis. The formation of creatinine has a direct relationship with total muscle mass and body weight and is reasonably constant throughout the day [33]. In patients with CKD, muscle wasting is prevalent. Therefore, urinary creatinine extraction can be used to relate arterial stiffness in CKD and subsequent relation between cardiovascular disease (CVD) and CKD [19]. Urinary albumin-

creatinine ratio can be used to diagnose Proteinuria (excess amount of protein in the urine) [18, 36], metanephrine-creatinine ratio for Pheochromocytoma and calcium-creatinine ratio for pregnancy induced hypertension [37, 38]. In addition, urinary creatinine excretion has been correlated with patients with CKD stages 3 and 4 [20].

Therefore, urinary uric acid and creatinine concentrations can be used as indicators to diagnose kidney condition.

2.3 Existing Detection Techniques for Urinary Uric Acid and Creatinine

Several analytical methods for detecting uric acid from human biofluids (urine, serum and saliva) have been reported. These techniques require sophisticated and expensive equipment such as, spectroscopy, chromatography, electrochemistry, membrane and capillary electrophoresis [22, 49-61]. The principle, technique, sample matrix and linear range of several detection methods by different research groups are described in Table 1. These techniques often require out sourcing/routing of samples to specialized laboratories, which makes these detection techniques expensive and time consuming.

Table 2 describes different detection methods for creatinine detection. Common detection techniques include spectroscopy, chromatography and capillary electrophoresis. As described in uric acid detection, these techniques require out sourcing of samples to specialized laboratories, which makes these detection techniques expensive and time consuming.

Hence, there is a need for instantaneous and low cost point-of-care detection technique capable for qualitative and quantitative detection of uric acid and creatinine in human biofluids. Paper based diagnostics, often used for health and environmental purposes [67-71]; offer an attractive option for detecting biomarkers such as uric acid and creatinine from biofluids such as urine, serum, sweat and/or saliva [68-71].

Conceptually, any colorimetric technique (spectrophotometric and chromatographic) can be re-modified and used to develop paper diagnostics [70, 95-98]. Among the techniques reported (Tables 1 and 2), the reduction of Fe (III)/ferricyanide into prussian blue in presence of uric acid [22] and standard Jaffe's reaction (production of orange pigment from sodium picrate in presence of creatinine) [33, 35] were selected for this study because of the low-cost and availability of the reactants and linear range of the resultant color signal produced.

Table 1: Different detection methods of uric acid from biofluids [22, 49-61]

Principle	Technique	Sample matrix	Linear range (ppm)
Enzymatic Uricase Method with 4-Aminodiphenylamine Diazonium Sulfate	Spectrophotometric	Urine	84-2185
Enzymatic Uricase Method with 4-Aminoantipyrine 1 mmol/l Peroxidase	Spectrophotometric	Urine, Serum	0-303
Enzymatic Uricase-Catalase Method	Spectrophotometric	Urine, Serum	0-319
Enzymatic Uricase-Peroxidase Method coupled with 3-methyl-2-benzothiazolinone hydrazone and N,N-dimethylaniline	Spectrophotometric	Serum	0-160
Enzymatic Uricase Method with Tribromophenol-Aminoantipyrine Chromogen	Spectrophotometric	Serum	0-319
Reduction of phosphotungstate ion to tungsten blue	Spectrophotometric	Urine, Serum	0-185
Reduction of Fe(III)/ferricyanide to Prussian blue	Spectrophotometric	Urine	0-17
Reduction of Cu(II) ions and complexation with 4,4'-dicarboxy-2,2'-bichinoline (BCA)	Spectrophotometric	Urine	2-17
Biosensor based on Uricase bound PVC membrane	Enzyme-membrane biosensor	Serum	0-100
Capillary Zone Electrophoresis	Capillary Electrophoresis	Urine	42-168
Electrochemical Detection	Electrochemical	Proof of concept	17-200
High-Performance Liquid Chromatography	Chromatographic	Saliva, Serum, Urine	1-27

Table 2: Different detection methods of creatinine from biofluids [62-66]

Principle	Technique	Sample matrix	Linear range (ppm)
O'Leary modified Jaffe	Spectroscopic	Serum	6.8-13.6 (male) 6.8-11 (female)
Compensated (rate blanked) kinetic Jaffe	Spectroscopic	Serum	7-12 (male) 5-9 (female)
Standard Kinetic Jaffe	Spectroscopic	Serum, Urine	8.4-12.4 (male) 6.6-10.9 (female)
Enzymatic formation of quinoneimine dye by the reaction between hydrogen peroxide and 4-aminoantipyrine and N-ethyl-N-sulfopropyl-m-toluidine in the presence of peroxidase.	Spectroscopic	Serum, Urine	0.5-1 (serum) 0.7-5 (urine)
Enzymatic Using creatininase/ creatinase/ sarcosine oxidase system	Spectroscopic	Serum, Urine	5-11 (male) 5-9 (female)
HPLC	Chromatographic	Serum, Urine	0-1131 (urine) 0-22624 (serum)
Separation of creatinine in a fused-silica capillary with H3PO4 (75 mmol/L, pH 2.5) as BGE, followed by UV detection at 200 nm.	Capillary Electrophoresis	Serum	0-100
Isotope dilution mass spectrometry	Spectroscopy	Serum	2-59.5

2.4 Paper-based Diagnostics (PADs) for Qualitative and Quantitative Detection of Uric Acid and Creatinine

Biosensors are defined as measurement devices that utilize chemical or biological reactions to detect and quantify a specific analyte or event [82]. An ideal biosensor should have high selectivity, specificity, stability and linearity, it should have simple calibration properties and it should be biocompatible [83]. According to the World Health organization, diagnostic devices should be ASSURED: affordable, sensitive, specific, user-friendly, rapid and robust [84, 85]. Recent developments in paper fluidics, microfluidics and nanotechnology have enabled scientists to create novel diagnostic devices which can be used on-spot and in low resource setting [83, 86-88]. Detection techniques based on enzymatic, immunoassay, flow cytometry and optofluidics have been used in these diagnostic devices [86, 87, 89-94]. Figure 3 illustrates some of the different platforms, detection techniques and diagnostic targets that have been developed recently for point of care diagnostics [83, 86, 87, 90-94].

Cellulosic paper can be a good option to fabricate low cost biosensors en-mass. Paper is lightweight, easy to process, inexpensive, biodegradable, biocompatible, combustible, sterilizable, allows passive liquid transport and easy to functionalize and fabricate [84, 92]. Consequently so, the interest on paper based biosensors has grown over the years. Paper has been used to produce diagnostic device to detect analytes from biofluids (blood, urine etc.), pesticide residue in food and feed products, water analysis and blood typing [70, 95-98].

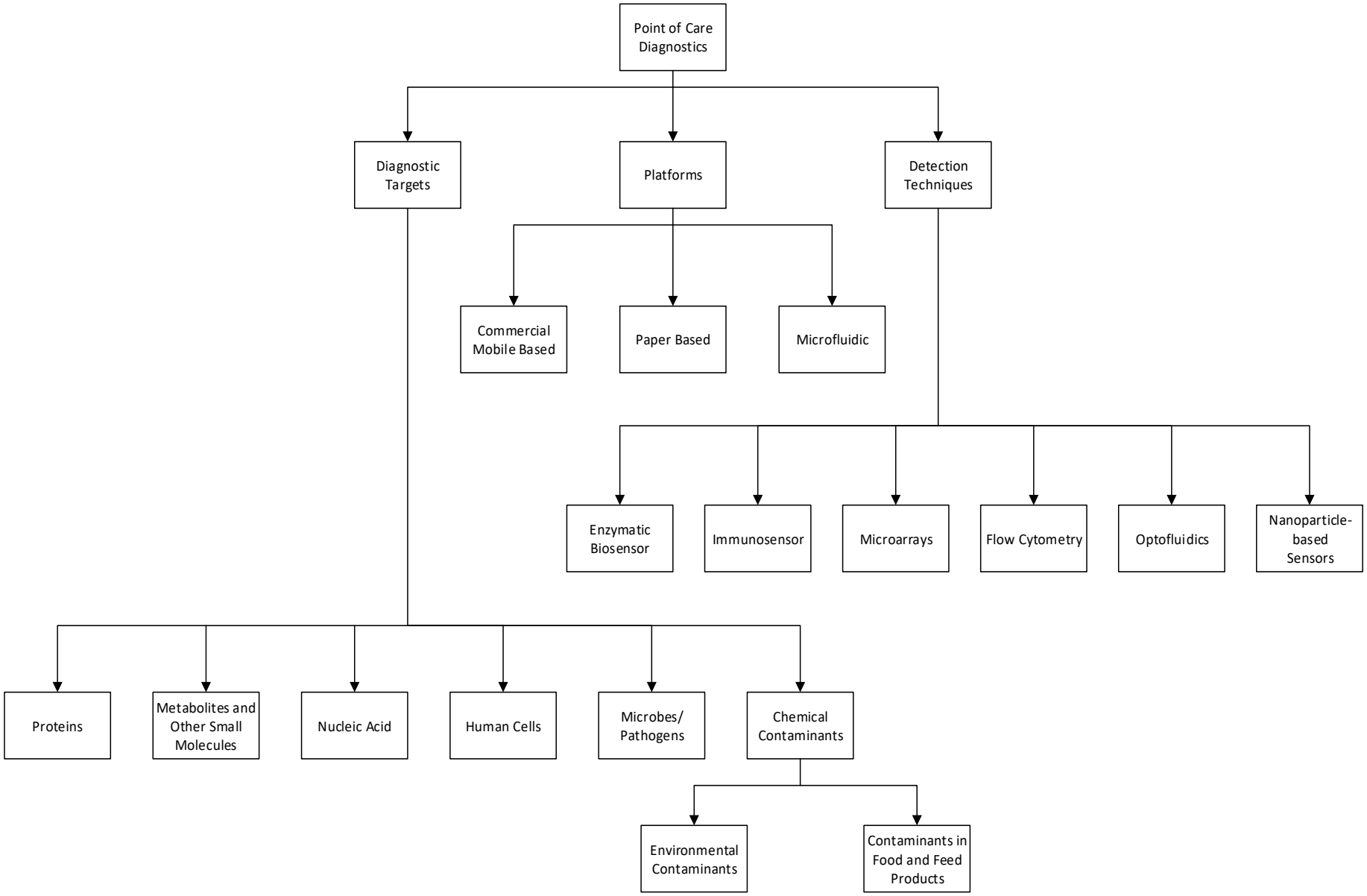


Figure 3: Platforms, detection techniques and diagnostic targets in point of care diagnostic devices [83, 86, 87, 90-94]

Using different fabrication techniques such as, lithography, wax patterning and printing, paper can be fabricated to produce 1-D, 2-D and 3-D structures which can be used as reactant reservoirs and reaction zones or can be utilized for fluidic manipulation such as transportation, sorting, mixing or separation [97, 99]. Chemical based reagents or biological reagents can be stored or immobilized into these structures by physical or chemical immobilization or through biochemical coupling [84, 97, 99]. These structures can then be used to detect infectious diseases, genetic conditions, environmental and food borne pathogens [84, 97, 99]. Paper based devices can also be utilized to detect biomarkers from biofluids such as blood and urine and can be used for DNA detection [84]. When coupled with electronic devices paper diagnostics can be used in telemedicine [90, 97]. Android or IOS based applications can be developed to quantify analytes on paper based devices such calculating the color intensity in a colorimetric reaction on paper [89, 90, 97].

Thus, paper can be used as a platform to develop low cost diagnostic device for rapid detection of kidney biomarkers such as urinary uric acid and creatinine. Colometric reactions can be used to detect uric acid and creatinine on paper surface. The paper surface can be functionalized with reagents for uric acid and creatinine. Urine samples can then be applied on the paper surface. The reagents will react with urinary uric acid and creatinine and will produce color signal. The higher the concentration of in the sample, the stronger the color signal it will produce on paper. This color signal can be quantified using image processing software. Based on the color intensity, calibration curves and gradient papers can be developed for qualitative and quantitative detection of urinary uric acid and creatinine.

2.5 Kidney Diagnosis through Paper-based Diagnostics

One of the major objectives of this study is to use the values of urinary uric acid and creatinine concentration to diagnose kidney condition. Graphical representation of urinary uric acid and creatinine ranges can be used to diagnose kidney condition. Results obtained from the proposed on-spot test can be compared with published concentration limits, illustrated in the graph and based on the comparison; decision about kidney condition can be made.

Different research groups have reported the range of human urinary uric acid concentration to be in-between 300 to 700 ppm [22, 24, 100]. According to Heil W. and Ehrhardt V., range creatinine concentration in human urine is 390 to 2590 ppm [101]. Using these values, figure 4 was created which can be used for on-spot kidney diagnosis.

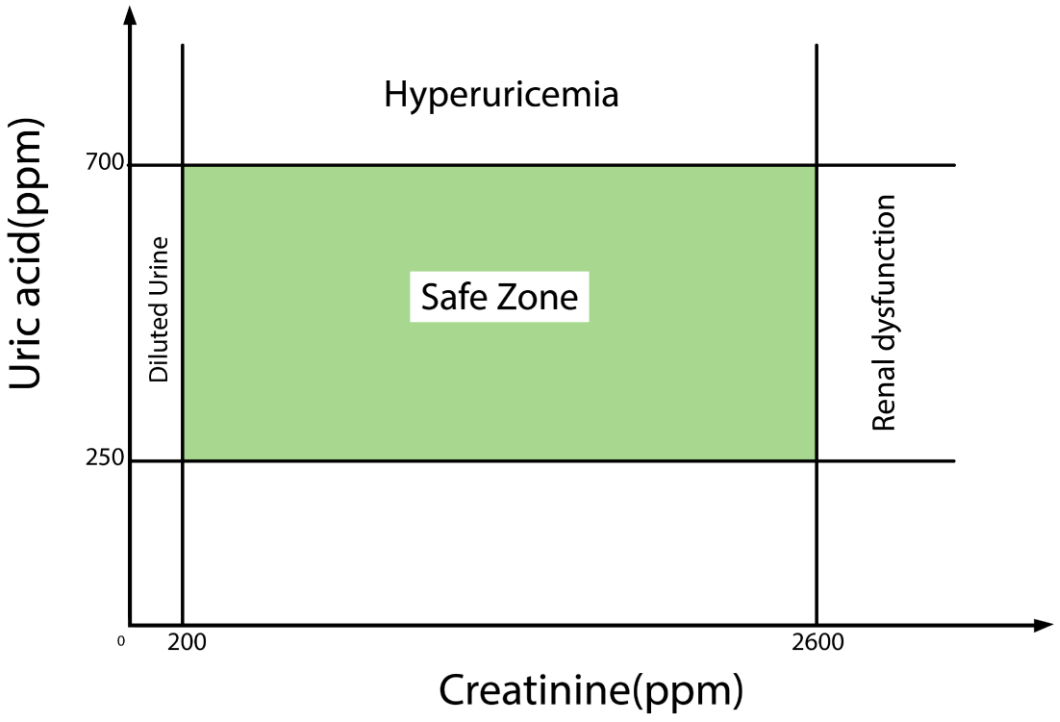


Figure 4: Diagnosis of kidney condition using urinary uric acid and creatinine concentration

In the figure, x-axis represents the urinary creatinine concentration (in ppm) whereas the y-axis represents concentration of uric acid in urine (in ppm). The region between 250 to 700 ppm uric acid and 200 to 2600 ppm creatinine concentration can be represented as the safe zone and in the figure is designated with green color. Any values outside of the safe zone should be interpreted as uncommon situation.

Urinary uric acid concentration higher than 700 mg/day is known as hyperuricemia [22-24, 27-30]. Hyperuricemia may disrupt renal function as well as accelerate existing renal diseases [23, 27-30]. In addition, hyperuricemia is a precursor of gout, and is also associated with the development of type 2 diabetes, obesity, Lesch-Nyhan Syndrome and arteriolar hypertension which may lead to cardiovascular disease [22-24, 27-32]. Therefore, patients with type 2 diabetes, Lesch-Nyhan Syndrome and gout, can use the proposed on-spot test and graph (Figure 4) to monitor their kidney condition on a regular basis.

Urinary creatinine is an important indicator of kidney condition. The normal range for urinary creatinine in adult human being is between 390 to 2590 ppm [101]. Any values outside of this range may indicate renal disfunction such as necrosis tubulus, glomerulonephritis and low glomerular filtration rate (GFR) [19, 20, 34, 35]. Urine creatinine concentration declines gradually for patients with chronic kidney disease (CKD) stages 3 and 4 [20]. In addition, on-spot urinary creatinine concentration is widely used as an indicator of sample abuse for urine samples used in drug tests. A urinary creatinine concentration lower than 200 ppm indicates that the urine sample has been diluted and the sample cannot be used for further testing [39-42]. Therefore, patients with stage 3 and 4 CKD, low GFR and other renal complications can use figure 16 to monitor their urine creatinine level. In addition, law enforcement agencies can use the proposed on-spot test to measure urine creatinine level and determine whether the sample was diluted or not and can be used for further analysis.

Furthermore, doctors treating patients with renal complicacies in resource scarce areas can use the proposed on-spot test and graph (figure 4) to monitor the kidney condition of their patients regularly in a rapid and cost-effective manner.

Based on the reasoning explained above, the proposed tests can be used as simple, rapid and cost-effective alternative for existing tests to monitor kidney condition.

2.6 Urinary Uric Acid: Creatinine Ratio as a Marker of Other Diseases

2.6.1 Diagnosis of Perinatal Asphyxia

Hypoxemia can be defined as lack of oxygen in blood supply [102]. Perinatal Asphyxia is a special type of hypoxemia which occurs in new born babies. It is a condition characterized by an impairment of exchange of the respiratory gases (oxygen and carbon dioxide) resulting in hypoxemia accompanied by metabolic acidosis [102]. It is one of the major causes of stillbirth, the third most common cause of newborn death (23%) and may cause permanent neuropsychological handicaps in the form of learning disabilities, epilepsy, cerebral palsy, and with or without associated mental retardation [43, 44, 102]. Therefore, a rapid test to detect Perinatal Asphyxia will be invaluable for doctors, pediatricians and parents around the world. Recent studies have indicated close relationship between urinary uric acid to creatinine ratio with Perinatal Asphyxia [43-46]. Asphyxia causes tissue injury which in turn initiates various metabolic activities that increases uric acid production. Therefore, children suffering from Perinatal Asphyxia will have a higher urinary uric acid to creatinine ratio than normal children. Works published by Patel et al., Choudhary et al. and Bhongir et al. have all indicated higher urinary uric acid to creatinine ratio in newborns suffering from Perinatal Asphyxia than normal newborns [43-45]. Table 3 indicates their findings.

