A MOBILE URINE ANALYSIS SYSTEM FOR HOMECARE

by

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ABSTRACT

A MOBILE URINE ANALYSIS SYSTEM FOR HOMECARE

Urinalysis is a remarkable diagnostic technique and an essential part of physical examination used frequently in kidney and urinary tract diseases. Urine reagent strips which are widely used in urinalysis are impregnated with a number of colored reagent blocks or pads separated from each other by narrow bands. The reagents in each block react with specific components of urine in such a way that the block changes color if the component is present, and the color change produced is proportional to the concentration of the component being tested for. Recent analysis of test strips is perfomed via refractometric devices of the corresponding test strips. In this thesis, a mobile urine strip analyzer called BUSA which can be controlled via both GUIs of MATLAB and Android operation system was designed by using Parallax's color sensor, TCS3200-DB. The fundemental idea behind this design was to form a database which included color data of control solutions of CombiScan 500, 40 patients analyzed by CombiScan 500 in Yeditepe University Hospital and prepared solutions of known pH and glucose amount. 15 patients analyzed by CombiScan 500 were compared to BUSA. Specificity of the results of the comparison for bilirubin, urobilinogen, glucose, protein, blood, nitrite and leukocyte was 1 and for ketone was 0.933. Sensitivty of the results of the comparison for glucose, protein and blood was 1, 0.2 and 1 respectively. pH and specific gravity were analyzed via Blant Altman method which means and standard deviations were 0.133, 0.5156 and 0.0017, 0.0059 respectively. In conclusion, BUSA is succesful at measuring pH and specific gravity of urine and negativeness of bilirubin, urobilinogen, ketone, glucose, protein, blood, nitrite and leukocytes. In addition, BUSA is successful at detecting positiveness of glucose and blood, except protein.

Keywords: Urinalysis, Color sensor, Android, homecare.

ÖZET

EVDE TEDAVİ İÇİN MOBİL BİR İDRAR ANALİZ SİSTEMİ

İdrar tahlili, böbrek ve idrar yolu hastalıklarında sıklıklı kullanılan kayda değer bir teşhis tekniği ve fiziksel muayenenin temel bir ögesidir. İdrar tahlilinde büyük ölçüde kullanılan idrar stripleri, birbirlerinden dar şeritlerle ayrılmış birçok renkli reaktif bloklardan ya da pedlerden oluşmktadır. Her bloktaki reaktif madde idrarda belli içeriklerle reaksiyona girer ve eğer o içerik idrarda var ise blok renk değiştirir, oluşan renk değişimi, test edilen maddenin idrardaki konsantrasyonuyla doğru orantılıdır. Test stripleri günümüzde ilgili test stripine ait refraktometrik cihazla analiz edilir. Bu tezde, Parallax'ın TCS3200-DB renk sensörü kullanılarak hem MATLAB'in hem de Android işletim sisteminin GUI'si vasıtasıyla kontrol edilebilen BUSA isimli mobil bir idrar strip okuyucusu tasarlanmıştır. Bu dizaynın arkasında temel fikir, CombiScan 500'ün kontrol solüsyonlarının, Yeditepe Üniversitesi Hastanesi'nde CombiSCan 500 tarafından analiz edilen 40 hastanın ve pH ve glikoz miktarları bilinen hazırlanmış solüsyonları renk verileri içeren bir veritabanı oluşturmaktır. CombiScan 500 tarafından analiz edilen 15 hasta BUSA ile karşılaştırılmıştır. Karşılaştırmanın kesinliği bilirubin, ürobilinojen, glikoz, protein, kan, nitrit ve lökosit için 1 ve keton için 0.933'tür. Karşılaştırmanın duyarlılığı glikoz, protein ve kan için sırasıyla 1, 0.2 ve 1'dir. pH ve dansite (özgül ağırlık) fark ortalama ve standard sapma sonuçları sırasıyla 0.133, 0.5156 ve 0.0017, 0.0059 olan Bland Altman metotu ile analiz edilmiştir. Sonuç olarak, BUSA idrarın pH'ını ve dansitesini ve bilirubin, ürobilinojen, keton, glikoz, protein, kan, nitrit ve lökositin negatifliğini ölçmede başarılıdır. Ayrıca, BUSA protein dışında glikoz ve kanın pozitifliğini ölçmede de başarılıdır.

Anahtar Sözcükler: İdrar tahlili, renk sensörü, Android, evde tedavi.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

RGB	Red Green Blue
MCU	Microcontroller
BUSA	Bogazici University Urine Strip Analyzer

1. INTRODUCTION

Urinalysis is a remarkable diagnostic technique and an essential part of physical examination used frequently in kidney and urinary tract diseases. Indeed, it can prodive and reveal significant events which happen and had happened historically in kidneys. Since early 1600s, researches and physicians had focused on urinalysis and its interpretations [2]. Beginning with microscopy, urinalysis has attained its last version by urine reagent strips in 1956 by Clinistix (from Ames Company, today Bayer Diagnostic) [2, 3].

Urine reagent strips are impregnated with a number of colored reagent blocks or pads separated from each other by narrow bands. When the strip is manually immersed in the urine specimen, the reagents in each block react with specific components of urine in such a way that the block changes color if the component is present, and the color change produced is proportional to the concentration of the component being tested for [4]. Recent analysis of test strips is perfomed via visual readings comparing to color charts or refractometric devices of the corresponding test strips [5]. Visual readings are based on the perception of individuals, hence it is relative. Considering refractometric devices, patients have to go to hospitals for giving urine samples, on the other hand, this procedure is quite hard for those who are too old to leave their homes and bedridden.

Biosense Technologies Pvt. Ltd. developed uChek which is a semi-automated system using iPhone's camera to automatically read urine strips, peripheral called the Cuboid and color mat. Results of urinalysis can be recorded on the phone, analyzed for trends and emailed. uChek application processes the image of color mat and strips. Cuboid maintains the light intensity during the photo capture [6]. As a results, patients do not need to leave their homes, instead, they can buy uChek kit and download the iPhone application and get their urine samples analyzed.

2. BACKGROUND

2.1 Urinary System

The kidneys are both regulatory and excretory organs whose primary function are regulation of the extracellular fluid (plasma and interstitial fluid) environment in the body [7, 8]. Formation of urine which is a modified filtrate of plasma enables the kidneys to accomplish this purpose [8]. The kidneys have some major functions such as:

- Regulation of body fluid and osmolality and volume.
- Regulation of electrolyte balance.
- Regulation of acid-base balance.
- Excretion of metabolic products and foreign substances.
- Production and secretion of hormones [7].