The proposed paper based technique offers a simple, rapid and cost effective way to detect urinary uric acid and creatinine. Doctors and pediatricians, therefore, can use this technique to detect urinary uric acid and creatinine in newborns and diagnose Perinatal Asphyxia.

Table 3: Comparison of urinary uric acid to creatinine ratio in newborns suffering from Perinatal Asphyxia with normal newborns

Normal Infants [43-45]	Infants With Perinatal Asphyxia [43-45]
0.64±0.48 to 1.89±0.59	2.58±0.48 to 3.1±1.3

2.6.2 Diagnosis of Sleep Hypoxemia

Another important application for the proposed detection technique is the diagnosis of sleep hypoxemia. Studies have shown a positive change in overnight uric acid to creatinine ratio occurs in urine samples of patients suffering from sleep hypoxemia (Lack of oxygen in blood during sleep) [47, 48]. As the proposed diagnostic technique offers a rapid and cost effective alternative to common nocturnal hypoxemia detection technique [47], the paper based technique can be used to monitor overnight uric acid to creatinine ratio. The subsequent rate of change of this ratio can be used to diagnose sleep hypoxemia.

Chapter 3: Reaction Kinetics

3.1 Uric Acid

The formation of prussian blue from ferric chloride and potassium ferricyanide in the presence of uric acid can be explained by the following mechanism [72]:

At first, potassium ferricyanide reacts with uric acid ($C_5H_4N_4O_3$) to form potassium ferrocyanide [103]

$$C_5H_4N_4O_3 + K_3[Fe(CN)_6] \longrightarrow K_4[Fe(CN)_6]$$

Uric acid	Potassium ferricyanid	Potassium ferrocyanide

Potassium ferrocyanide then reacts with ferric chloride to form prussian blue [104, 105]. This reaction takes place in several steps. At first, potassium ferrocyanide reacts with Fe^{3+} ion to form $HFe[Fe(CN)_6]$. There are major and minor paths for $HFe[Fe(CN)_6]$ formation [104, 105].

Major paths:

$$Fe^{3+} + [Fe(CN)_6]^{4-} \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} Fe[Fe(CN)_6]^{-} \text{ (Fast)} \quad \ldots\ldots(1)$$

$$H^{+} + Fe[Fe(CN)_6]^{-} \xrightarrow{K_2} HFe[Fe(CN)_6] \text{ (Slow) Rate limiting} \quad \ldots\ldots(2)$$

Minor Paths:

$$H^{+} + [Fe(CN)_6]^{4} \underset{K_{-3}}{\overset{K_3}{\rightleftharpoons}} H[Fe(CN)_6]^{3-} \quad \ldots\ldots(3)$$

$$Fe^{3+} + H[Fe(CN)_6]^{3-} \xrightarrow{K_4} HFe[Fe(CN)_6] \text{ (Slow)} \quad \ldots\ldots(4)$$

After the formation of $HFe[Fe(CN)_6]$, three molecules of $HFe[Fe(CN)_6]$ and one Fe^{3+} ion slowly assemble to form insoluble prussian blue with a face centered cubic (FCC) structure [105].

$$3HFe[Fe(CN)_6] + Fe^{3+} \underset{K_{-5}}{\overset{K_5}{\rightleftharpoons}} \underset{\text{Prussian blue}}{Fe_4[Fe(CN)_6]_3} + 3H^+ \quad \text{.....(5)}$$

Based on the proposed mechanism, a rate equation for the formation of Prussian blue can be expressed as:

$$\frac{d[HFe[Fe(CN)_6]]}{dt} = K_2[H^+][Fe[Fe(CN)_6]^-] \quad \text{.....(6)}$$

Assuming quasi-equilibrium state in equations (1) and (3) [106], and assuming concentrations of H^+ and Fe^{3+} ions are in excess, equation (6) can be written as,

$$r = K\,[Fe(CN)_6]^{4-} \quad \text{.....(7)}$$

Where, the pseudo-first order reaction constant, $K = \frac{K_1 K_2}{K_{-1}}[H^+][Fe^{3+}]$

Thus, the reaction kinetics can be expressed as:

$$H^+ + Fe[Fe(CN)_6]^- \longrightarrow HFe[Fe(CN)_6]$$

t = 0	a	0
t = t	a-x	x

This model can be described by [107]:

$$2.303 \times \log (a-x) = -Kt + c$$

Hence, a straight line of log(a-x) vs. time will indicate that the formation of prussian blue from ferric chloride and potassium ferricyanide in the presence of uric acid follows first order reaction kinetics [107].

3.2 Creatinine

A proposed reaction mechanism for the reaction between urinary creatinine and sodium picrate (NaOH and picric acid solution) can be expressed by the following reactions [33, 73, 74]:

At first, picric acid reacts with NaOH to form sodium picrate

$$\text{Picric acid} + (\text{OH})^- \xrightarrow{K_0} (\text{Picrate} - \text{OH}^-)^* \text{ [Rapid]}\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

This sodium picrate then reacts with creatinine to produce a picrate-creatinine complex which gives the orange-red color.

$$(\text{Picrate} - \text{OH}^-)^* + \text{Creatinine} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} (\text{Picrate} - \text{Creatinine Complex}) + (\text{OH}^-)$$

[Rate Limiting Step]……...(2)

One of the major challenges of this reaction mechanism is the determination of the structure of picrate-creatinine complex. Different research groups have proposed different structures for the picrate-creatinine complex [33, 73, 108, 109]. Some of which are indicated in figure 5.

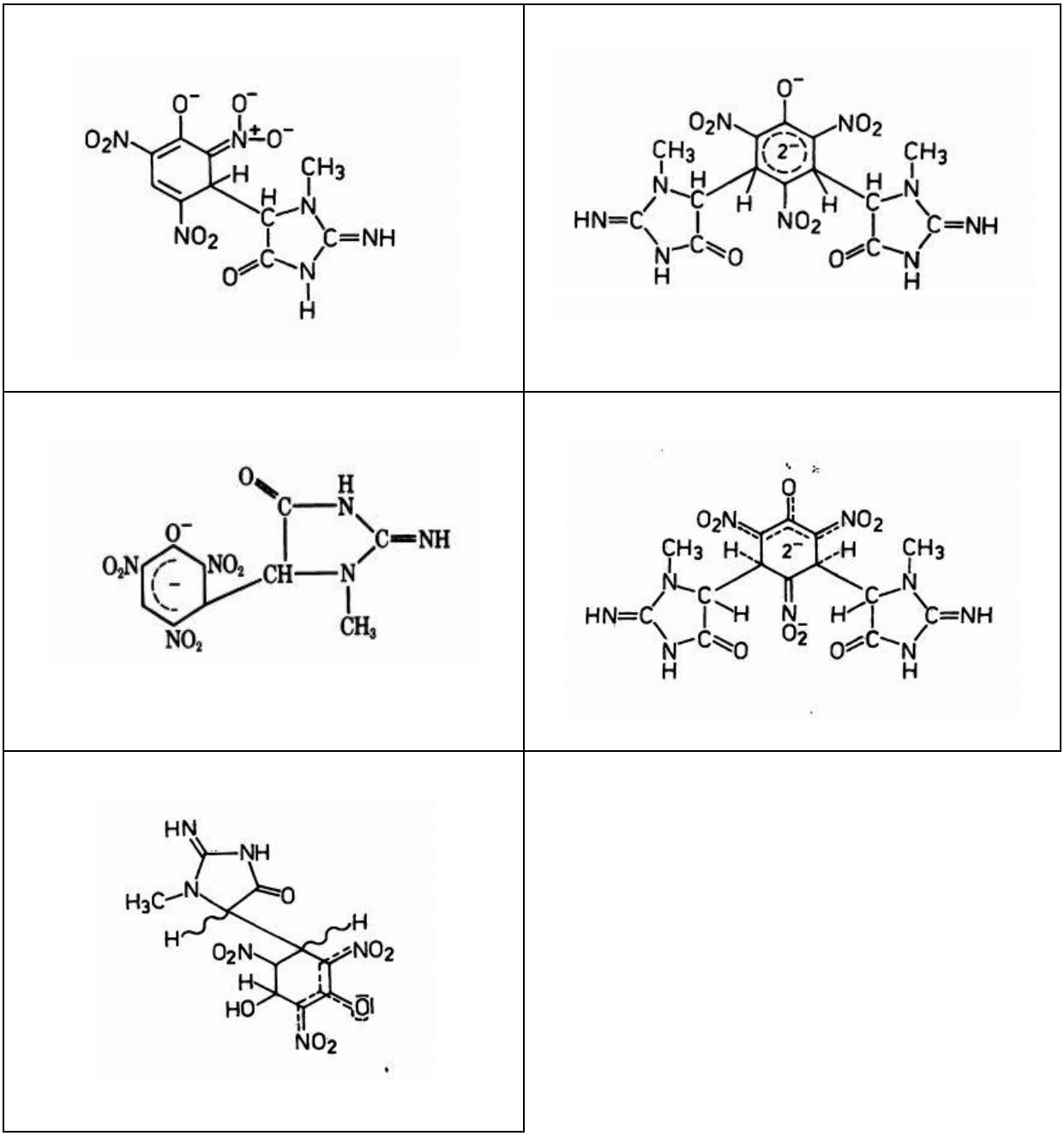


Figure 5: Different Structures of picrate-creatinine complex as described by different groups [33, 73, 108, 109]

The overall reaction can be written as:

$$\text{Picric acid} + (OH)^- \xrightarrow{K_0} (\text{Picrate} - OH^-)^* + \text{Creatinine} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} (\text{Picrate} - \text{Creatinine Complex}) + (OH^-)$$

t=0	a	0
t=t	a-x	x

Where, ‘a’ is the concentration of sodium picrate and ‘x’ is the product (picrate-creatinine complex) concentration at time ‘t’. As the reaction between sodium picrate and creatinine is the rate limiting reaction, so the concentration of sodium picrate will limit and govern the reaction rate [33, 73, 74].

This model can be described by [107]:

$$2.303 \times \log (a-x) = - K t + c$$

So if the slope of 2.303log(a-x) vs time plot is a straight line, we can say that the formation of picrate-creatinine complex from sodium picrate in the presence of creatinine follows first order reaction kinetics [107].

Chapter 4: Experimentation

4.1 Materials

For experimentation, reagent grade uric acid, creatinine (Sigma Aldrich, Germany), potassium ferricyanide (Carl Roth GmbH, Germany), ferric chloride (Loba-chimie, India), picric acid, sodium hydroxide (Merck, Germany) and ultra-high purity de-ionized water (18.2MΩ.cm, Purite, UK) were used. The artificial urine was prepared as described by Martinez et al. [110, 111]. Reaction kinetics images were captured using a Sony Alpha 57 DSLR camera (Sony Corp., Japan). The temperature and humidity was measured using Palmer Hygrometer (Palmer, USA). For calibration curve, the color produced on the paper surface was measured at 600 dpi using a standard scanner (canon lide 120). The images were analyzed using ImageJ software (ImageJ 1.47t). ImageJ calculates the weighted average gray value within a selected section of an image which can be related to the concentration of uric acid on the activated paper. Adobe Photoshop Lightroom 3.6 (Adobe systems, USA) was used to create the gradient paper. A phoenix RSM 65H hot plate (Phoenix instruments, Germany) was used to measure relationship between changes in color intensity with temperature. Throughout the experiments, Whatman 1 filter paper was used.

4.2 Experimental Steps

4.2.1 Determination of Reaction Kinetics and Order

To determine the reaction order and kinetics, images were captured using a Sony Alpha 57 DSLR camera on video mode. The video clips were transferred to a computer and converted into JPEG files (30 frames per second) using a video to JPEG converter (v. 5.0.99, DVDVideoSoft Ltd. USA). The converted images were analyzed using ImageJ software (ImageJ 1.41t). ImageJ calculates the gray values of RGB (red-green-blue) images. For any selected area, the ImageJ software calculates the weighted average gray value within the

selection, which can be related to the activity of the treated paper. Figure 6 indicates the steps followed to measure reaction rate.

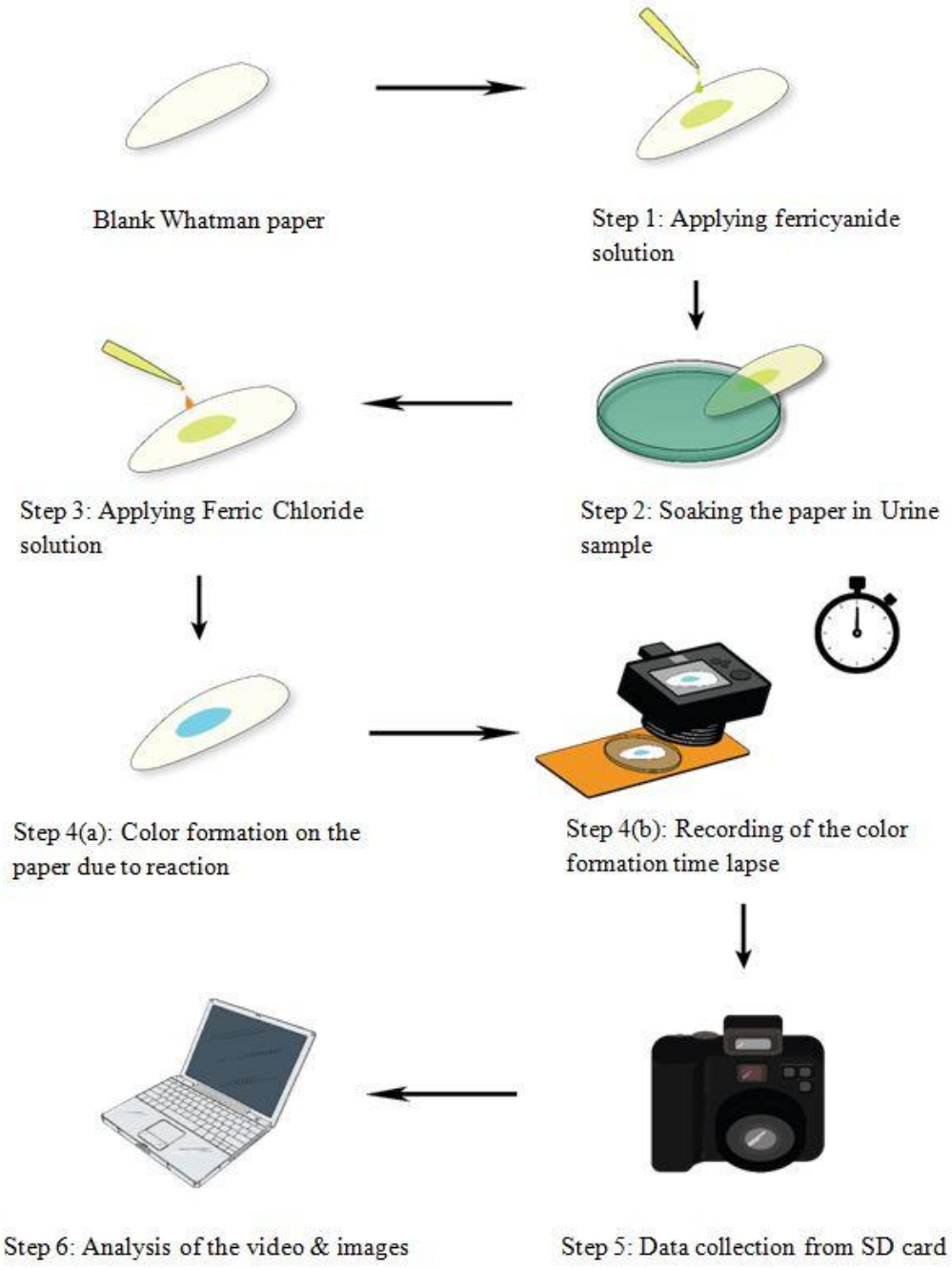


Figure 6: Schematic representation of measurement of reaction kinetics

4.2.2 Uric Acid Detection

Figure 7 illustrates the schematic representation of uric acid detection using paper diagnostic technique. Using a micropipette, potassium ferricyanide solution was applied on Whatman 1 filter paper (Diameter: 2cm). Then, the treated paper was socked in urine sample (5 mL). Potassium ferricyanide reacts with uric acid in urine to form potassium ferrocyanide. Finally, ferric chloride solution was applied on the treated paper. Ferric chloride reacts with potassium ferrocyanide to form Prussian blue and produce color signal.

For the qualitative and quantitative determination of uric acid in urine, potassium ferricyanide treated paper samples were dipped into artificial urine samples containing different concentrations of uric acid. The artificial urine samples were prepared as described by Martinez et al. [110, 111]. Then, ferric chloride was applied on the treated paper samples. The paper samples developed color signals. These color signals were scanned and analyzed using standard image processing software (ImageJ 1.41t). The intensity of the color signals produced from the reaction of uric acid and activated paper samples were varied according to the strength of the uric acid in urine solution. Finally, a calibration curve and a gradient paper were produced for the qualitative and quantitative detection of uric acid in urine.

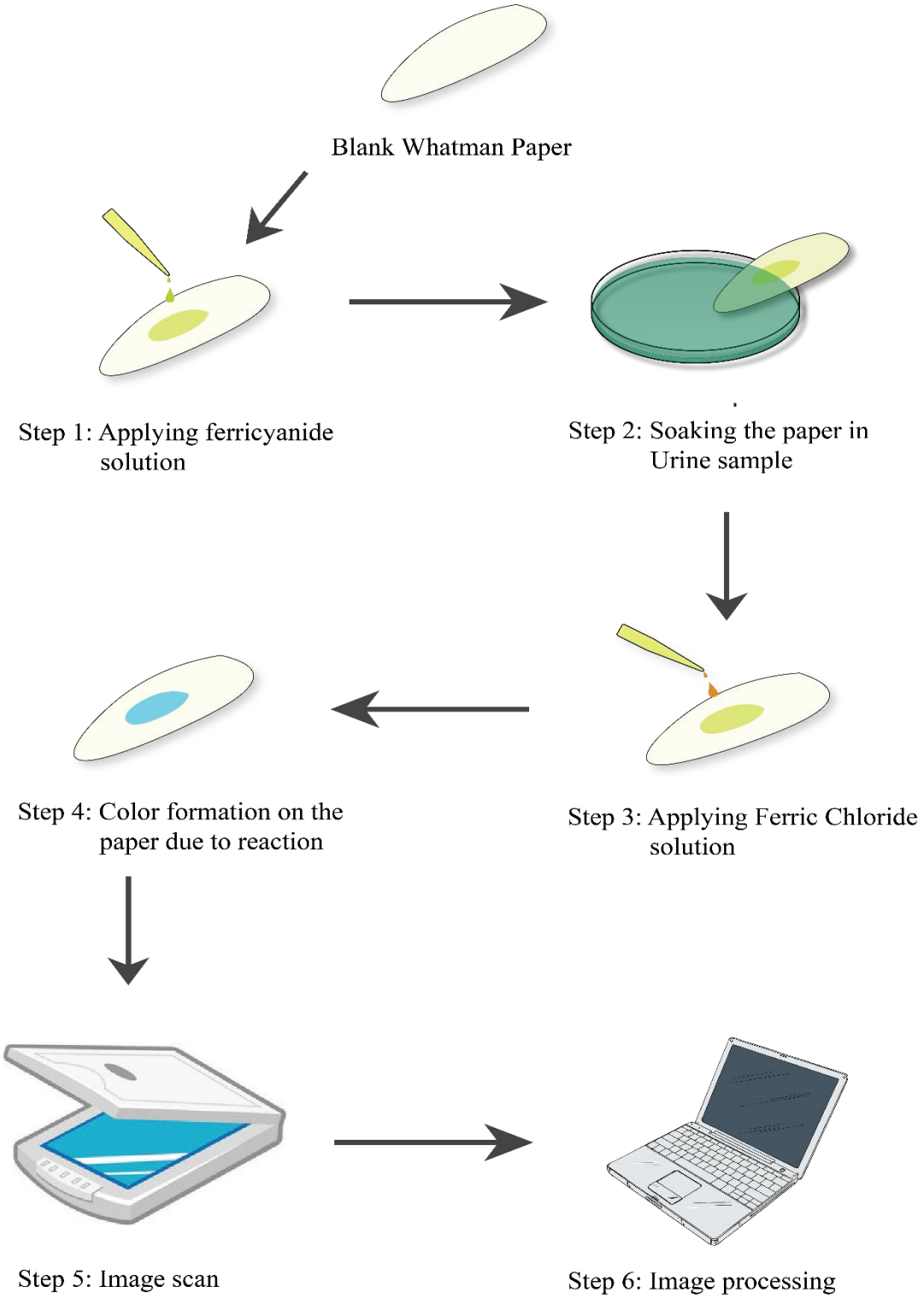


Figure 7: Schematic representation of uric acid detection using paper diagnostic technique

4.2.3 Creatinine Detection

Figure 8 illustrates the schematic representation of creatinine detection using paper diagnostic technique. Picric acid solution was mixed with Sodium Hydroxide. Using a micropipette, this solution was applied on the paper surface (Diameter: 2cm). Then, the treated paper was socked in urine sample (5 mL). Sodium picrate reacted with urinary creatinine to form picrate-creatinine complex and produce orange color signal.

Creatinine in urine sample was determined qualitatively and quantitatively by following the steps as described in uric acid detection.

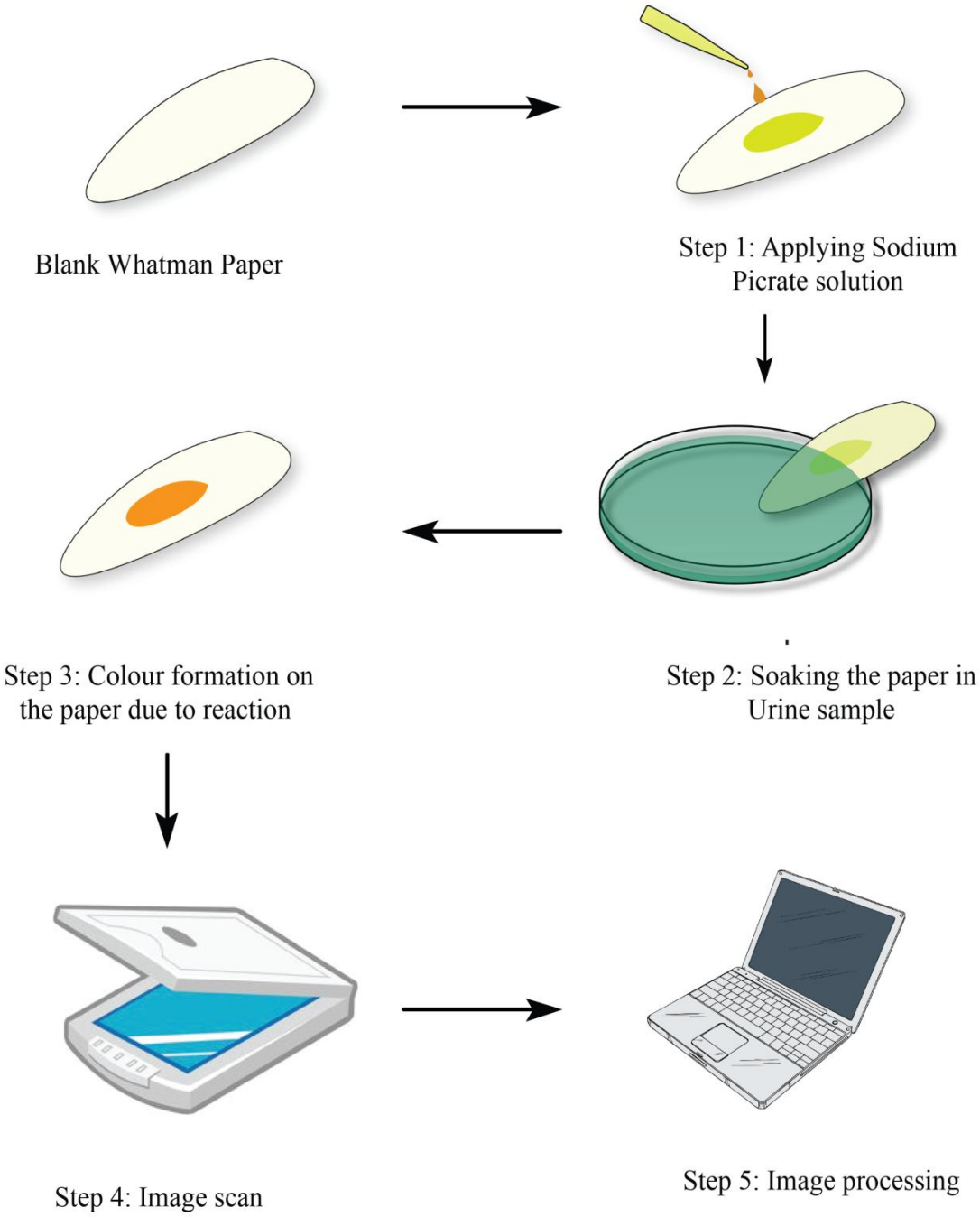


Figure 8: Schematic representation of creatinine detection using paper diagnostic technique

4.3 Digital Analysis for Telemedicine

Patients in remote and underdeveloped areas and patients, who need to monitor their uric acid level regularly, can use their mobile phone to take picture of the diagnostic paper and send it to established medical facilities for further analysis. To demonstrate this concept, a computer code was developed for digital analysis of proposed paper based diagnostic kit. MATLAB (Mathworks, r2013a) was used to develop the code. Using a mobile phone (SAMSUNG core 2), images of the color developed on paper surface was taken. Image analysis tool of MATLAB can analyze the color intensity of any image and produce a three dimensional matrix, which quantifies the color intensity at different points of the image in RGB (Red, green and blue). A code was generated to calculate uric acid concentration from this matrix.

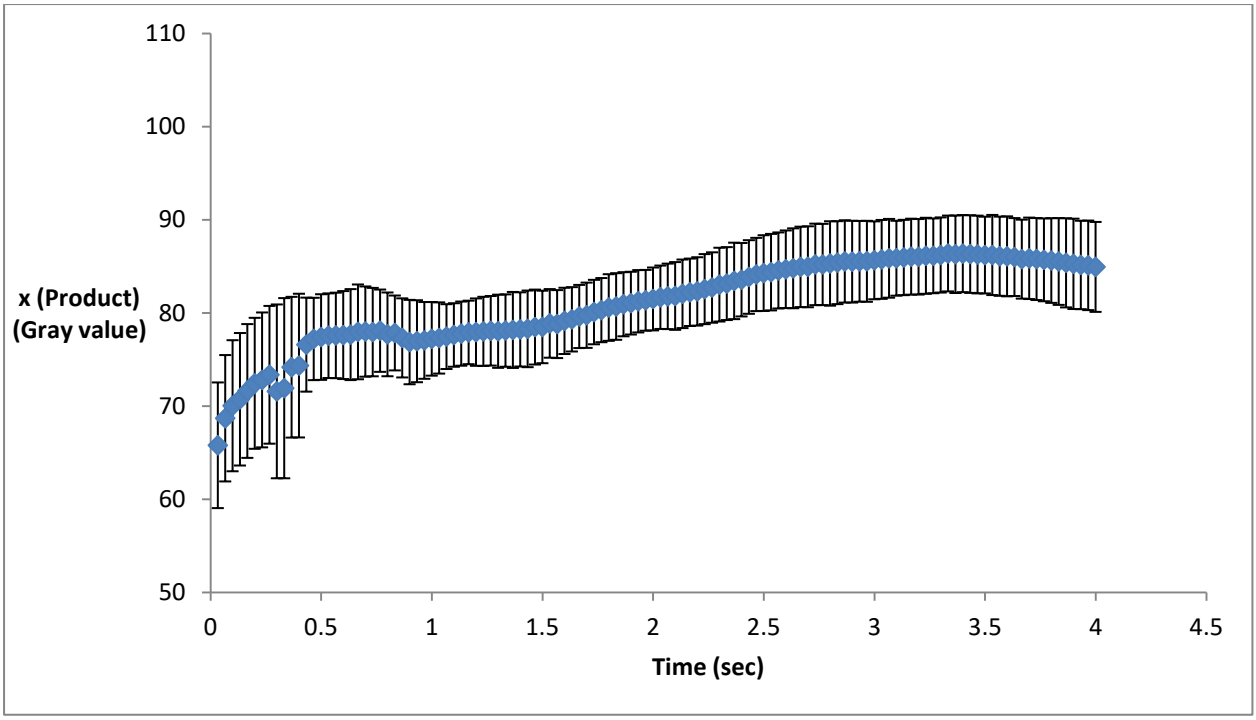
Chapter 5: Experimental Determination of Reaction Rate Constants

5.1 Uric Acid

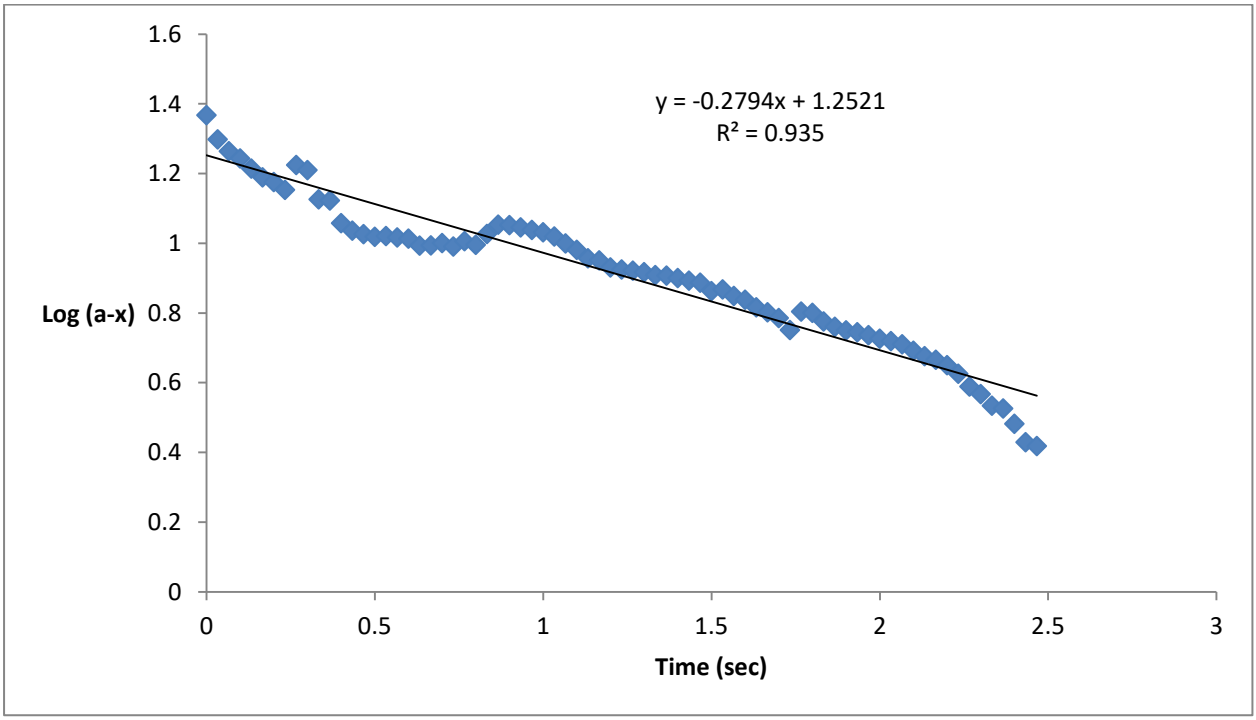
Figure 9 illustrates the reaction kinetics of color formation on paper surface. Figure 9 (a) and 9 (c) indicates the formation of prussian blue (x) on activated paper as a function of time. The color intensity of the product increased non-linearly as a function of time. Figure 9 (b) represents the relationship between log (a-x) as a function of time for the formation of prussian blue color on activated paper. The straight line from Figure 9 (b) conforms that the reaction follows first order reaction mechanism. From the Figure 9 (b), the reaction rate constant, *K* can be calculated as 0.64 sec^{-1} (Temperature: 30^0C). The calculated value is slightly higher than reported result based on liquid solution (Table 4) [72], which indicates that the reaction is slightly faster on paper than in liquid solution. This increase in reaction rate is due to the presence of paper channel networks. These networks facilitate reactions by providing large interfacial areas for the reactants to react with each other [85, 112].

Table 4: Comparison of calculated value and published value of *K* (Temperature: 30^0C)

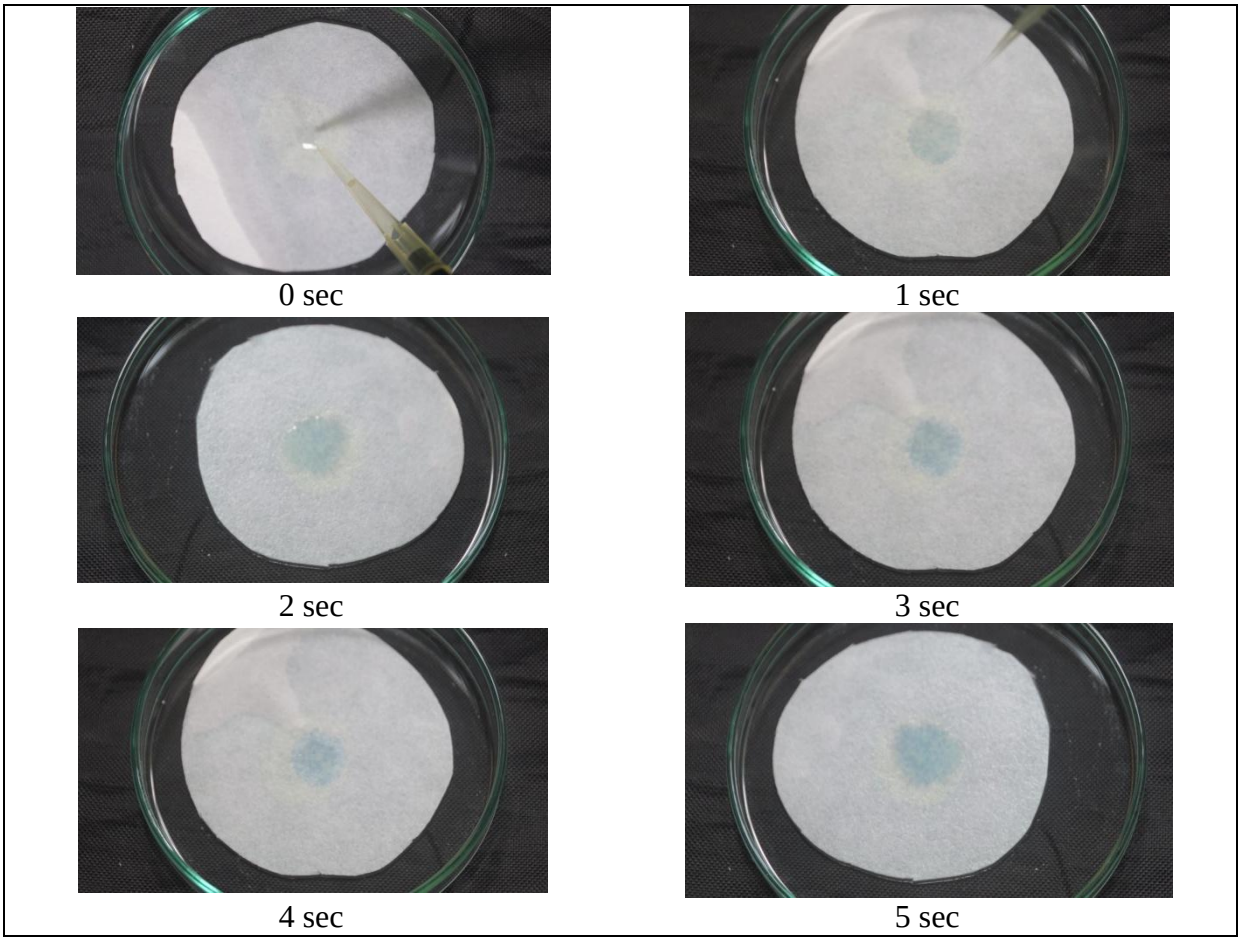
Calculated value (sec^{-1})	**Published value (sec^{-1}) [72]**
0.64	0.47