The functional unit of the kidneys is the nephron. Each human kidney contains approximately 1.2 million nephrons [7]. The nephron consists of a glomerular capsule, a proximal convoluted tubule, a loop of Henle and a distal tubule [8]. The glomerular (Bowman's) capsule contains an inner visceral layer of epithelium around the glomerular capillaries and an outer parietal layer. The space between these two layers is continuous with the lumen of the tubule and receives the glomerular filtrate. Filtrate that enters the glomerular capsule passes into the lumen of the proximal convoluted tubule. The wall of the proximal convoluted tubule consists of a single layer of cuboidal cells containing millions of microvilli. These microvilli increase the surface area for reabsorption. In the process of reabsorption, salt, water and other molecules needed by the body are transported from the lumen, through the tubular cells and into the surrounding peritubular capillaries [8]. The paired kidneys lies on either side of the vertebral

column below the diaphragm and liver. Urine produced in the kidneys is drained into a cavity known as the renal pelvis (basin) and then it is channeled from each kidney via long ducts - the ureters - to the urinary bladder. Urinary bladder is a storage sac for urine and its shape is determined by the amount of urine it contains. An empty urinary bladder is pyramidal; as it fills, it becomes ovoid and bulges upward into the abdominal cavity [8]. The kidneys play an essential role in regulating the amount of several important inorganic ions in the body, including Na^{+}, K^{+}, Cl^{-}, HCO_3^{-}, H^{+}, Ca^{+2}, Mg^{+2}, and $PO4^{-3}$. In order to maintain appropriate balance, the daily intake of these electrolytes must be equal to the excretion. If intake exceeds excretion, the amount of a particular electrolyte in the body increases and the individual is in positive balance with respect to that electrolyte. Conversely, if excretion exceeds intake, the amount decreases and the individual is in negative balance with respect to that electrolyte [7].

2.2 History of Urinalysis

In 1844, Golding Bird who was one of the physician of the great Guy's Hospital's wrote the rediscovery of the art of microsccoping the urine. He wrote that Provençal astronomer and polymath Nicolas-Claude Fabricius de Pieresc (1580-1637) described urinary crystals as "a heap of rhomboidal bricks" in 1630. This may be the first record of urine microscopy, but the researchers began to examine the urine regulary after the kidney tissue was first examined by microscope, in the late 1830. The founder of French nephrology, Pierre Rayer (1793-1867), and his young associate Eugene Napoléon Vigla introduced the regular urine microscopy to clinical practice in 1837. Rayer noticed that normal (clear) urine should not contain lots of red cells, which is the first microscopic haematuria. Alfred Becquerel noted in his classic book on the analysis of urine that clear urine may contain only sheets of epithelium wtih mucus, globules of this substance, closely resembling globules of pus, red blood cells, sperm, calcium and magnesium carbonate, and phosphates including ammonium magnesium phosphate in 1841. Golding Bird's book published after he died was the first book on described the presence and the meaning of urine sediment and crystals. His book enabled the urine

microscopy to place in routine clinical diagnostics [2].

Studies of urine sediment reached the top by means of Thomas Addis's (1881-1949)studies. He examined the urines of the patients who had various renal disease and recorded the differences of them over two decades. He was the first who informed the apperances of the renal failure cylinders. He offered that over night collected acidic urine should be studied both urine sediment and proteinuria [2].

Urinary test papers were emerged in early 1880's by some practitioners and pharmacists. The first test paper measuring sugar and albumin originated in England, in 1883. Afterwards it had been a methodological breakthrough that Austrian Fritz Feigel utilized the capillary properties of filter paper in improving color reactions. In 1956, Clinistix (from Ames Company, today Bayer Diagnostic) initialized "stick tests" [3]

Colorimetric test strips are undoubtly the fastest and the least expensive analytical methods available. Test strips are able to be read via reference color chart, visual reading, or via reflectometer, reflectometric readings [5].

2.3 Urinalysis

In general, urine consists of urea, uric acid, creatinine, soduim, potassium, chloride, calcium, magnesium, phosphates, sulfates, ammonia and 95 percent water [9, 10]. Table 2.1 shows selective filtration, reabsorption and excretion by the urinary system [9].

Normally, 700-2500 mL of urine is voided daily by an adult and this amount depends on nature of fluids and food intake [10]. Urine is usually acidic in nature and is amber or straw-colored. The specific gravity of urine normally varies between 1.015-1.020, pyhsiological conditions, such that dilute urine has a lower specific gravity than concentrated urine, changes this value [10]. Urinalysis can be conducted both by

Table 2.1
Selective filtration, reabsorption and excretion by the urinary sistem

Constituent	Filtered (g/24 h)	Reabsorbed (g/24 h)	Excreted (g/24 h)
Sodium	540	537	3.3
Chloride	630	625	5.3
Bicarbonate	300	300	0.3
Potassium	28	24	3.9
Glucose	140	140	0.0
Urea	53	28	25
Creatinine	1.4	0.0	1.4
Uric acid	8.5	7.7	0.8

the traditional methods and dipstick analysis [9].

2.3.1 Bilirubinuria

Detection of bilirubin in urine is called bilirubinuria [10]. Bilirubin is formed the degredation of hemoglobin [9]. The urine of a healthy individual does not contain bilirubin normally.

Foam test and Fouchet's test are the tests used in the detection of bile pigments. In Foam test, the formation of a yellow foam on the top service indicates the presence of bile segments. In Fouchet's test may be carried out in suspected jaundice. The test involves the conversion of barium chloride to barium sulfate, which in turn complexes with bilirubin and bilivirdin to form yellow and green products respectively, when react with ferric chloride in the presence of trichloroacetic acid. Moreover, Hay's test is used in the detection of bili salts in urine. The presence of bile salts in excessive quantity results in reduction of urine's surface tension. When sulfur flowers are sprinkled on such urine samples, they sink. On the contrary, in normal urine they keep floating [10].

Procedure of Foam Test (for Bilirubin)

- The urine sample is shaked and the forming of a yellow colored foam which is an indicator of the presence of bilirubin is observed [10].

Procedure of Fouchet's Test (for Bile Pigments)

- 10% $BaCl_2$ is prepared in distilled water and Fouchet's reagent by dissolving 25 g trihloroacetic acid in 10 mL of 10% $FeCl_3$ solution. Then the volume is adjusted with distilled water to 100 mL.
- If the pH of urine is alkaline, it is acidified with 33% acetic acid.
- 10 mL urine is added to 5 mL of 10% barium chloride and mixed. The presence of a precipitate is observed. A drop od dilute sulfric acid can be added to increase the amount of precipitate.
- Filtered through Whatman No.1 filter paper.
- A drop of Fouchet's reagent is added to the filtered precipitate. Green color is an indicaator of the presence of bile pigments [10].

Procedure of Hay's Test (for Bile salts)

- A few flower of sulfur is sprinkled lightly on the surface of a urine sample.
- If these flowers sink to the bottom, it means that the presence of bile salts in urine [10].