110
100
90
80
70
60
50
x (Product)
(Gray value)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Time (sec)

(a)


y = -0.2794x + 1.2521
R² = 0.935
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Log (a-x)
0
0.5
1
1.5
2
2.5
3
Time (sec)

(b)



(c)

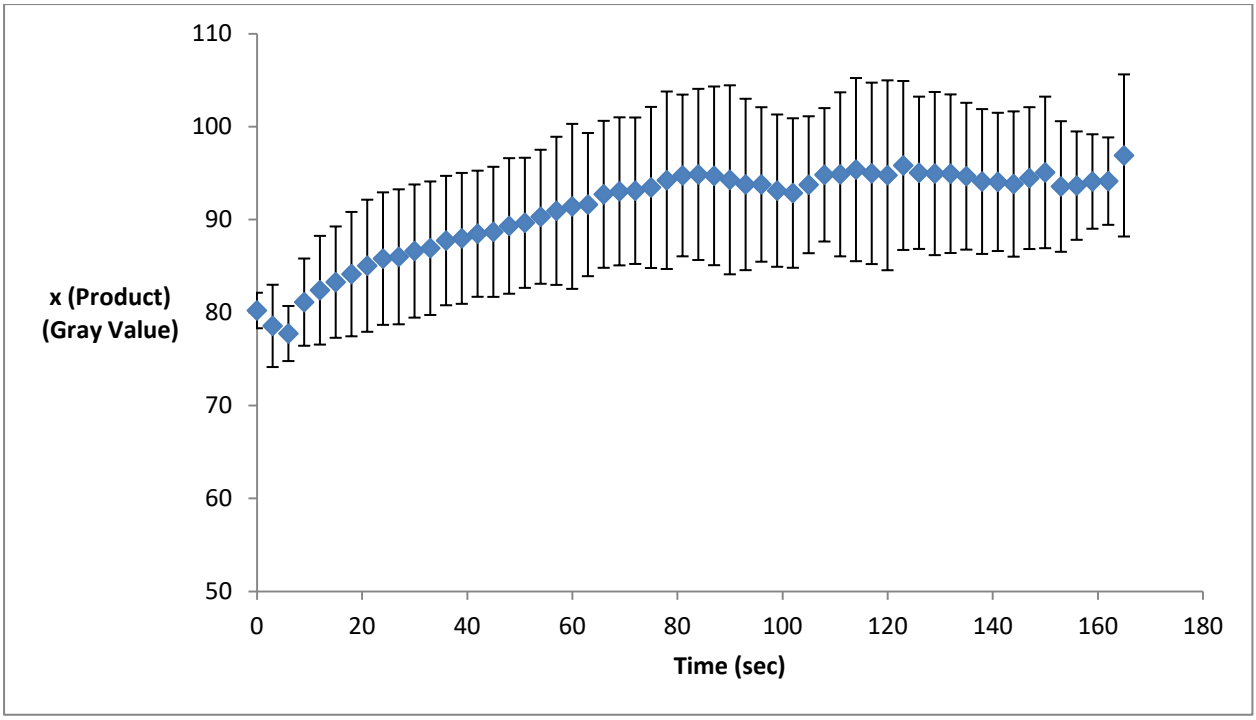
Figure 9: (a) Formation of Prussian blue on activated paper as a function of time. (b) Prussian blue reaction kinetics on activated paper (Standard deviation bars for n=5 samples). (c) Formation of Prussian blue on activated paper at different times.

5.2 Creatinine

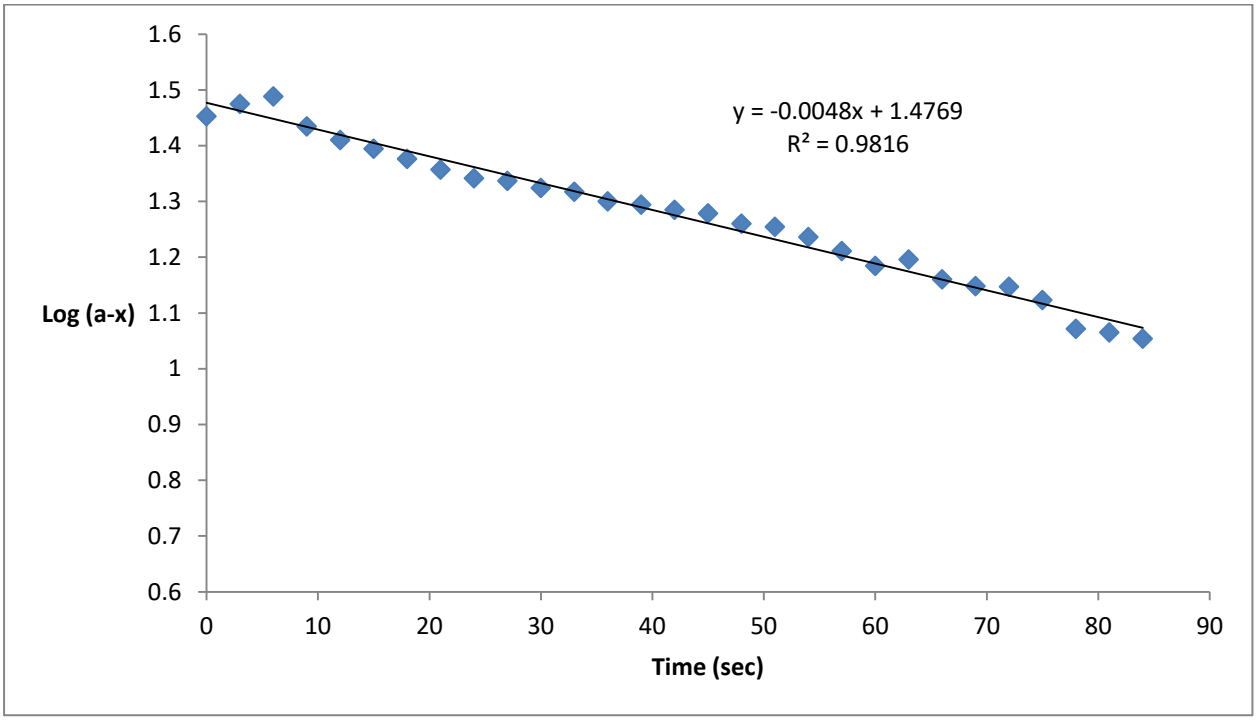
Figure 10 illustrates the reaction kinetics of color formation on paper surface. Figure 10 (a) indicates the formation of picrate-creatinine complex (x) on activated paper as a function of time. The color intensity of the product increased non-linearly as a function of time. Figure 10 (b) represents the relationship between log (a-x) as a function of time for the formation of prussian blue color on activated paper. The straight line from Figure 10 (b) conforms that the reaction follows first order reaction mechanism. From the Figure 10 (b), the reaction rate constant, *K* can be calculated as 0.011 sec^{-1} (Temperature: 30^0C). The calculated value is higher than reported result based on liquid solution (Table 5) [73], which indicates that the reaction is faster on paper than in liquid solution. This increase in reaction rate is due to the presence of paper channel networks. These networks facilitate reactions by providing large interfacial areas for the reactants to react with each other [85, 112].

Table 5: Comparison of calculated value and published value of *K* (Temperature: 30^0C)

Calculated value (sec^{-1})	**Published value (sec^{-1}) [73]**
0.011	0.005



(a)



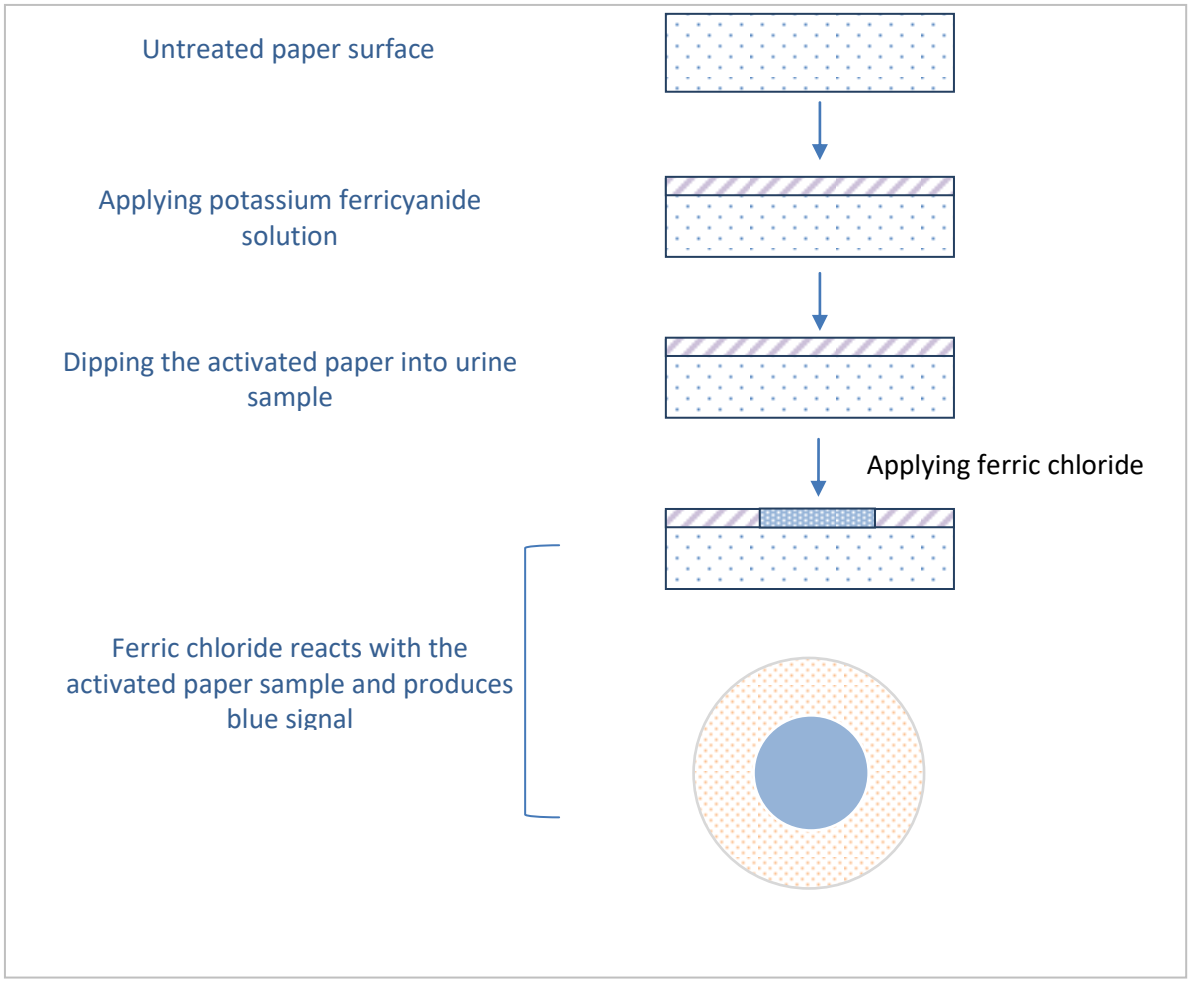
(b)

Figure 10: (a) Formation of picrate-creatinine complex on activated paper as a function of time. (b) Picrate-creatinine reaction kinetics on activated paper (Standard deviation bars for n=5 samples).

Chapter 6: Qualitative and Quantitative Determination

6.1 Qualitative Detection of uric acid in urine sample

To develop point-of-care paper diagnostics paper samples were treated with different reagents to make it reactive with uric acid in urine. Uric acid reacted with reagent treated paper samples and produced blue color signals on the paper surface; the higher the uric acid concentration in the sample, the stronger the color signal it produced on paper. Figure 11 represents schematic diagrams of treating paper surface with different reagents to develop paper diagnostics for uric acid detection (Figure 11 a), and experimental results of detecting of uric acid in urine sample using paper diagnostics (Figure 11 b).



(a)

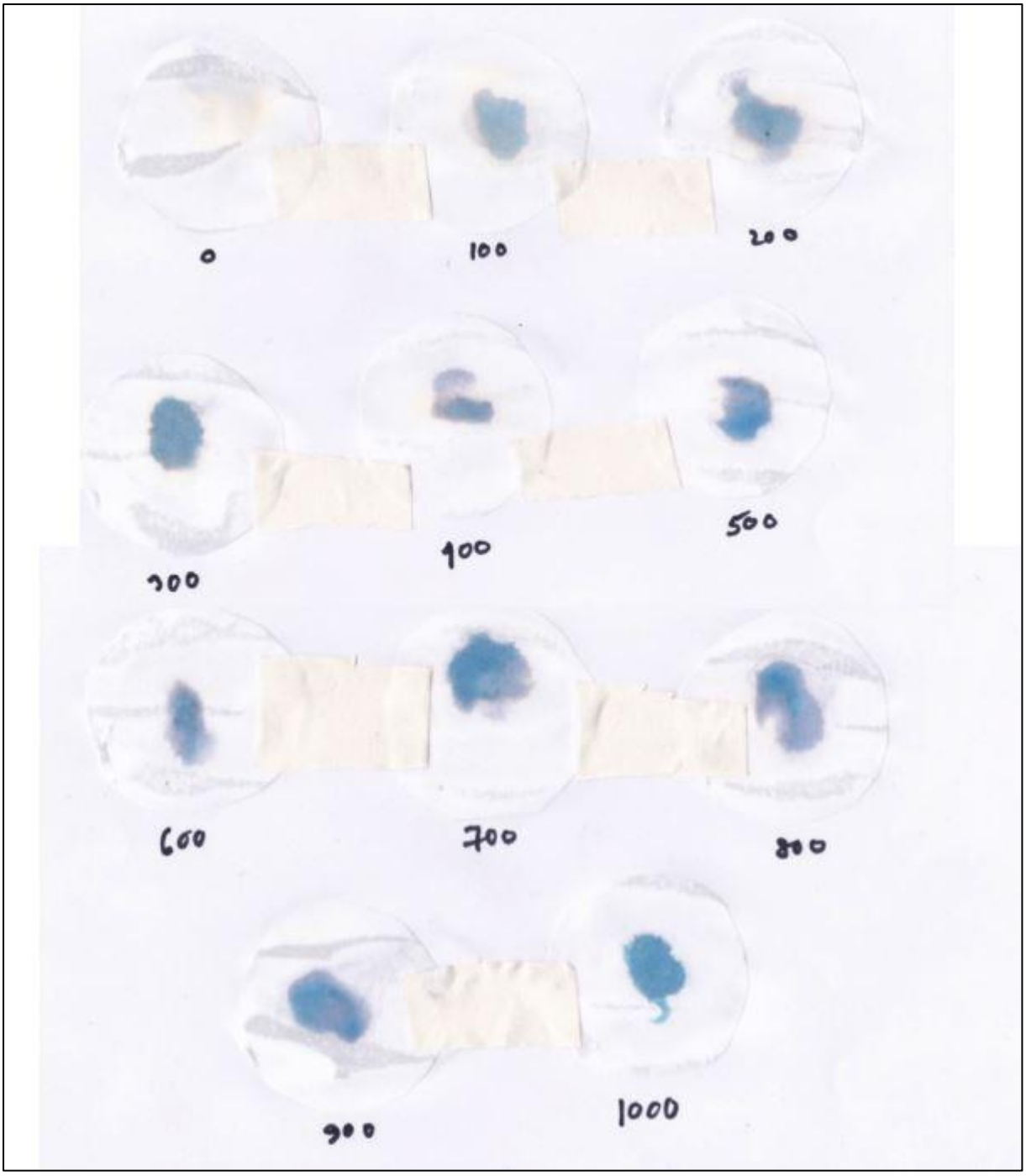
Blank With Uric Acid

(b)

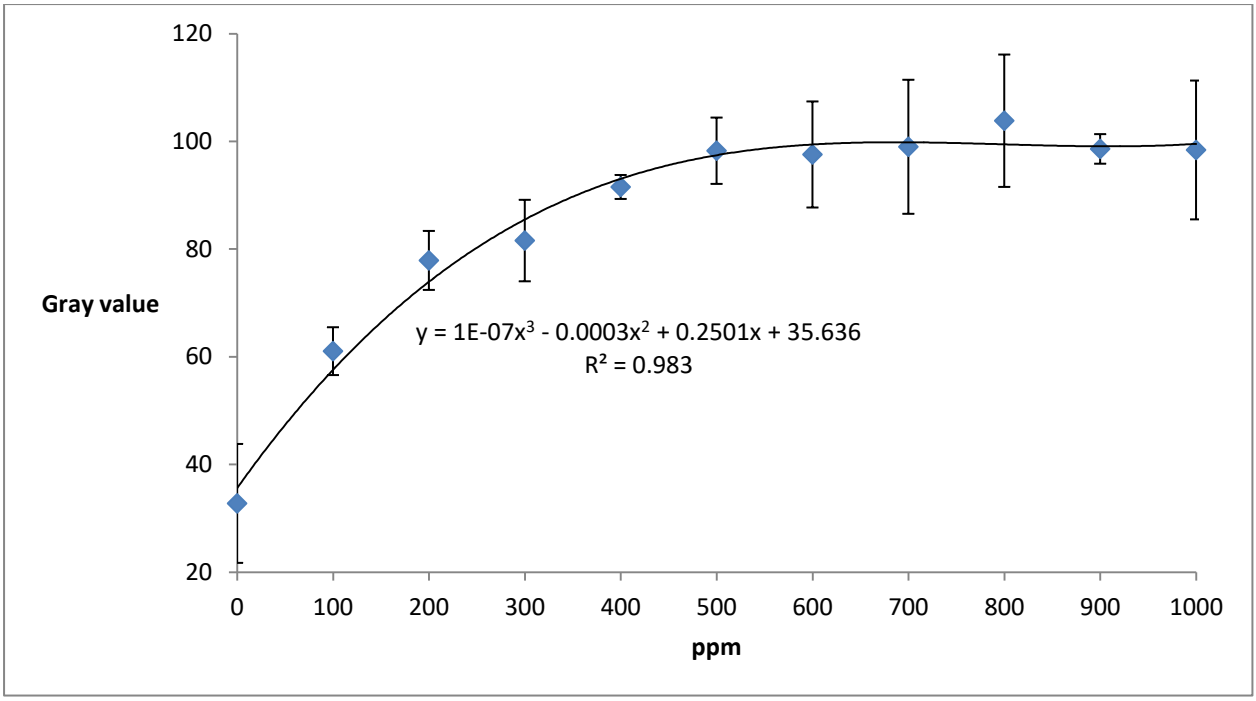
Figure 11: (a) Schematic representation of uric acid detection using paper diagnostic technique; (b) qualitative detection of uric acid solution using paper based detection technique

6.2 Quantitative Detection of uric acid in urine sample

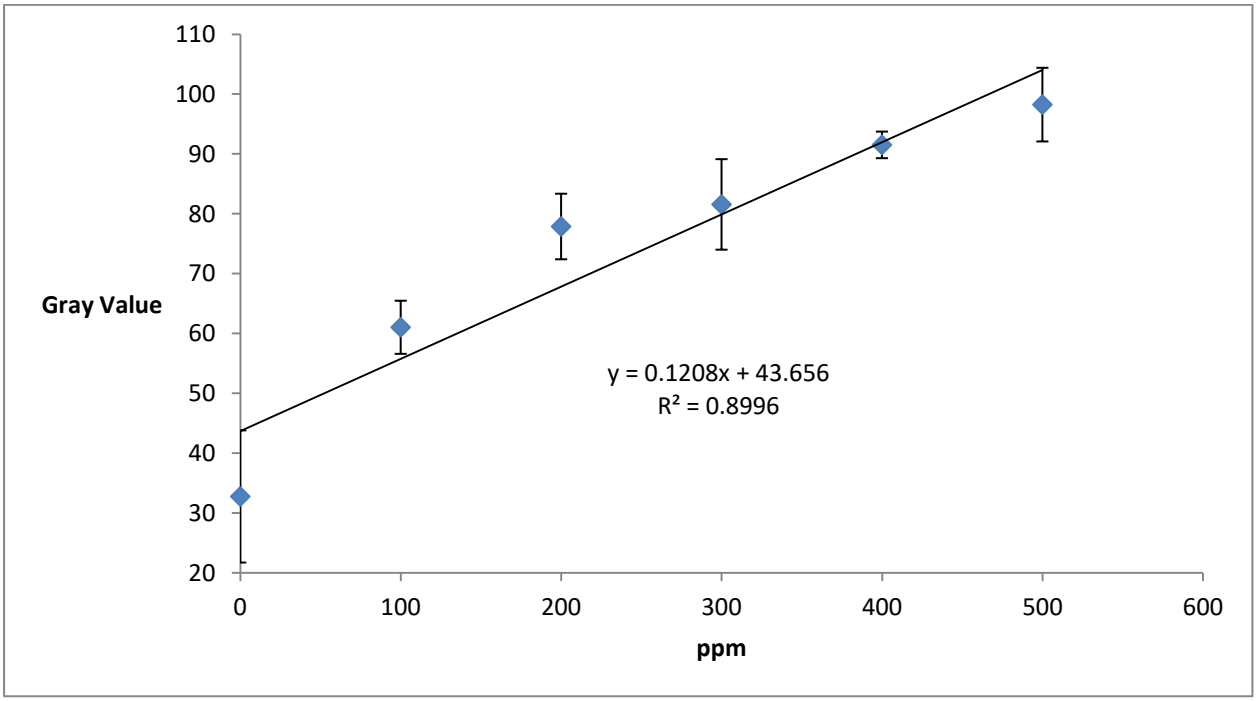
Different concentrations of uric acid in urine solutions were detected using proposed paper diagnostics. Figure 12 (a) shows the experimental results of detecting uric acid concentrations, ranging from 0 ppm to 1000 ppm using the paper detection technique. The color signals formed for different concentrations of uric acid in urine solutions were scanned and analyzed using ImageJ software. To understand the change in color intensity with respect to the change in uric acid concentration, the corresponding CMY values of the color signals were plotted against the uric acid concentrations (Figure 12 b). From figure 12 (b), it is clear that the color intensity reaches saturation at around 500 ppm of uric acid concentration. Therefore, the calibration curve must not exceed 500 ppm. Figure 12 (c) shows the calibration curve. The experimental results also produce a color gradient indicating uric acid concentration (Figure 12 d): the higher the concentration, the darker the color product formation.



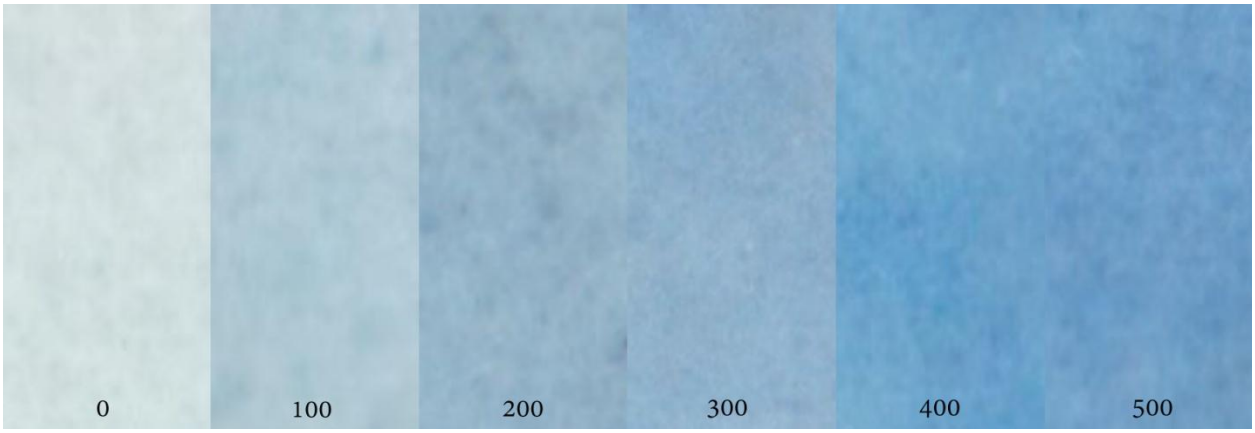
(a)


Gray value
120
100
80
60
40
20
0
100
200
300
400
500
600
700
800
900
1000
ppm
y = 1E-07x³ - 0.0003x² + 0.2501x + 35.636
R² = 0.983

(b)


Gray Value
110
100
90
80
70
60
50
40
30
20
0
100
200
300
400
500
600
ppm
y = 0.1208x + 43.656
R² = 0.8996

(c)

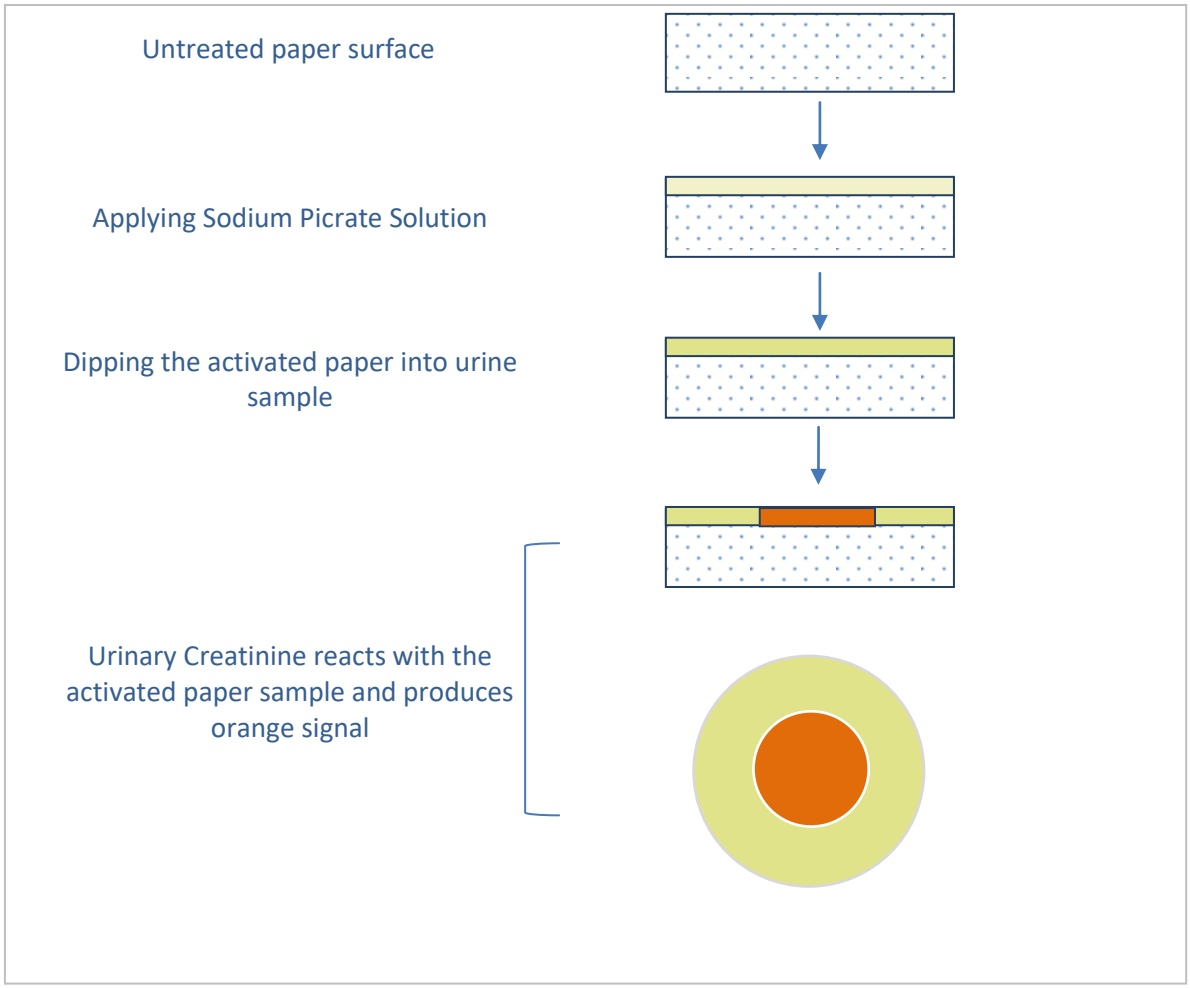


(d)

Figure 12: (a) Experimental detection of uric acid concentrations. (b) Change in color intensity (measured in gray value) with respect to the change in uric acid concentration (Temperature: 29.44^{0}C, R_H: 80.4%). (Standard deviation bars for n=4 samples) (c) Calibration for uric acid concentrations from 0 ppm to 500 ppm. (Temperature: 29.44^{0}C, RH: 80.4%, Standard deviation bars for n=4 samples). (d) The color gradient. (Temperature: 29.44^{0}C, RH: 80.4%)

6.3 Qualitative Detection of creatinine in urine sample

To develop point-of-care paper diagnostics paper samples were treated with different reagents to make it reactive with creatinine in urine. Creatinine reacted with reagent treated paper samples and produced orange color signals on the paper surface; the higher the creatinine concentration in the sample, the stronger the color signal it produced on paper. Figure 5 represents schematic diagrams of treating paper surface with different reagents to develop paper diagnostics for creatinine detection (Figure 13 a), and experimental results of detecting of creatinine in urine sample using paper diagnostics (Figure 13 b).



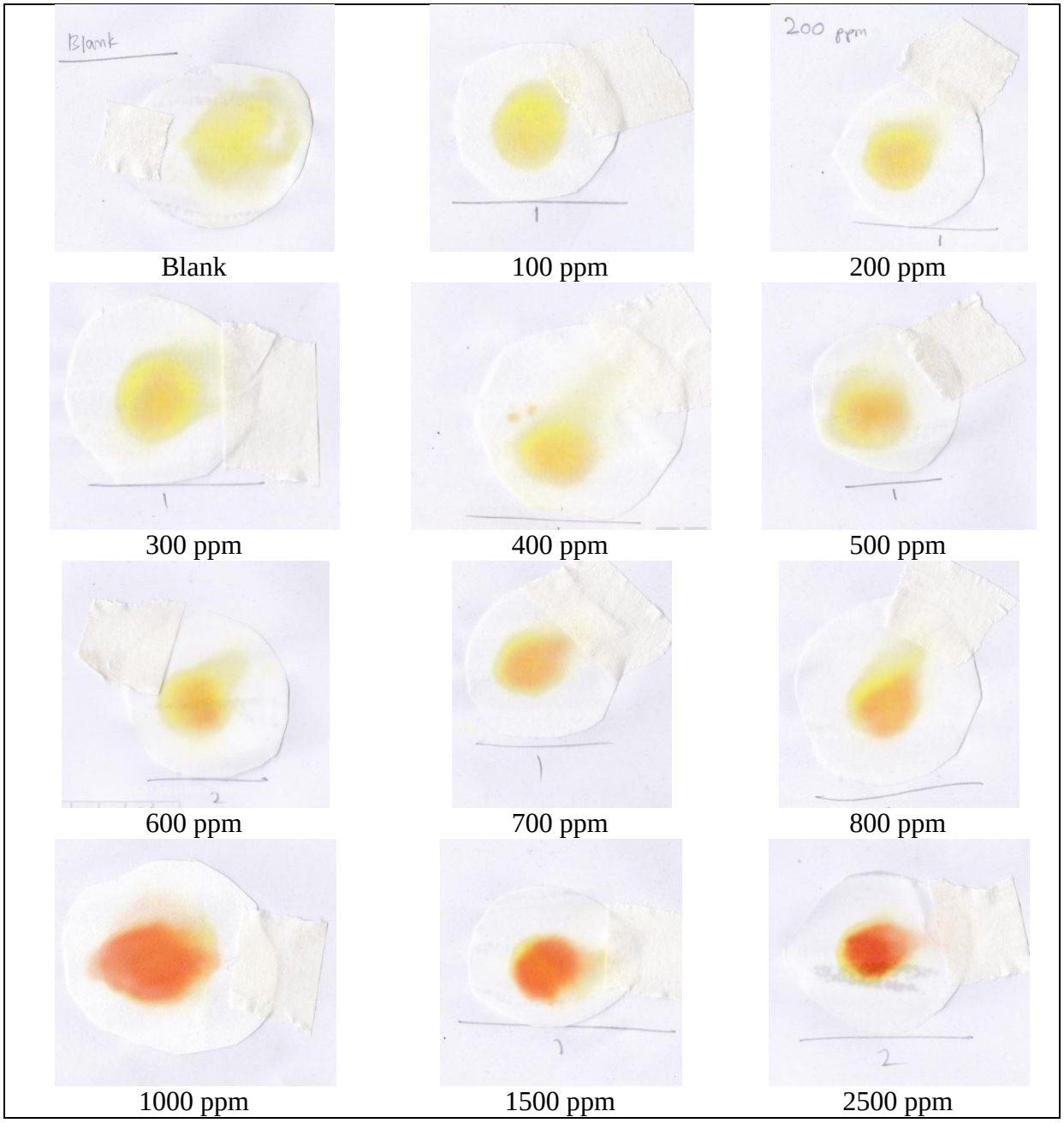
(a)

(b)

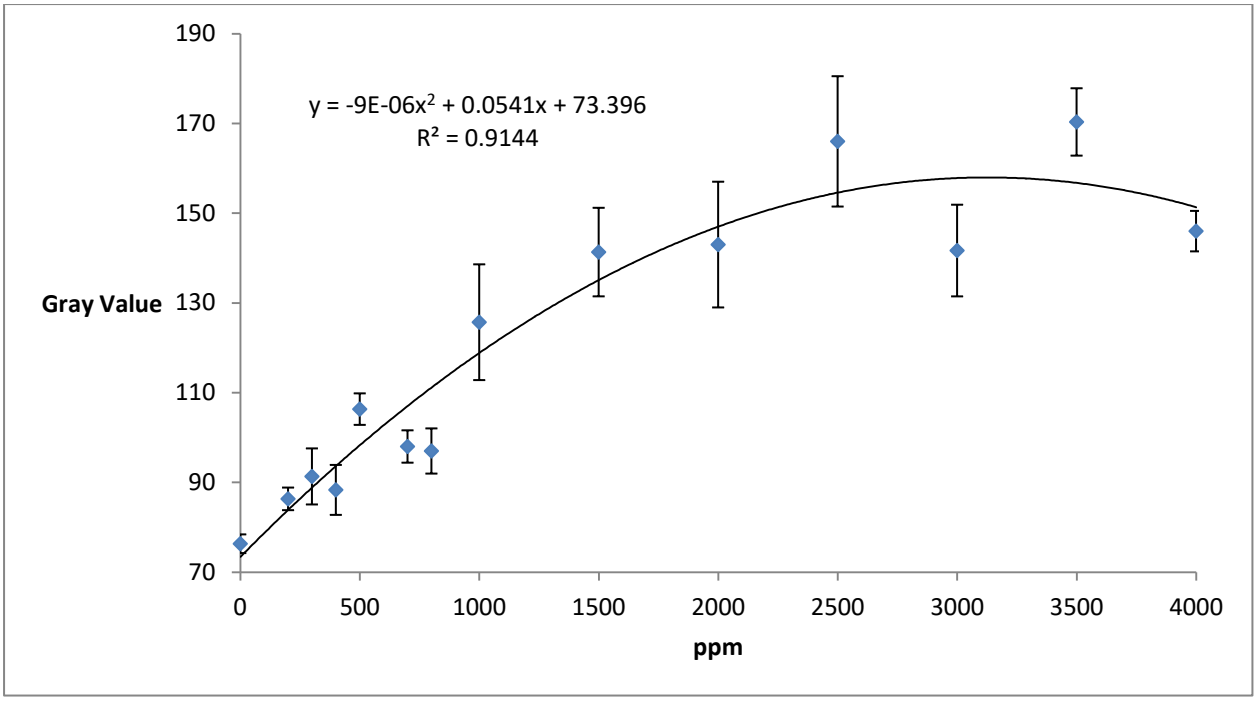
Figure 13: (a) Schematic representation of creatinine detection using paper diagnostic technique; (b) qualitative detection of creatinine solution using paper based detection technique.