Price of conjugated bilirubin for 25 mg, 100 mg, 1g and 5g are $50.00, $125.00, $200.00 and $825.00 resspectively [11].

2.3.2 Urobilinogen In Urine

Urobilinogen and urobilin are the reduced forms of bilirubin due to action of bacterial enzymes after it enters the intestines [10, 9]. Some part of urobilinogen formed in the intestines is excreted as part of the feces, where it is oxidized to urobilin. Another part is absorbed into the portal bloodstream and carried out to the liver, where it is excreted in the bile. Urobilinogen which escapes removal by the liver is carried out to the kidneys and excreted in the urine. Unlike biliburin, urobilinogen is colorless [9]. The presence of urobilinogen in urine is an indicator of assesing the stages of jaundice and monitoring liver function impairment. Considering liver cirrhosis and hemolysis, large amount of urobilinogen is secreted in the urine, whereas in the case of obstructive jaundice, it is absent from urine. [10].

When urobilinogen and urobilin reacts with Ehrlich's aldehyde reagent (p-dimethylaminober
in pure hydrochloric acid), pink or faint red color occurs.

Procedure of Ehrlich's Test

- Ehrlich's reagent is prepared and 2g of p-dimethylaminobenzaldehyde is dissolved in 20 mL conc. hydrochloric acid. The voluem is adjusted to 100 mL by distilled water.
- 1 mL of Ehrlich's reagent is added to 10 mL of urine and a parallel control is set up by adding 1 mL of 20% HCl to 10 mL of urine.
- The contents of both tubes are mixed by inverting them and left for 3-5 min at room temperature. The contents are warmed if no pink color is produced [10].

Normal values

In random specimen, urobilinogen should be less than 1 mg/dL. In 2-hour specimen, urobilinogen should be less than 1 mg/2 hours.

Price of urobilinogen for 10 mg, 25 mg, 100 mg and 500 mg are $45.00, $75.00, $150.00 and $450.00 respectively [12].

2.3.3 Ketonuria

Detection of ketone in urine is called ketonuria [10]. Screening for ketonuria is done in hospitalized patients, presurgical patients, pregnant women, children and persons with diabetes [9].

Ketone bodies are the products of fat metabolism when fat serves as a major energgy source in the absence of glucose or in case of improper glucose metabolism, such as untreated diabetes, starvation and dehydration. Normally, ketone bodies are not present in urine or in blood and their presence indicates serious physiological conditions. Ketone bodies consist of three substances, namely, acetoacetic acid, CH3-C(=O)-CH2-COOH, β-hydroxybutyric acid (CH3-CHOH-CH2-COOH) and acetone(CH3COCH3). Among these, acetone and acetoacetic acid may cause brain damage if present in case of high concentration in blood. Acetoacetic acid and β-hydroxybutyric acid also decrease the pH of blood by transferring excess H+ to blood, resulting in acidosis. In such case, the body tries to compensate pH of blood by excreting more H+ ions in urine. This results in a change in the pH of urine in patients with ketosis as low as 4.5 [10].

A freshly voided urine sample should be used in ketonuria, since in case of storage, acetoacetic acid and β-hydroxybutyric acid are converted to acetone which is volatile and dissappears from sample. The urine sample shoul be kept in refrigerator if there is a delay in performing the test [10].

Apart from reagent strips, two clinical tests, Gerhardt's test and Rothera's test, can be used in detection of ketone bodies in urine. In Gerhardt's test, FeCl3 solution reacts with acetoacetic acid and forms red-brown precipitate. In Rothera's test, ketone bodies are treated with nitroprusside under alkaline condition and a purple color is formed [10].

Procedure of Gerhardt's test

- 5 mL of fresh urine is transferred to a clean test tube and 10 percent FeCl3 solution is added dropwise. Any red-brown precipitate formation is observed [10].

Procedure of Rothera's test

- 10 g of ammonium sulfate, 0.3 g sodium nitroprusside and 5 g anhydrous sodium carbonate are mixed and grinded.
- 0.5-1.0 g of reagent is transferred to test tube and a drop of fresh urine is added.
- Under alkaline conditions, ketone groups react with nitroprusside and give a purple color after 1 min [10].

Normal values

Negative result of ketone in urine: ($<$0.3 mg/dL or $<$0.05 mmol/L) Negative result of ketone in serum or plasma:

- Acetone: $<$2.0 mg/dL or 0.34 mmol/L
- Acetoacetic acid: $<$1 mg/d Lor $<$0.1 mmol/L
- β-hydroxybutyric acid: 0.21-2.81 mg/dL or 20-270 umol/L [2].

β-hydroxybutyric acid is a useful indicator for monitoring insulin therapy in patients with diabetic ketoacidosis [2].

Price of acetone for 16 oz (473.176 mL) is \$13.95 [13]. Price of acetoacetic acid for 1g, 5g, 10g and 25g are \$60.00, \$215.00, \$300.00 an \$625.00 respectively [14].

2.3.4 Glycosuria

Detection of glucose in urine is called glycosuria which is an indication of diabetes mellitus. The presence of significant levels of glucose in urine may be detected by Benedict's test. In this process, glucose reduces cupric ions of Benedict's reagent to cuprious ions further reacting with potassium thiocyanate to produce a white precipitate of cuprous thiocyanate as follows [10]:

$$Cu^{+2} + CuSO_4 \longrightarrow^{Hightemperature}_{Reducingsugar} Cu^{+} \longrightarrow^{OH^-} CuOH \longrightarrow^{KCNS} CuCNS$$

Procedure of Benedict's test

- 100 g of sodium citrate, 37.5 g of anhydrous sodium carbonate nd 62 g of potassium thiocyanate are dissolved in 300 mL of distilled water. The mixture is heated and then cooled to room temperature and filter through an ordinary filter paper. 50 mL of 1.8 % $CuSO_4$ is addeed to this solution. Also 2.5 mL of 5% potassium ferrocyanide is added and mixed well and total volume adjusted to 500 mL.
- 25 mL of reagent is transferred to a beaker to add 10 g of anhydrous sodium carbonate and the mixture is boiled.
- The urine sample is added to this boiling mixture and the time which blue color is fades is recorded.
- Volume of the sample is used for calculating the concentration of glucose. Since 25 mL of Benedict's reagent is reduced by 50 mg of glucose, the glucose present in mg per 100 ml of urine= $(50 * 100)/Volumeofurineused$ [10].

Glucose does not appear in the urine of healthy individuals [8].

2.3.5 Proteinuria

Detection of protein in urine is called proteinuria. The presence of increased amount of protein in urine might be an indicator of renal disease. However other physiological conditions such as fever, can cause the presence of protein in urine. Also there are some renal disease in which proteinuria is absent [9].