6.4 Quantitative Detection of creatinine in urine sample

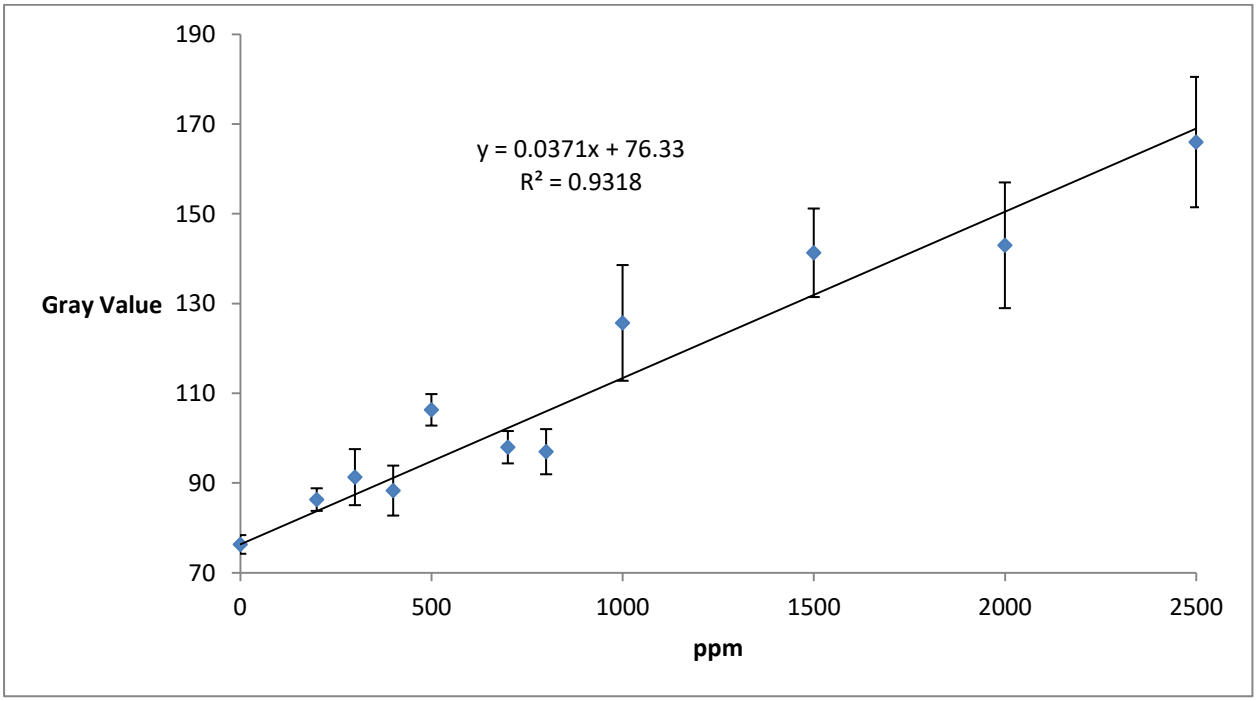
Different concentrations of uric acid in urine solutions were detected using proposed paper diagnostics. Figure 14 (a) shows the experimental results of detecting creatinine concentrations, ranging from 0 ppm to 2500 ppm using the paper detection technique. The color signals formed for different concentrations of creatinine in urine solutions were scanned and analyzed using ImageJ software. To understand the change in color intensity with respect to the change in creatinine concentration, the corresponding CMY values of the color signals were plotted against the creatinine concentrations (Figure 14 b). From figure 14 (b), it is clear that the color intensity reaches saturation at around 2500 ppm of creatinine concentration. Therefore, the calibration curve must not exceed 2500 ppm. Figure 14 (c) shows the calibration curve. The experimental results also produce a color gradient indicating creatinine concentration (Figure 14 d): the higher the concentration, the darker the color product formation.


Blank
200 ppm
Blank
100 ppm
200 ppm
300 ppm
400 ppm
500 ppm
600 ppm
700 ppm
800 ppm
1000 ppm
1500 ppm
2500 ppm

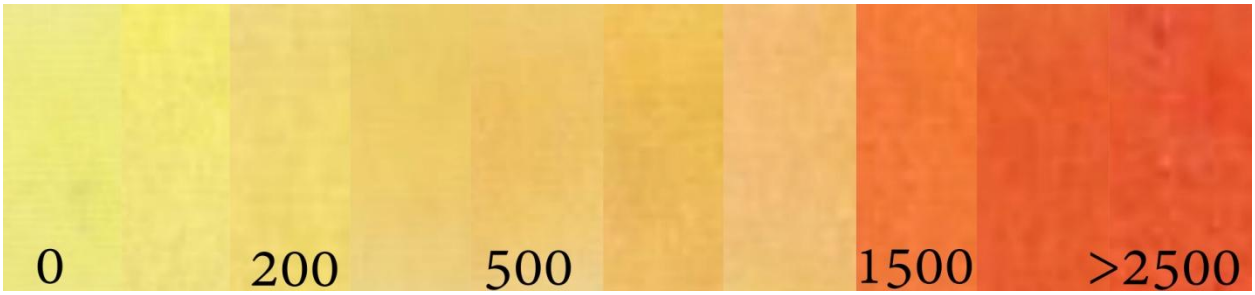
(a)


y = -9E-06x² + 0.0541x + 73.396
R² = 0.9144
Gray Value
190
170
150
130
110
90
70
0
500
1000
1500
2000
2500
3000
3500
4000
ppm

(b)


y = 0.0371x + 76.33
R² = 0.9318
Gray Value
190
170
150
130
110
90
70
0
500
1000
1500
2000
2500
ppm

(c)



(d)

Figure 14: (a) Experimental detection of creatinine concentrations, ranging from 0 to 2500 ppm. (b) Change in color intensity (measured in gray value) with respect to the change in creatinine concentration (Temperature: 29.44^0C, R_H: 80.4%). (c) Calibration curve for creatinine concentrations from 0 ppm to 2500 ppm. (Temperature: 29.44^0C, RH: 80.4%, Standard deviation bars for n=3 samples). (d) The color gradient. (Temperature: 29.44^0C, RH: 80.4%)

Chapter 7: Effect of Temperature on Paper-based Diagnostics

7.1 Uric Acid

To determine the effect of temperature on resultant color intensity, potassium ferricyanide treated paper samples were kept at different temperatures and uric acid detection tests were performed on them. The color intensity was found to increase with the increase in temperature (Figure 15). An increase in temperature from 30^0C to 55^0C changes the RGB value from 82 to 96. Therefore, a test performed at a temperature different than that used for determining the calibration curve will render in erroneous result. Temperature specific calibration curve or correction factor for temperature change should be used to render accurate results. Figure 15 can be used to determine the correction factor.

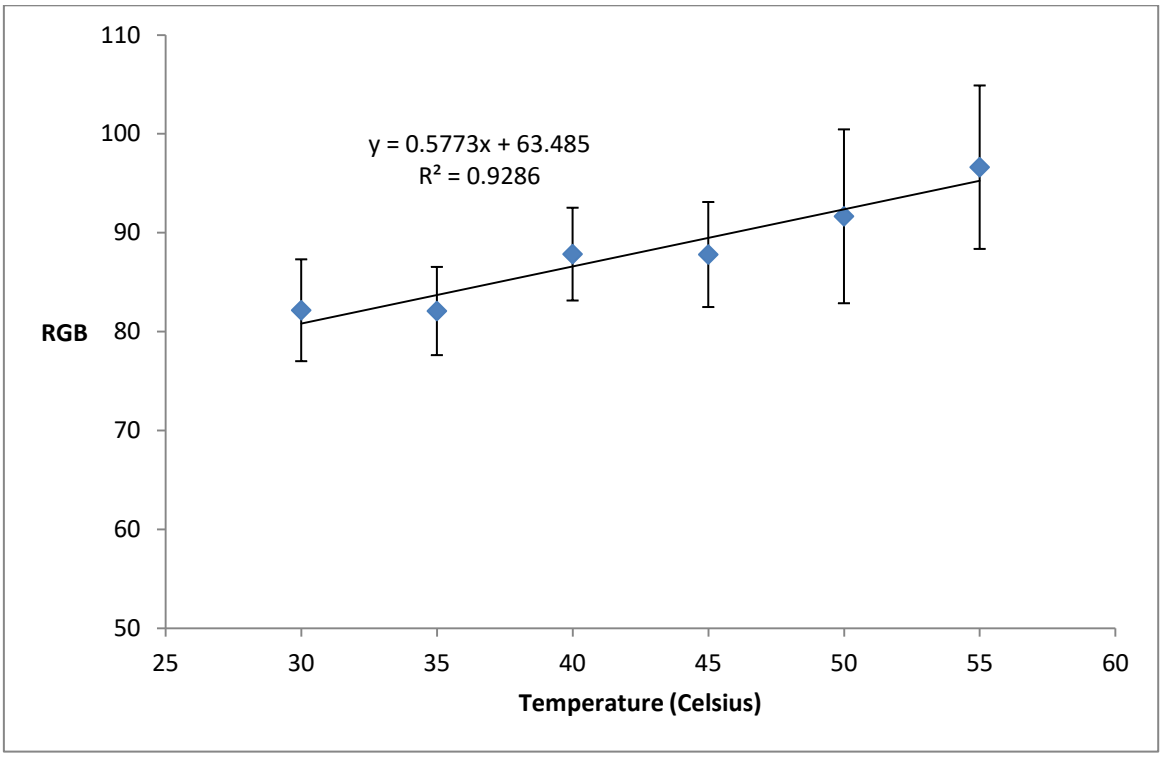


Figure 15: Change in color intensity with respect to temperature (Standard deviation bars for n=4 samples)

7.2 Creatinine

To determine the effect of temperature on resultant color intensity, sodium picrate treated paper samples were kept at different temperatures and creatinine detection tests were performed on them. The color intensity was found to increase with the increase in temperature (Figure 16). An increase in temperature from 25^0C to 50^0C changes the RGB value from 74 to 86. Therefore, a test performed at a temperature different than that used for determining the calibration curve will render in erroneous result. Temperature specific calibration curve or correction factor for temperature change should be used to render accurate results. Figure 16 can be used to determine the correction factor.

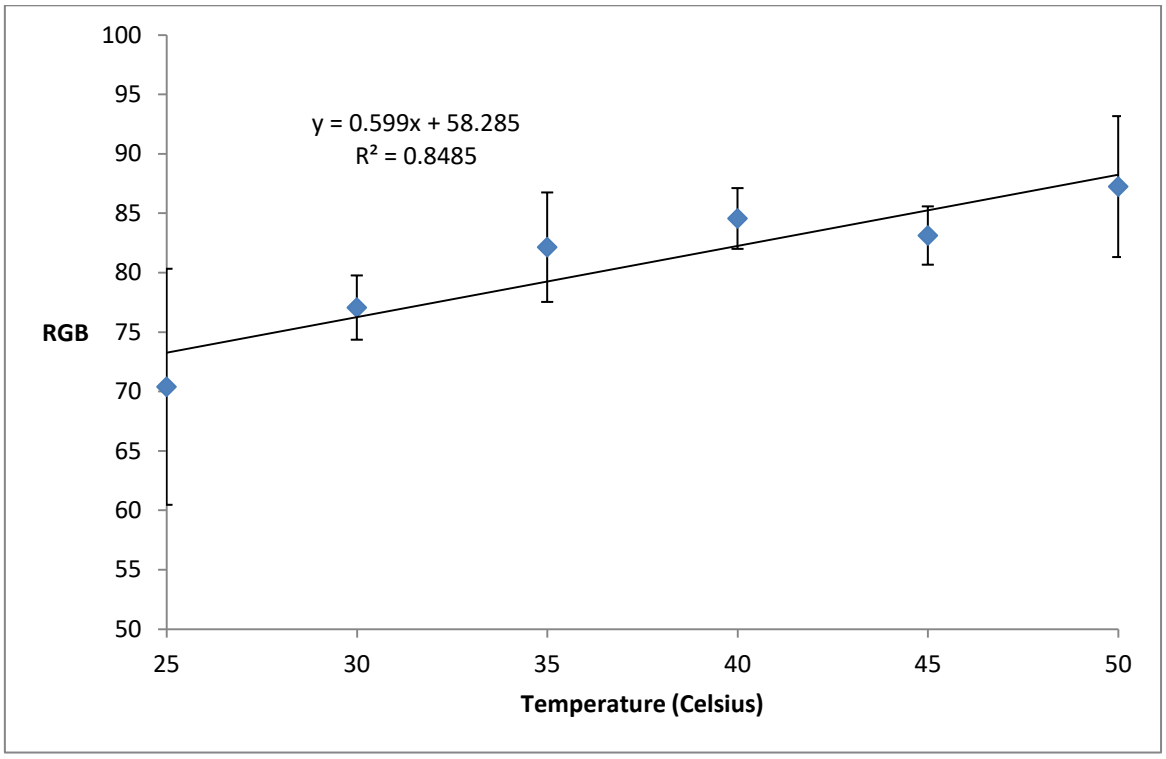


Figure 16: Change in color intensity with respect to temperature. (Standard deviation bars for n=4 samples)

Chapter 8: Pathological Trial

8. Laboratory Trial

The paper diagnostic technique was used to detect uric acid and creatinine concentrations in human urine. Twenty-four hour urine samples were collected from three healthy male subjects aged between 20 to 25 years. The uric acid and creatinine concentration of these urine samples were then measured using proposed paper based techniques. Uric acid and creatinine present in urine samples produced color signal on paper diagnostics. Calibration curves were used to quantify uric acid and creatinine concentrations for corresponding color signals. In addition, the uric acid to creatinine ratio for the urine samples was also determined. The results produced by the paper diagnostic technique were compared with published data [22, 24, 100, 101]. The comparison shows that urinary uric acid and creatinine concentrations and their ratio determined by paper based technique were within acceptable limit.

Table 6 compares the uric acid concentration determined by paper based technique with that of published data. According to the table, values obtained by paper based technique (442 to 558 ppm) fall within the published range (300 to 700 ppm).

Table 6: Comparison of the paper based uric acid detection technique with published data

No.	Paper Based Technique (ppm)	Published Data (ppm) [22, 24, 100]
1.	550±56	300-700
2.	558±17	
3.	442±27	

Table 7 compares the creatinine concentration determined by paper based technique with that of published data. As with uric acid, values obtained by paper based technique (392 to 839 ppm) also fall within the published range for creatinine concentration (390 to 2590 ppm).

Table 7: Comparison of the paper based creatinine detection technique with published data

No.	Paper Based Technique (ppm)	Published Data (ppm) [101]
1.	859±296	390-2590
2.	834±143	
3.	853±260	

Finally, table 8, indicates the uric acid to creatinine ratio for the three subjects. The values varied between 0.52 and 0.67. In addition, laboratory trials were performed to compare the results produced on paper surface with that of established diagnostic techniques. The values obtained from paper based technique are slightly higher than laboratory trials (0.3 to 0.63) and the published data (0.21 to 0.59). The ratio in one sample is significantly higher than the published results for both laboratory trial and paper based technique. According to Kaufman et al. a higher uric acid to creatinine ratio is an indicator of renal disease such as hyperuricemia and phosphoribosyltransferase (PRT) deficiency [113]. Therefore, the higher uric acid to creatinine ratio in this sample may be an indicator of gout for that patient.

To examine this assumption, serum uric acid level of the patient was measured at combined military hospital (CMH), Dhaka, Bangladesh. The serum uric acid level for this person was higher than the reference range (Appendix, Figure: B). In addition, visual inspection of hallux (big toe) was performed to inspect swelling and redness. The hallux was found to be swollen and redder than normal condition. Therefore, measurement serum uric acid concentration and observation of for that patient indicates the presence of urate crystals and confirms gout. Figure 17 indicates the hallux of the sample with higher uric acid/creatinine ratio and confirms gouty toe.

Table 8: Comparison of the uric acid to creatinine ratio of proposed paper based technique with that of published data.

No.	Paper Based Technique	Laboratory Trial	Published Data [113, 114]
1.	0.64±0.18	0.57	0.21-0.59
2.	0.67±0.1	0.63	
3.	0.52±0.14	0.3	

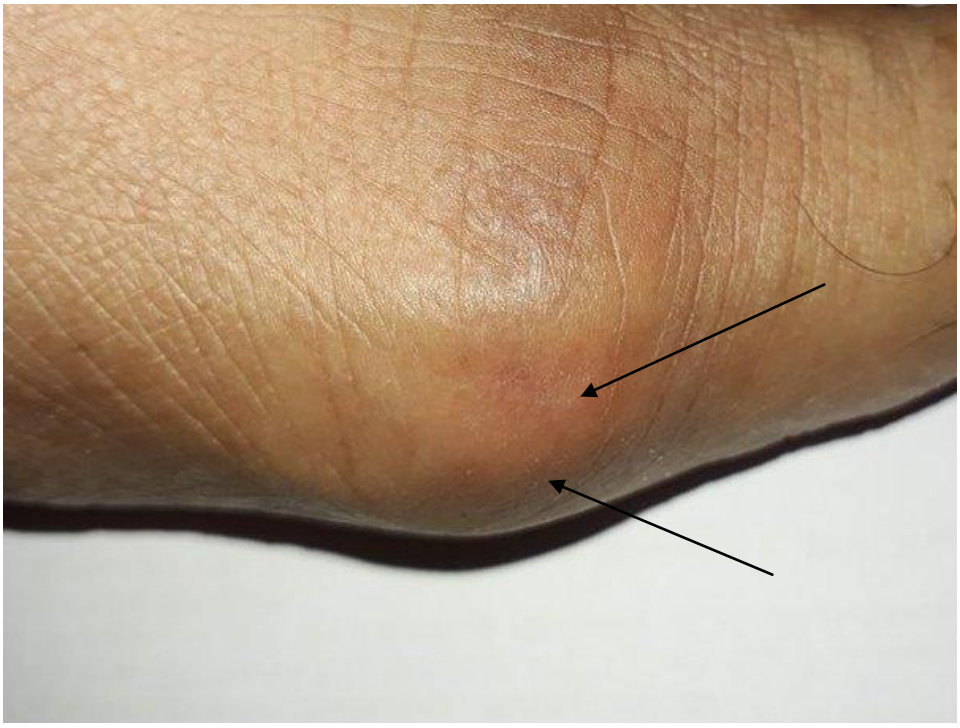


Figure 17: Hallux (big toe) for the sample with higher uric acid to creatinine ratio. Swelling and redness of hallux indicates initiation of gout.