Trace amount of protein consists of albumin, one third of normal urine protein, and globulin from the plasma [9]. Boiling and coagulation test, Heller's test and Sulfosalicyclic test detects protein in urine [10].

Procedure of Boiling and Coagulation Test

- 5 mL of urine is transferred into a tube and boiled. The precipitate occuring due to boiling may be the indicator of the presence proteins, phosphates and carbonates in urine.
- To confirm whether the precipitate is due to proteins, 3-5 drops of dilute acetic acid is added and boiled. The presence of a precipitation due to acetic acid indicates the presence of protein in urine [10].

Procedure of Heller's Test

- 5 mL of HNO_3 is transferred to a test tube and a few drops of urine sample is added. The presence of a precipitation is an indicator of the presence of protein in urine [10].

Procedure of Sulfosaliyclic Acid Test

- 2-3 volumes of 3% sulfosaliyclic acid is added to 1 mL of urine sample and any cloudy formation is observed [10].

In qualitative measurement, normal urine sample does not contain protein [9]. Prices of albumin for 1 mg, 2mg and 5 mg are $115.00, $150.00 and $325.00 respectively [15].

2.3.6 Hematuria

The presence of increased number of red blood cells in the urine is called hematuria where as the presence of only blood pigments without corpuscles is called hemoglobinuria[9, 10]. Erythrocytes occasionally appear in urine in healthy persons. On the other hand, persistent findings of even small numbers of RBCs can be an indicator of renal disease or glomerular disease [9]. The commonly used method to detect blood in urine is Benzidine test [10]. Hemoglobin peroxides acts on the hydrogen peroxide present in Benzidine reagent, therefor, the reagent is oxidized to give blue colored solution. Apart from Benzidine test, microscopic examination is also done [9].

$$2H_2O_2 \longrightarrow^{Hbperoxidase} 2H_2O + O_2$$

$$Benzidine + O_2 \longrightarrow Bluecolor$$

Procedure of Benzidine Test

- A saturated solution of benzidine in glacial acetic acid is prepared.
- 3 mL of urine sample is added to 3mL of benzidine followed by 1 mL of H_2O_2.
- The presence of green or blue color is a indicator of the presence blood in urine [10].

Procedure of Microscopic Examination

- Both the low-power field (lpf) and the high-power field (hpf) are used. Low

power is used to find and count casts; RBCs, WBCs and bacteria show up and are counted under high power [9].

Normal values

Negative limit is < 0.03 mg free Hb/dL or $< 10 Ery/\mu L$ [9].

Prices of erythrocyte for 1 mL, 5mL, 25 mL and 50 mL are \$20.00, \$75.00, \$150.00 and \$250.00 respectively [16].

2.3.7 Nitrituria

The detection of nitrite is an indirect method for detecting bacteria in the urine. Common gram-negative organisms contain enzymes that reduce the nitrate in the urine to nitrite. The nitrite test can also be used to evaluate the success of antibiotic therapy [9]. Nitrites react with *p*-arselinic acid, forming a diazonium compound that reacts with a colorimetric indicator. It is a qualitative test resulting in either positive or negative [17]. Price of sodium nitrite for 100 g is \$11.95 [18].

2.3.8 Leukocyturia

The detection of leukocytes in urine is called Leukocyturia. Usually, the presence of leukocytes indicates an urinary tract infection [9]. The leukocyte esterase, a bacteria-related enzyme, test detects esterase released by the lysed neutrophills into the urine [9, 17]. When the leukocyte esterase reacts with ester, it produces 3-hydroxy-5-phenyl pyrrole which, in turn, reacts with diazo salt and color change occurs. The test is usuallay positive if the number of white blood cells is greater than 5 [17]. Price of leukocyte esterase used in urine test strips for 1 mL, 5 mL nd 25 mL are \$50.00, \$125.00 and \$375.00 respectively [19].

2.3.9 Specific Gravity

Specific gravity measures relative density by comparing the weight of urine to that of distilled water in a constant volume. The specific gravity depends on both the weight and number of particles in solution. Specific gravity is a convenient and rapid measure of osmolality, and, hence, urine concentration and ranges from 1.001 N/cm^3 to 1.035 N/cm^3. Specific gravity of distilled water is 1.000 N/cm^3 and that of plasma is 1.010 N/cm^3, meaning, low specific gravity indicates dilute urine whereas, high spesific gravity indicates concentrated urine [17].

The oldest method to measure the specific gravity is by measuring the buoyancy of a hydrometer in urine, on the other hand, reproducibility of this method is poor and inaccurate. Most laboratories use a refractometer that measures the refractive index and reflects the quantity of solids in a liquid. However, if substances (e.g., glucose and protein) of higher molecular weight than the normal urine constituents are present in the urine, there is a disproportionate rise in the urine specific gravity as compared to urine osmolality as measured by refractometer or hydrometer. In this case, specific gravity is outside of the normal range, greater than 1.040 N/cm^3 [17].

2.3.10 pH

The pH of urine can fluctuate between 4.5 and 8.0. In general, pH of normal urine is between 5.0 and 6.0, meaning, the usual obligate excretion of acids generated from metabolism. Urine pH is measred by the double indicators methyl red and bromthymol blue, which give a broad range of colors at different pH. If a more accurate measurement is required, urine should collected under oil to prevent CO_2 escape and measured via a glass pH electrode. The urine pH tends to be lower in the first-void sample and rise after meals. If urine is left at room temperature, the pH becomes more alkaline, due to breakdown of urea by bacteria which liberates ammonia. pH is an indicator of acid-base disorders [17].

2.3.11 Reagent Strips

Biochemical testing of urine involves the use of commercially available disposable strips. Each strip is impregnated with a number of colored reagent 'blocks' separated from each other by narrow bands. When the strip is manually immersed in the urine specimen, the reagents in each block react with specific components of urine in such a way that the block changes color if the component is present, and the color change produced is proportional to the concentration of the component being tested for [4].

- Fresh urine is collected into a clean dry container.
- The sample is not centrifuged.
- The disposable strip is briefly immersed in the urne specimen; care must be taken to ensure that all reagent blocks are covered.
- The edge of the strip is held against the rim of the urine container to remove any excess urine.
- The strip is then held in a horizontal position for a fixed length of time that varies from 30 second to 2 minutes.
- The color of the test areas are compared with those provided on a color chart.the strip is held close to the color blocks on the chart and matched carefully and then discarded [4].

The range of the components routinely tested for in commonly available commercial urinalysis strips is extensive ad includes glucose,bilirubin,ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite and leukocytes [4].

3. MATERIALS

The thesis consists of two parts, namely, hardware and software. Hardware part includes PIC 16F877A Microcontroller, Parallax's TCS-3200-DB ColorPal Color Sensor, Single Axis Linear Slide, Bluetooth Module, USB to Serial Converter and Step Motor. Software part, on the other hand, includes Proton BASIC, MATLAB, Eclipse and Android SDK.

3.1 Microcontroller

Microcontrollers are single-chip microcomputer used in controlling and automating of machines and processes [20]. A microcontroller contains microprocessor and also one or more of the following components [1].

- Memory
- Analog to Digital (A/D) converter
- Digital to Analog (D/A) converter
- Parallel I/O interface
- Serial I/O interface
- Timers and Counters [1]

The first 4 bit microcontrollers was develeoped by different companies like Hitachi, National, Toshiba, etc. Afterwards, 8 bit microcontrollers were developed by Intel, Motorola, Ziolog, Philips, Microchip technology, etc [1].

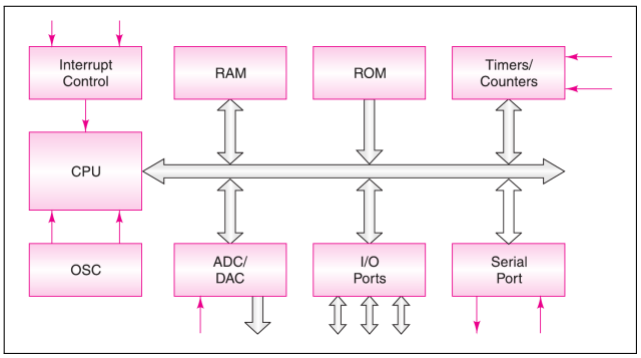


Figure 3.1 Block diagram of a typical microcontroller [1].

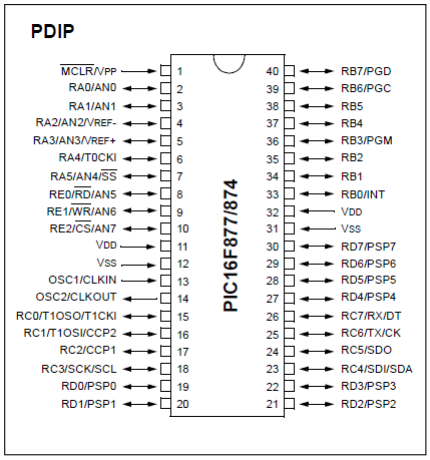


Figure 3.2 Pin descriptions of 16F877A microcontroller

3.2 TCS3200-DB Color Sensor

Parallax's TCS3200-DB color sensor consists of TAOS TCS3200 RGB sensor, two white LEDs to illuminate the object, 5.6 mm collimator lens to focus the object and standoffs to adjust the optimum distance between the object and the sensor. The TCS3200 includes photo detectors with red, green, blue filter and no filter. These filters enable the sensor to eliminate the location bias of colors. The frequency of square-wave output is proportional to the intensity of the color of the object. Features of the sensor can be listed as:

- Sensor provides the RGB color information as a digital frequency.
- Two white LEDs placed in two sides of the sensor can be gated on and off for ambient light substraction.

- Sensor needs 3.3V or 5V logic supplies and plus 5V for the LEDs.

Recommended application can be listed as:

- Test strip reading.
- Sorting by color.
- Ambient light sensing and calibration.
- Color matching

TCS3200-DB has four photodetector arrays, namely, red, green, blue filter and no filter (clear). The filters are distributed evenly thoroughout the array in order to eliminate the location bias among the colors. The device has a oscilltor which produces the square wave output proportional to the intensity of th chosen color. There is one output from this oscillator and the color which is read is selected using S2 and S3 adress lines. Moreover, the divide rate can be programmed via S0 and S1 adress lines. The settings for S0, S1, S2 and S3 can be summarized in Table 3.1.

Table 3.1
The settings for S0, S1, S2 and S3 and their functions.

S0	S1	Divide	S2	S3	Color
0	0	Power down	0	0	Red
0	1	1:50	0	1	Blue
1	0	1:5	1	0	Clear
1	1	1:1	1	1	Green

Considering a subject distance of 1" (25mm) from the front surface of the circuit board, 5.6mm lens enables the sensor module to see a square area approximately 9/64" (3.5mm) on a side, meaning, any color variation within an area having that size will be averaged by the TCS3200-DB. The Spectral response of the sensor is a function of the

sensor's device response and the system's optics and illumination and also by taking into account the lens' IR filter.

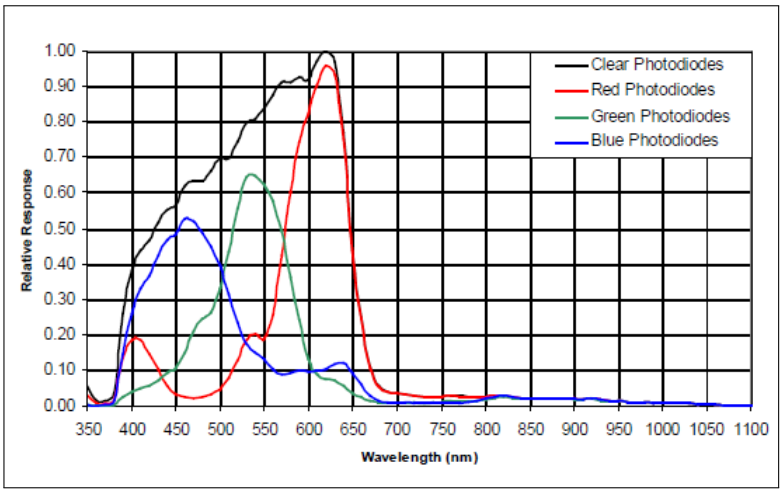


Figure 3.3 The spectral response of the TCS3200-DB with IR filter.

Response curve should be combined with the spectrum of the white LEDs for reflecive objects.

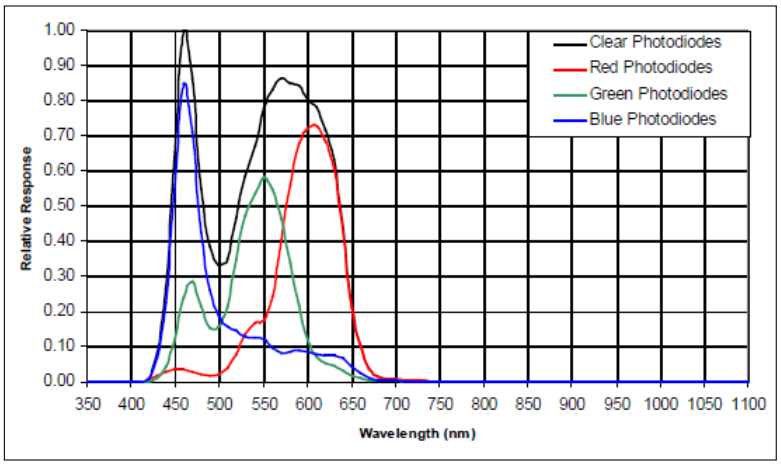


Figure 3.4 The spectral response of the TCS3200-DB with IR filter and white LEDs

3.3 Single Axis Linear Slide

Linear slide moving along x axis was designed in CAD programme called ThinkDesign by A.Murat Ağır worked in Numeric Control Group and engraved in IMES.