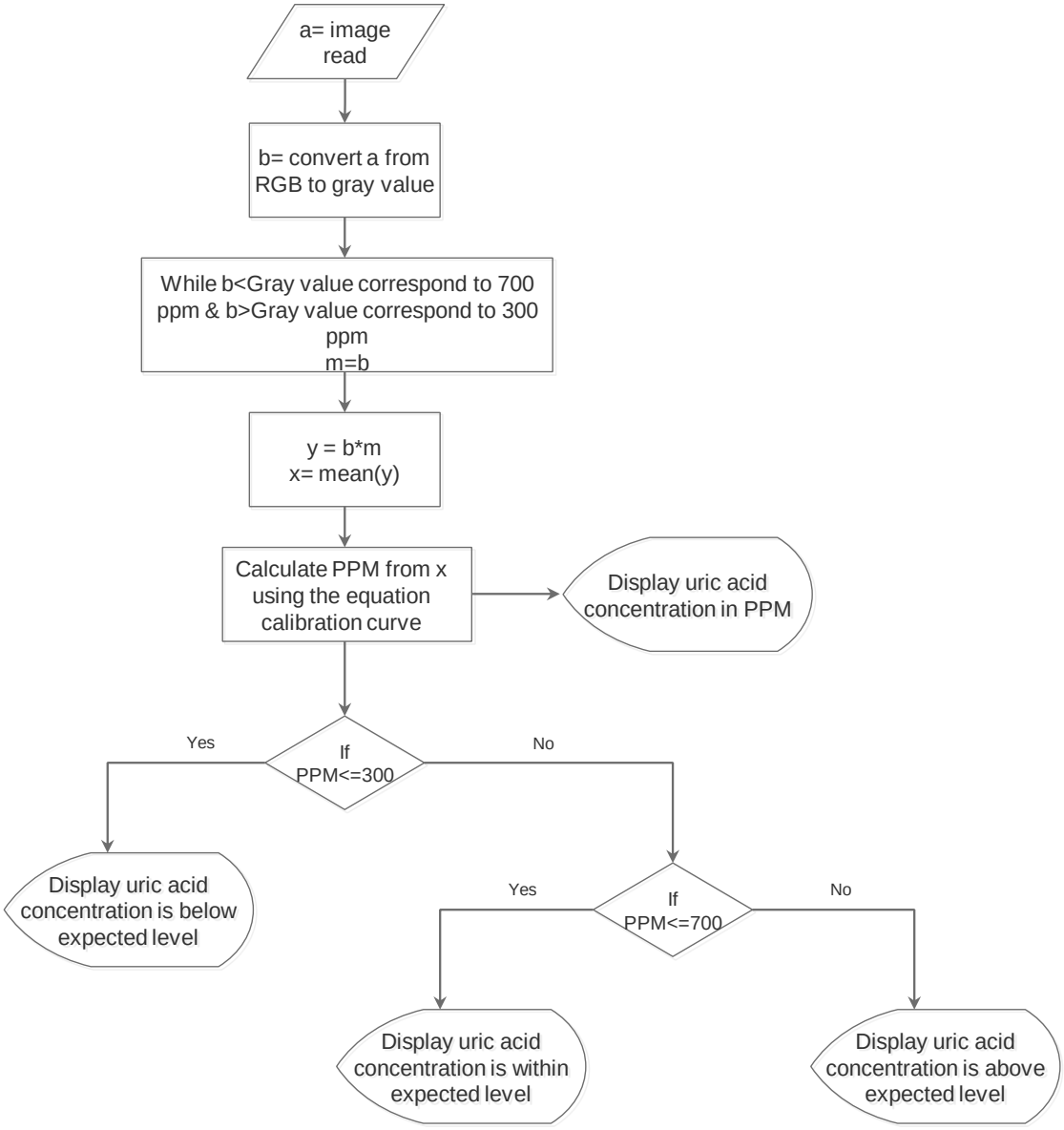
Chapter 9: Application in Telemedicine: Digital Approach

9. Application in Telemedicine

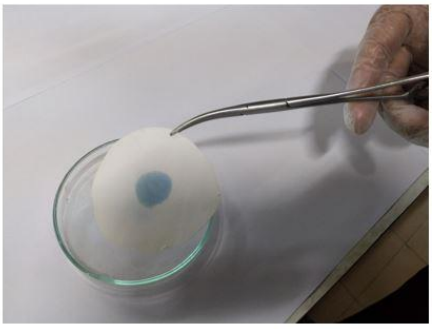
The proposed diagnostic technique can be used in telemedicine which could be highly useful to provide health services at remote and underdeveloped areas. This technique can also be used to monitor the urinary uric acid and creatinine level of patients with chronic renal disease. The color signal produced on the paper surface can be analyzed with a gradient paper or with the help of a camera enabled mobile device. The results can then be sent to an established medical facility for further analysis. If a mobile platform is used then an android or IOS application can be developed which will show the user the uric acid concentration in ppm or in any other desired unit. Figure 18 (a) illustrates a MATLAB code which can be used as a baseline to develop any mobile application; Figure 18 (b) describes the algorithm for the code. According to the algorithm, the image is at first inserted into the MATLAB program. MATLAB reads the image in RGB (Red, Green and Blue) format, which is displayed as a 3-dimentionl matrix [115]. This 3D matrix is then converted into a 2-D matrix by converting the RGB image to Gray image (Black and White image). Outliers from this matrix are then sorted and eliminated. The mean value from the resulting matrix is taken for further calculation. Equation developed from the calibration curve is used to convert the color value (mean value) into concentration value as parts per million (ppm) of uric acid. Finally, this value is displayed with a concluding remark. Figure 18 (c) shows the estimation of urinary uric acid using a mobile device and figure 18 (d) illustrates a MATLAB code that can be used to determine urinary creatinine concentration.

```
Clc
clear all
a=imread('G:\Nazibul\drive-download-20170412T040354Z-
001\sample1.jpg');
b=rgb2gray(a);
m=(b<193.6203&b>149.1396);
% Limiting the value to be evaluated within a pre-
% determined range (zero to five-hundred PPM)
y=b(m);
x=mean(y);
PPM=(x-43.656)/0.1208
% Calculating the uric acid level in PPM from the
% calibration curve developed in experimental section
if PPM<=300
    disp('!!!Your uric acid level is below the expected
range!!!')
elseif PPM<=700
    disp('Your uric acid level is within the excepted
range')
else
    disp('!!!Your uric level is above the excepted
range!!!')
end
```

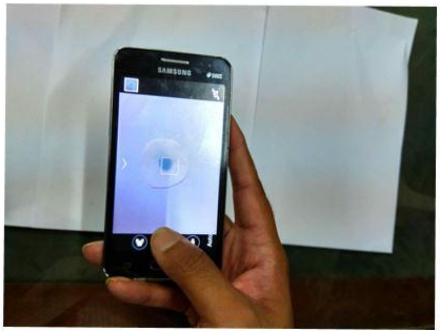
(a)


a= image read
b= convert a from RGB to gray value
While b<Gray value correspond to 700 ppm & b>Gray value correspond to 300 ppm
m=b
y = b*m
x= mean(y)
Calculate PPM from x using the equation calibration curve
Display uric acid concentration in PPM
Yes
If PPM<=300
No
Display uric acid concentration is below expected level
Yes
If PPM<=700
No
Display uric acid concentration is within expected level
Display uric acid concentration is above expected level

(b)

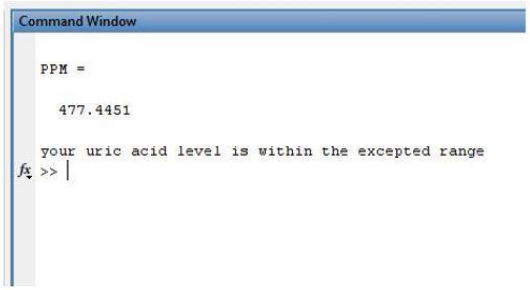


Step 1: Formation of color signal on paper surface



Step 2: Capturing the color signal using a camera unbaled mobile device

Step 3: Running a MATLAB code to analyze the captured image



Step 4: Result

(c)

```
Clc
clear all
a=imread('G:\Nazibul\drive-download-20170412T040354Z-
001\sample1.jpg');
b=rgb2gray(a);
m=(b<131.904&b>64.6238);
% Limiting the value to be evaluated within a pre-
% determined range (200 to 2500 PPM)
y=b(m);
x=mean(y);
PPM=(x-76.33)/0.0371
% Calculating the uric acid level in PPM from the
% calibration curve developed in experimental section
if PPM<=200
    disp('!!!Your urine sample has been diluted!!!')
elseif PPM>= 200 && PPM<=390
    disp('Your uric acid level is below the excepted
range')
elseif PPM<=2590
    disp('Your uric acid level is within the excepted
range')
else
    disp('!!!Your uric level is above the excepted
range!!!')
end
```

(d)

Figure 18: (a) MATLAB code for color signal analysis (uric acid). (b) Algorithm for the MATLAB code. (c) Estimation of urinary uric acid using a mobile device. (d) MATLAB code for color signal analysis (creatinine).

Chapter 10: Conclusion

10. Conclusion

This study presents a simple, low cost, point of care, paper based detection technique for qualitative and quantitative detection of uric acid and creatinine in human urine. The urinary uric acid and creatinine concentration can be used to diagnose kidney condition. Reaction kinetics for color development due the presence of urinary uric acid and creatinine on paper was analyzed. Reaction rates on paper were found to be slower than that in liquid solution. Calibration curves were developed to measure the urine contents in parts per million from color intensity. Effect of temperature on color signal produced was analyzed. Graphs were developed for graphical analysis of uric acid and creatinine concentrations for kidney diagnosis. Use of uric acid to creatinine ratio for diagnosis of other diseases (Perinatal Asphyxia and Sleep Hypoxemia) was also explored. Using the calibration curves, the uric acid and creatinine content in human subjects was measured, three out of four of which were close to acceptable limit reported in literature. Existence of gout and subsequent increase in serum and urinary uric acid concentration was found to be the reason for anomaly in the other sample. Finally, a MATLAB code was developed to demonstrate the utility of the proposed diagnostic device as a form of telemedicine. The proposed paper based techniques can be used by patients especially in remote and underdeveloped areas and patients, who need to monitor their kidney condition regularly. Doctors and patients can use their mobile phone cameras to take picture of the diagnostic paper and mobile based applications can be used to quantify uric acid and creatinine concentration in urine and diagnose kidney condition on-spot.

10.1 Recommendations for Future Work

The following issues can be addressed in future works:

- Product design for mass consumption;
- Develop a paper based technique to quantify creatinine concentration from blood serum;
- To assess other biomarkers which can be detected using paper based diagnostics;
- To assess reactions other than colorimetry (electrochemical, cytometric etc.) that can be used on paper surface for analyte detection.

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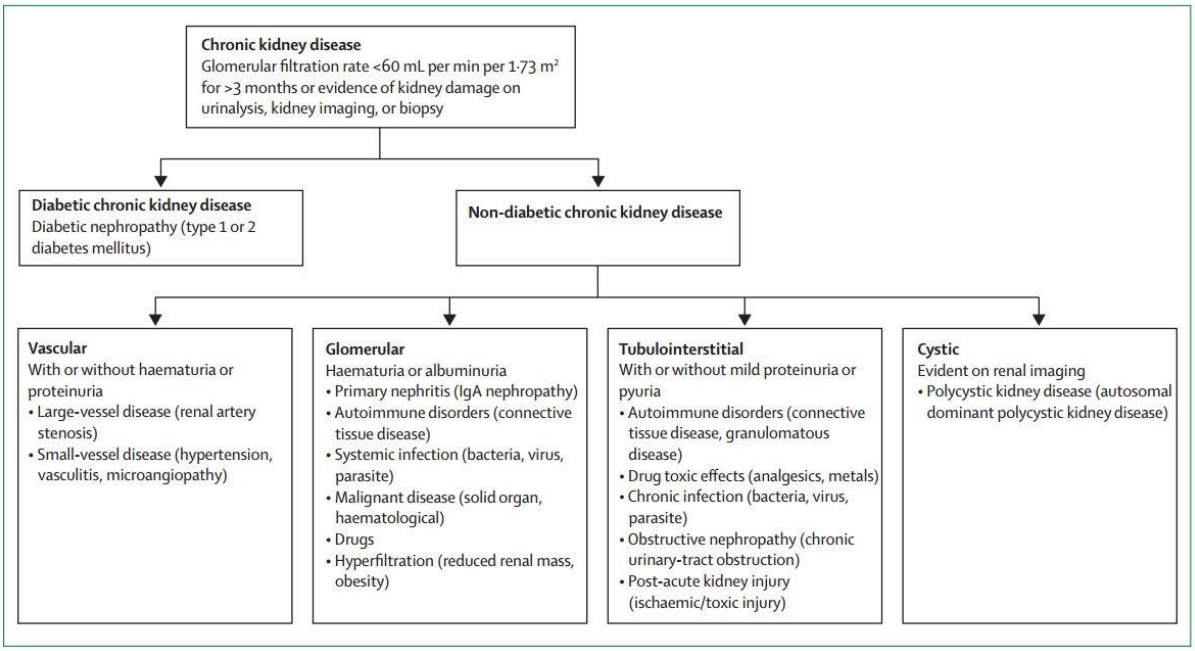
Appendix

Classification of Chronic Kidney Disease (CKD)



Distribution of Causes of CKD in Different Countries

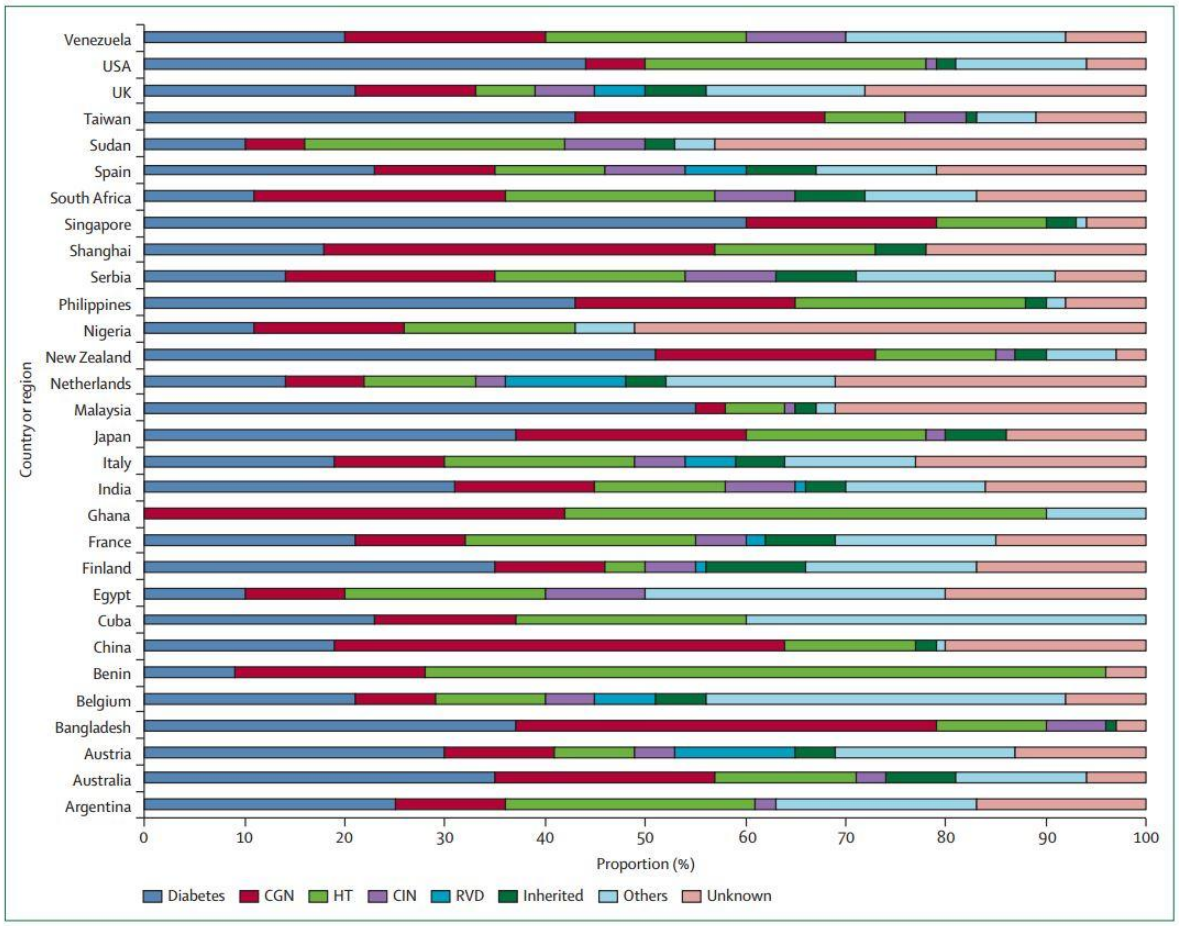
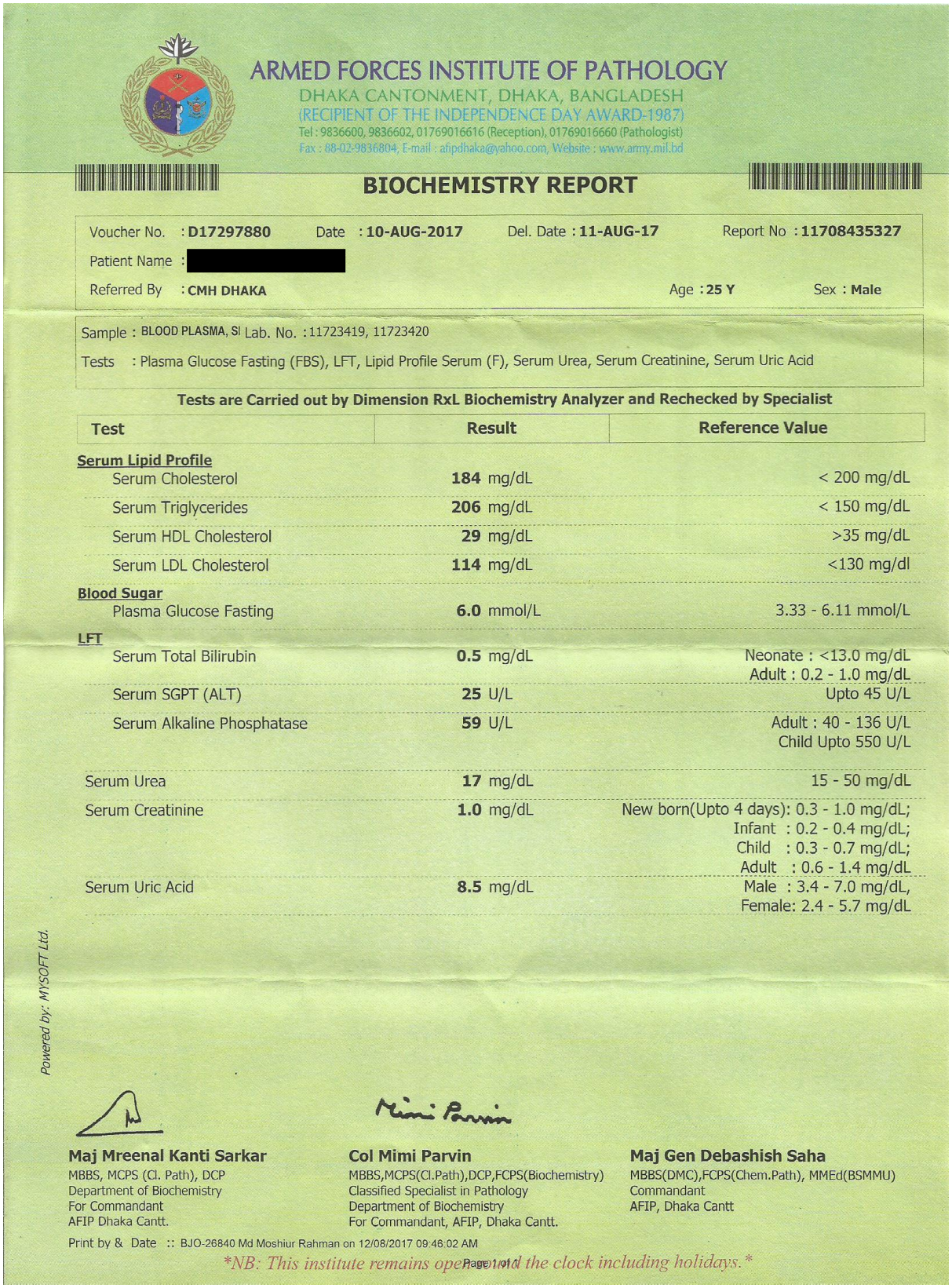