As seen in Figure 3.5, step motor was attached to a screw whose pitch was 1 mm.

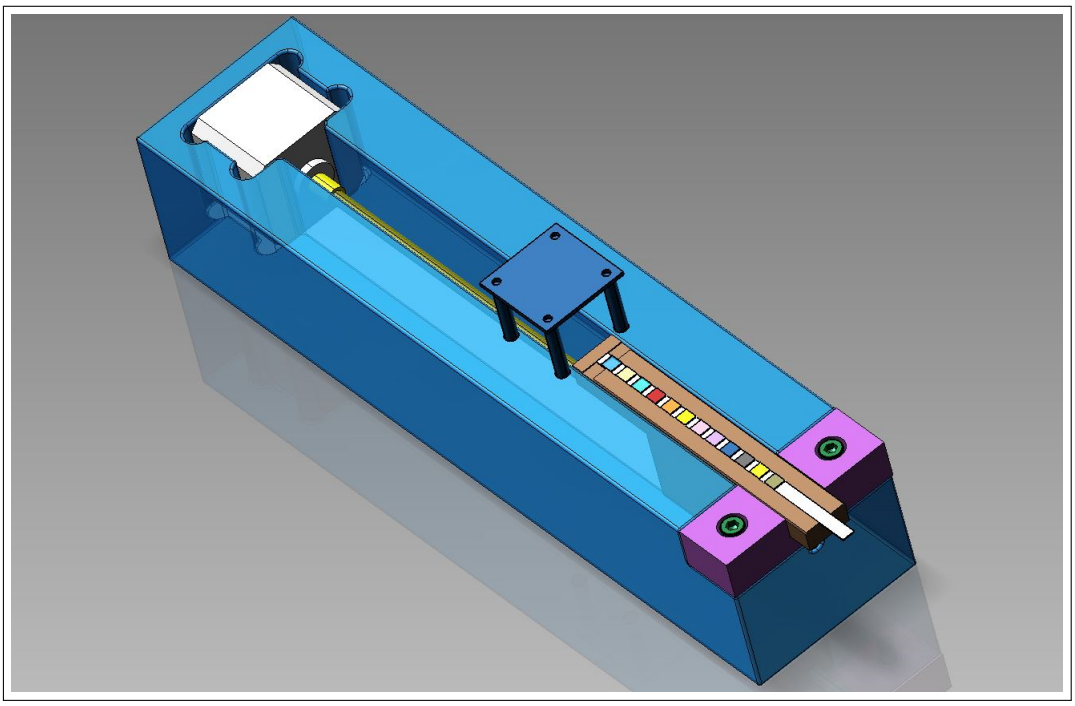


Figure 3.5 CAD of linear slide

The screw went through the part which the strip was moved. When the step motor rotated counterclockwise, the strip moved towards the sensor, on the other hand, when the step motor rotated couterclockwise, the strip moved away from the sensor. The realized version of the linear slide can be seen in Figure 3.6.

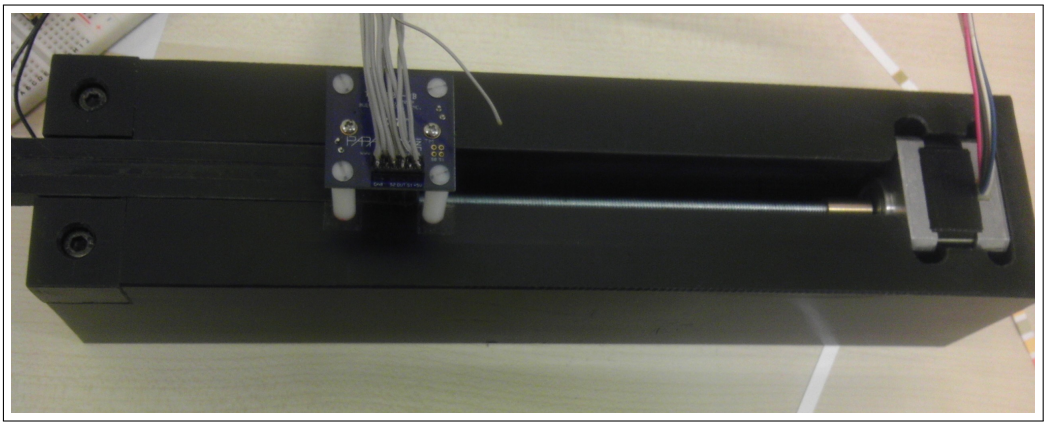


Figure 3.6 Realization of the linear slide

3.4 Bluetooth Module

EGBT-046S is generic bluetooth module for UART wireless cable replacement function. It is permenantly progammed as bluetooth slave device, meaning, it cannot connect to master bluetooth device, only neighbour master devices can connect to it.

EGBT-04 module is supplied between 3.1V to 4.2V DC. If it is supplied with 3.3V, it can be directly connnected to UART port of any mirocontroller running at 3.3V. When used with the microcontollers running at 5V, however, the scenerio changes. EGBT TXD is tolerant to 5V, hence it can be directly connected to the UART RXD of the microcontroller. On the contrary, 5V logic can damage EGBT RXD, thus level translation circuit should be added to protect RXD.

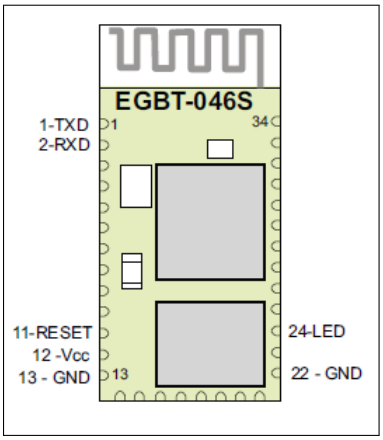


Figure 3.7 Pins of EGBT-046S bluetooth module

TXD in pin 1 is connected to RXD pin of the microcontoller. Similarly, RXD in pin 2 is connected to TXD pin of the microcontoller. but since 16F877A is supplied with 5V, these connections are protected via level translation circuit in Fig 3.9. LED in pin 24 is used for the indication of whether the module is connected to any device or not. If it is not connected, LED toggles. On the other hand, if it is connected, LED illuminates continuously. Other pin descriptions can be seen in Table 3.2.

As clarified above, EGBT-046S wiring varies according to Vcc supply of the microcontoller. If a microcontoller supplied with 3.3V is used, the connection should be as in Fig 3.8.

Table 3.2
Pin descriptions of EGBT-046S

Pin	ID	Description
1	TXD	UART TXD Output
2	RXD	UART RXD Input
11	RESET	RESET Input
12	Vcc	+3.1 to 4.2VDC Power Input
13	GND	Common Ground
22	GND	Common Ground
24	LED	LED Status Indicator Flashing

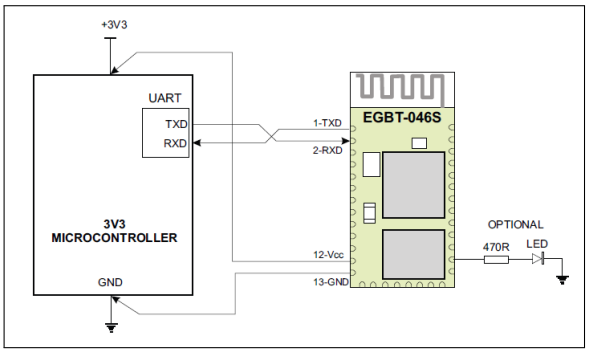


Figure 3.8 EGBT-046S connection with a microcontoller supplied with 3.3V

On the other hand, if a microcontroller supplied with 5V is used, the connnection should be as in Figure 3.9. Default configurations for EGBT-046S can be seen in Table 3.3.

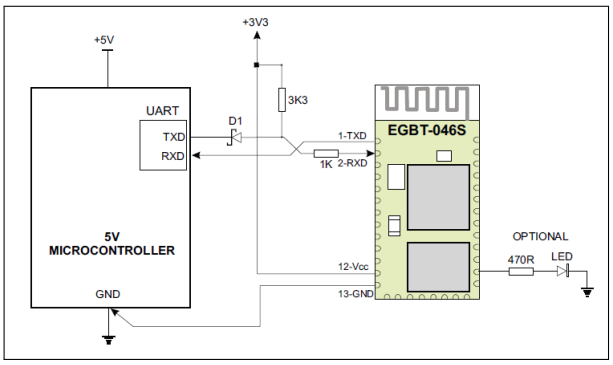


Figure 3.9 EGBT-046S connection with a microcontoller supplied with 5V

Table 3.3
Default configurations for EGBT-046S

Property	Value
Baud Rate	9600 bps
Data	8 bits
Stop Bits	1bit
Parity	None
Handshake	None
Passkey	1234
Device Name	Linvor

All these properties can be changed via AT command set.

3.5 Arduino USB to Serial Converter

Arduino USB to serial converter board converts a USB connection into 5V TX and RX that it can be connected straight to the Arduino Ethernet or any other boards with an FTDI cable compatible connector [21]. As seen in Figure 3.10, Arduino USB to serial converter has 6 pin but one is unused. Others are ground, +5V, TX, RX and reset. In order to connect it to a microcontroller one should connect TX pin of Arduino to RX pin of the microcontoller and RX pin of Arduino to TX pin of the microcontroller. GND pin should be connected to the ground of the board. It is not

necessary to connect +5V pin to the power of the board, computer provides the supply for Arduino.

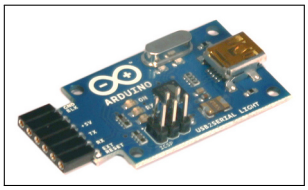


Figure 3.10 Arduino USB to serial converter

3.6 Step Motor

A step motor is an electromechanical device which converts electrical pulses into discrete mechanical movements. The shaft or spindle of a step motor rotates in discrete step increments when electrical command pulses are applied to it in the proper sequence. The motors rotation has several direct relationships to these applied input pulses. The sequence of the applied pulses is directly related to the direction of motor shafts rotation. The speed of the motor shafts rotation is directly related to the frequency of the input pulses and the length of rotation is directly related to the number of input pulses applied [22].

The advantages of the step motors can be listed as follows:

- The rotation angle of the motor is proportional to the input pulse.
- The motor has full torque at standstill (if the windings are energized).
- Precise positioning and repeatability of movement since good step motors have an accuracy of 3-5 percent of a step and this error is non cumulative from one step to the next.
- Excellent response to starting/stopping/reversing.
- Very reliable since there are no contact brushes in the motor. Therefore the life of the motor is simply dependant on the life of the bearing.

- The motors response to digital input pulses provides open-loop control, making the motor simpler and less costly to control.
- It is possible to achieve very low speed synchronous rotation with a load that is directly coupled to the shaft.
- A wide range of rotational speeds can be realized as the speed is proportional to the frequency of the input pulses [22].

The disadvantages of the step motors, on the other hand, can be listed as follows:

- Resonances can occur if not properly controlled.
- Not easy to operate at extremely high speeds [22].

3.7 Proton BASIC

Proton Development Suite combines the Proton Plus Compiler with a brand new IDE and Virtual Simulation Environment, creating a PIC® microcontroller BASIC development environment suitable for both hobbyists and professionals. The Proton Development Suite is the result of a collaboration between three British companies that lead their field, Crownhill Associates, Mecanique and Labcenter [23].

Once Proton Development Suite is downloaded, Proton IDE, Proton Plus Compiler and ISIS Virtual Simulation are also downloaded. Proton IDE developed by Mecanique, supports 16F870, 16F871, 16F873(A), 16F874(A), 16F876(A), 16F877(A), 16F87, 16F88, 18F242, 18F248, 18F252, 18F258, 18F442, 18F448, 18F452, 18F458, 18F1220, 18F1320, 18F2220, 18F2320, 18F4220, 18F4320, 18F6620, 18F6720, 18F8620 and 18F8720. It also enables real time simulation via Proteus. Moreover, serial communicator allows user to transmit and receive data via a serial cable connected to your PC and development board [24]. Proton Plus Compiler takes full advantage of each type of PIC® micro available, and offers a friendly and intuitive language that allows

very complex operations to be carried out with a minimum of fuss, and provides a flexibility and functionality that is unparalleled in the world of PIC® micro programming. The PROTON+ compiler is functionally compatible with the language of the Parallax BASIC Stamp modules and the PICBASIC Pro Compiler from microEngineering labs [25]. Labcenter ISIS contains a microprocessor (a PIC® 12, PIC® 16 or PIC®18 variant) and many of electrics and electronics components enabling users to simulate their system on PC [26].

Command called HSEROUT and HSERIN were used for serial communication. Data sent by HSEROUT was able to be seen in Serial Communicator program and data sent by other devices can be read via HSERIN [23].

3.8 MATLAB

MATLAB® is a programming language enabling data analysis by MathWorks Company [27]. MATLAB allows the users to develop algorithms faster than current languages such as C/C++ and Java. MATLAB has variety of toolboxes which are used in many fields [28]. Instrument Control Toolbox which can be found in Test and Measurement section lets users connect MATLAB directly to instruments such as oscilloscopes, function generators, signal analyzers, power supplies, and analytical instruments. With Instrument Control Toolbox, one can generate data in MATLAB to send out to an instrument, or read data into MATLAB for analysis and visualization [29]. One of the applications of Instrument Control Toolbox in MATLAB is providing serial port communication. This feature connects two devices by the RS-232 standard [30].