Figure A: Classification and distribution of causes of CKD worldwide

Diagnostic Report of Serum Uric Acid



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(RECIPIENT OF THE INDEPENDENCE DAY AWARD-1987)
Tel : 9836600, 9836602, 01769016616 (Reception), 01769016660 (Pathologist)
Fax : 88-02-9836804, E-mail : afipdhaka@yahoo.com, Website : www.army.mil.bd

BIOCHEMISTRY REPORT

Voucher No. : **D17297880** Date : **10-AUG-2017** Del. Date : **11-AUG-17** Report No : **11708435327**
Patient Name : [redacted]
Referred By : **CMH DHAKA** Age : **25 Y** Sex : **Male**

Sample : BLOOD PLASMA, SI Lab. No. : 11723419, 11723420
Tests : Plasma Glucose Fasting (FBS), LFT, Lipid Profile Serum (F), Serum Urea, Serum Creatinine, Serum Uric Acid

Tests are Carried out by Dimension RxL Biochemistry Analyzer and Rechecked by Specialist

Test	Result	Reference Value
Serum Lipid Profile		
Serum Cholesterol	**184** mg/dL	< 200 mg/dL
Serum Triglycerides	**206** mg/dL	< 150 mg/dL
Serum HDL Cholesterol	**29** mg/dL	>35 mg/dL
Serum LDL Cholesterol	**114** mg/dL	<130 mg/dl
Blood Sugar		
Plasma Glucose Fasting	**6.0** mmol/L	3.33 - 6.11 mmol/L
LFT		
Serum Total Bilirubin	**0.5** mg/dL	Neonate : <13.0 mg/dL Adult : 0.2 - 1.0 mg/dL
Serum SGPT (ALT)	**25** U/L	Upto 45 U/L
Serum Alkaline Phosphatase	**59** U/L	Adult : 40 - 136 U/L Child Upto 550 U/L
Serum Urea	**17** mg/dL	15 - 50 mg/dL
Serum Creatinine	**1.0** mg/dL	New born(Upto 4 days): 0.3 - 1.0 mg/dL; Infant : 0.2 - 0.4 mg/dL; Child : 0.3 - 0.7 mg/dL; Adult : 0.6 - 1.4 mg/dL
Serum Uric Acid	**8.5** mg/dL	Male : 3.4 - 7.0 mg/dL, Female: 2.4 - 5.7 mg/dL

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Maj Mreenal Kanti Sarkar
MBBS, MCPS (Cl. Path), DCP
Department of Biochemistry
For Commandant
AFIP Dhaka Cantt.

Col Mimi Parvin
MBBS,MCPS(Cl.Path),DCP,FCPS(Biochemistry)
Classified Specialist in Pathology
Department of Biochemistry
For Commandant, AFIP, Dhaka Cantt.

Maj Gen Debashish Saha
MBBS(DMC),FCPS(Chem.Path), MMEd(BSMMU)
Commandant
AFIP, Dhaka Cantt

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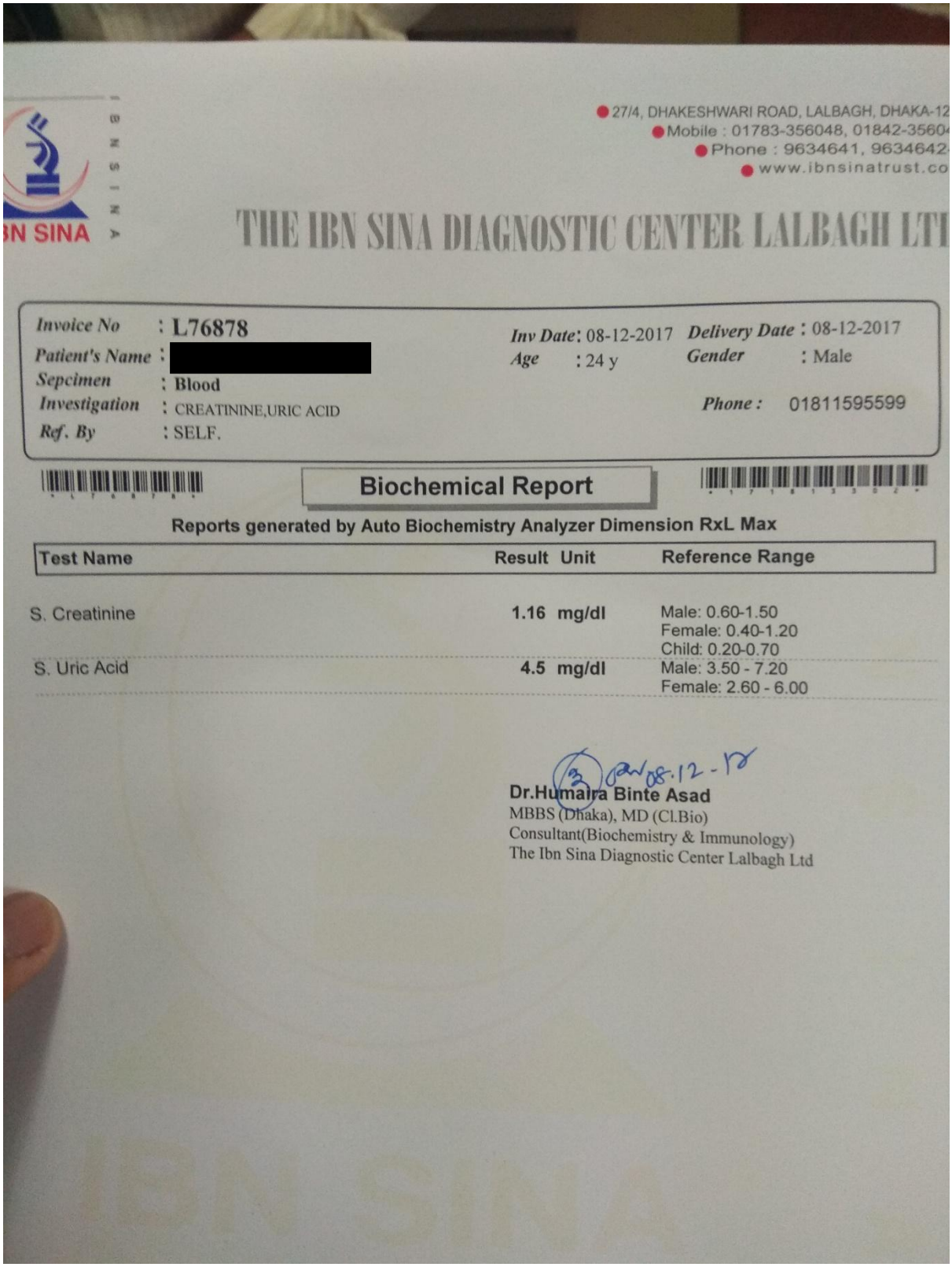

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THE IBN SINA DIAGNOSTIC CENTER LALBAGH LTI

Invoice No	: L76878	*Inv Date* : 08-12-2017	*Delivery Date* : 08-12-2017
Patient's Name	: [redacted]	*Age* : 24 y	*Gender* : Male
Sepcimen	: Blood		
Investigation	: CREATININE,URIC ACID		*Phone :* 01811595599
Ref. By	: SELF.		

Biochemical Report

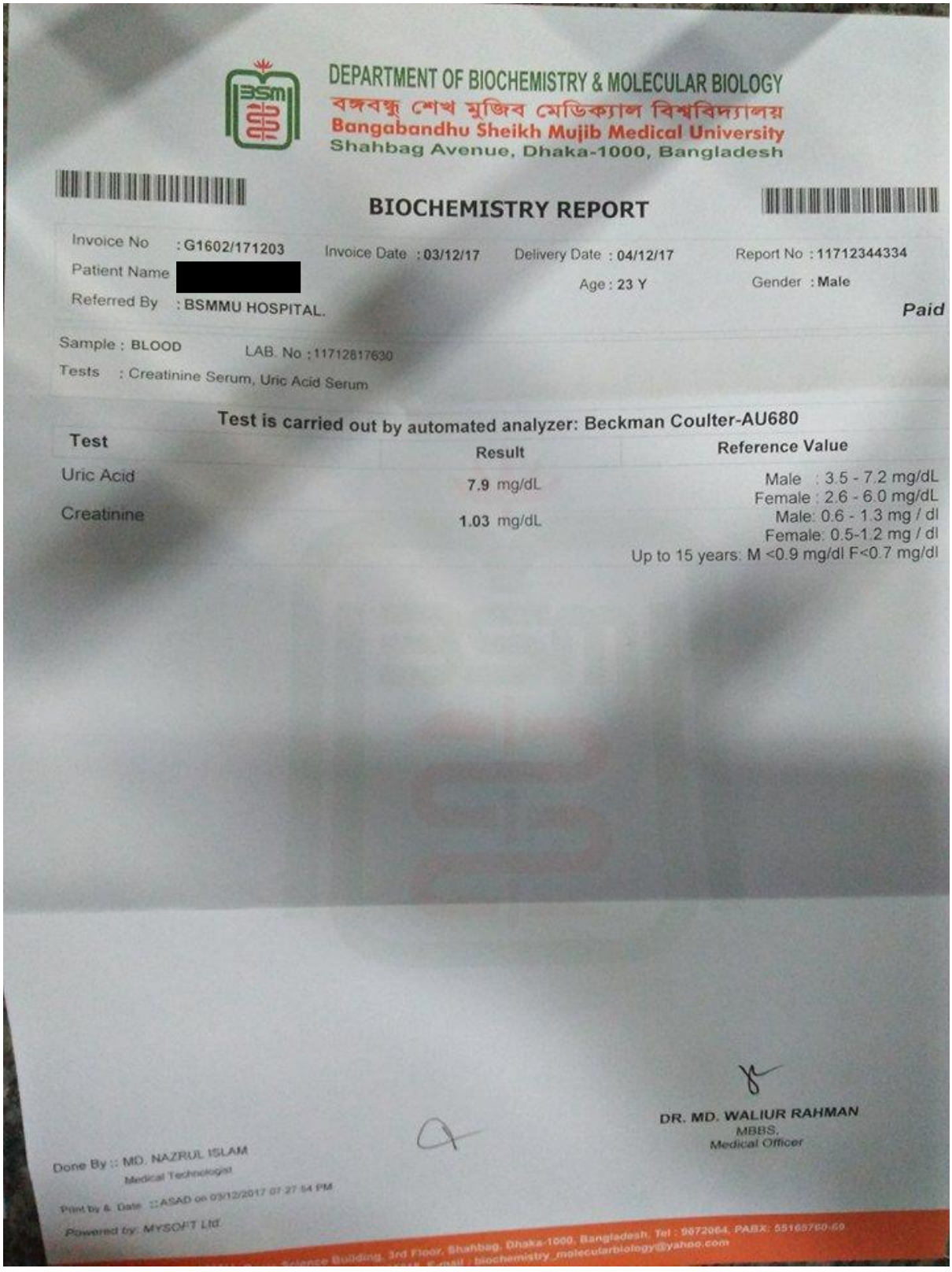
Reports generated by Auto Biochemistry Analyzer Dimension RxL Max

Test Name	Result	Unit	Reference Range
S. Creatinine	1.16	mg/dl	Male: 0.60-1.50 Female: 0.40-1.20 Child: 0.20-0.70
S. Uric Acid	4.5	mg/dl	Male: 3.50 - 7.20 Female: 2.60 - 6.00

08.12.17

Dr.Humaira Binte Asad
MBBS (Dhaka), MD (Cl.Bio)
Consultant(Biochemistry & Immunology)
The Ibn Sina Diagnostic Center Lalbagh Ltd

(b)



(c)

Figure B: Laboratory report from different pathological laboratories

Artificial Urine Constituents

Table A: Constituents of artificial urine sample

Name	Concentration
Lactic Acid	1.1 mM
Citric Acid	2.0 mM
Sodium Bicarbonate	25 mM
Urea	170 mM
Calcium Chloride	2.5 mM
Sodium Chloride	90 mM
Magnesium Sulfate	2.0 mM
Sodium Sulfate	10 mM
Potassium Dihydrogen Phosphate	7.0 mM
Dipotassium Hydrogen Phosphate	7.0 mM
Ammonium Chloride	25 mM

The pH of the solution was adjusted to 6.0 by addition of 1.0 M hydrochloric acid.

Range of Urinary Uric Acid to Creatinine Ratio among Normal People and Patients with Lesch-Nyhan Syndrome

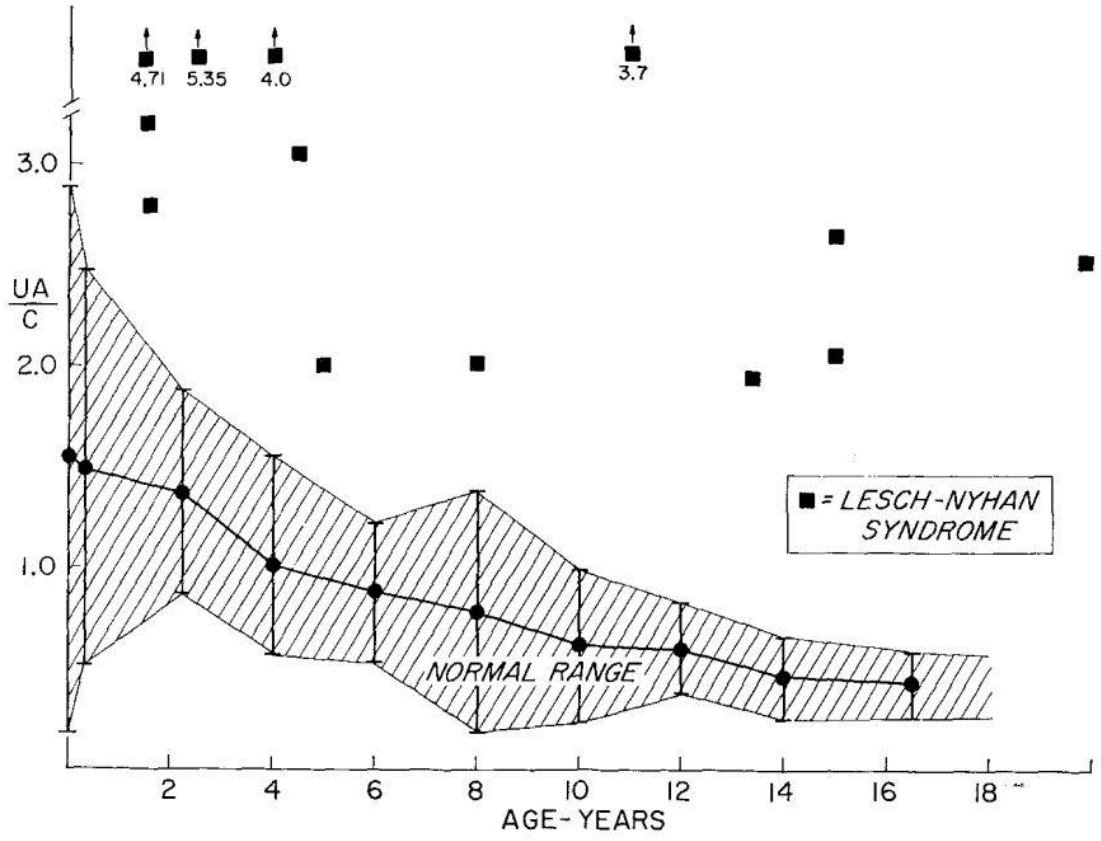


Figure C: Range of Urinary Uric Acid to Creatinine Ratio among different population groups