3.9 Android

Android is a mobile operating system that is based on a modified version of Linux. It was originally developed by a startup of the same name, Android, Inc. In 2005, as part of its strategy to enter the mobile space, Google purchased Android and took over its development work (as well as its development team) [31].

Google wanted Android to be open and free; hence, most of the Android code was released under the open-source Apache License, which means that anyone who wants to use Android can do so by downloading the full Android source code. Moreover, vendors (typically hardware manufacturers) can add their own proprietary extensions to Android and customize Android to differentiate their products from others. This simple development model makes Android very attractive and has thus piqued the interest of many vendors [31].

Android has gone through a quite number of updates since its first release. Android versions can be seen in Table 3.4.

Some features of Android can be listed as follows:

- Storage - Uses SQLite, a lightweight relational database, for data storage.
- Connectivity - Supports GSM/EDGE, IDEN, CDMA, EV-DO, UMTS, Bluetooth (includes A2DP and AVRCP), WiFi, LTE, and WiMAX.
- Messaging - Supports both SMS and MMS.
- Web browser - Based on the open-source WebKit, together with Chrome's V8 JavaScript engine.
- Media support - Includes support for the following media: H.263, H.264 (in 3GP or MP4 container), MPEG-4 SP, AMR, AMR-WB (in 3GP container), AAC, HE-AAC (in MP4 or 3GP container), MP3, MIDI, Ogg Vorbis, WAV, JPEG, PNG, GIF, and BMP.

Table 3.4
Android versions

Versions	Codename	API
1.5	Cupcake	3
1.6	Donut	4
2.1	Eclair	7
2.2	Froyo	8
2.3.3	Gingerbread	10
3.0	Honeycomb	11
3.1	Honeycomb	12
3.2	Honeycomb	13
4.0	Icecream	14
4.0.3	Icecream	15
4.1.2	Jelly Bean	16
4.2.2	Jelly Bean	17

- Hardware support - Accelerometer Sensor, Camera, Digital Compass, Proximity Sensor, and GPS.
- Multi-touch - Supports multi-touch screens.
- Multi-tasking - Supports multi-tasking applications.
- Flash support - Android 2.3 supports Flash 10.1.
- Tethering - Supports sharing of Internet connections as a wired/wireless hotspot [31].

3.10 CombiScreen-Plus (Lab U11Plus) Urine Test Strips

CombiScreen-Plus test strips has 11 pads which measure bilirubin, urobilinogen, ketone, ascorbic acid, glucose, protein, blood, pH, nitrite, leukocytes and specific

gravity respectively. The reason of ascorbic acid, known as Vitamin C, pad is involved in test strip is that ascorbic acid in urine causes false negative results of the blood and glucose pads [32].

Figure 3.11 CombiScreen Plus test strips

Lab U11Plus test strips has exactly the same features with CombiScreen-Plus except the compensation area pad between leukocytes and specific gravity pad.

Figure 3.12 Lab U11 Plus test strips

Reagent compositions of each pad can be seen in Table 3.5.

3.11 CombiScan 500 Urine Analyzer

CombiScan 500 is a semi automated urine test strip analyzer for use with CombiScreen Plus. It is designed for bigger laboratories and for hospitals [33]. Feature of CombiScan 500 can be listed as follows:

Table 3.5
Regent compositions of CombiScreen Plus and Lab U11 Plus test strips

Pad	Reagents	Percentile
Bilirubin	Diazonium salt	3.1 %
Urobilinogen	Diazonium salt	3.6 %
Ketones	Sodium nitroprusside	2.0 %
Ascorbic acid	2,6-dichloro-phenol-indophenol	0.7 %
Glucose	Glucose oxidase	2.1 %
	Peroxidase	0.9 %
	O-Tolidine hydrochloride	5.0 %
Protein	Tetra-bromophenol blue	0.2 %
Blood	Isopropylbenzol-hydroperoxide	21.0 %
	Tetramethylbenzidine-dihydrochloride	2.0 %
pH	Bromthymol blue	10.0 %
	Methyl red	2.0 %
Nitrite	Sulfanilic acid	1.9 %
	Tetrahydrobenzol[h]quinolon-3-ol	1.5 %
Leukocytes	Carboxylic acid ester	0.4 %
	Diazonium salt	0.2 %
Specific gravity	Bromthymol blue	2.8 %

- Modern CCD technology
- Interface for barcode reader and keyboard
- Automatic test strip recognition and standby
- Variable format selection for printout of test results and time/data
- Integrated printer
- Automatic transport, measurement of test strips
- Bi-directional interface for laboratory and hospital EDP [33]

Figure 3.13 CombiScan 500 urine strip analyzer

3.12 Negative and Positive Control Solutions

Analyticon® produces control solutions to control CombiScreen urine test strip and CombiScreen urine analyzers. These solutions are regulated to the labs who use CombiScreen urine test strips and CombiScan urine analyzers [34]. Table 3.6 shows the result chart of negative and positive control solutions.

Figure 3.14 Negative control solution of CombiScreen Plus

Table 3.6
Result chart of negative and positive control solutions

Combi Scan 500	Level 1 Negative Control	Level 2 Positive Control	Measured Values
Specific Gravity	1.010-1.030	1.005-1.020	1.005-1.030
Leukocytes	Negative	25-500 Leu/uL	25-75-500
Nitrite	Negative	Positive	Negative-Positive
pH	5.0-6.0	6.0-9.0	5-6-7-8-9
Erytrocytes	Negative	10-300 Ery/uL	10-50-300
Protein	Negative	30-100 mg/dL	30-100-500
Glucose	Negative	100->1000 mg/dL	50-100-250-500->=1000
Ascorbic Acid	Negative	Negative	20-40
Ketone	Negative	10-300 mg/dL	10-25-100-300
Urobilinogen	Normal	2-12 mg/dL	2-4-8-12
Bilirubin	Negative	1-4 mg/dL	1,2,4

3.13 Atago Manual Refractometer

ATAGO Clinical Refractometers use special scales for specific gravity of urine, and total protein in serum or plasma by using the principles of light refraction. Its urine specific gravity scale range is between 1.000 to 1.060 with 0.001 measurement accuracy [35].

3.14 Gray Scale Calculation

Conversion of RGB to gray scale is performed by the formulation below:

$I = W_R * R + W_G * G + W_B * B$ where I is intensity and W_R, W_G, W_B are weight coefficients for red, green and blue respectively. In general sum of W_R, W_G, W_B

Figure 3.15 Positive control solution of CombiScreen Plus

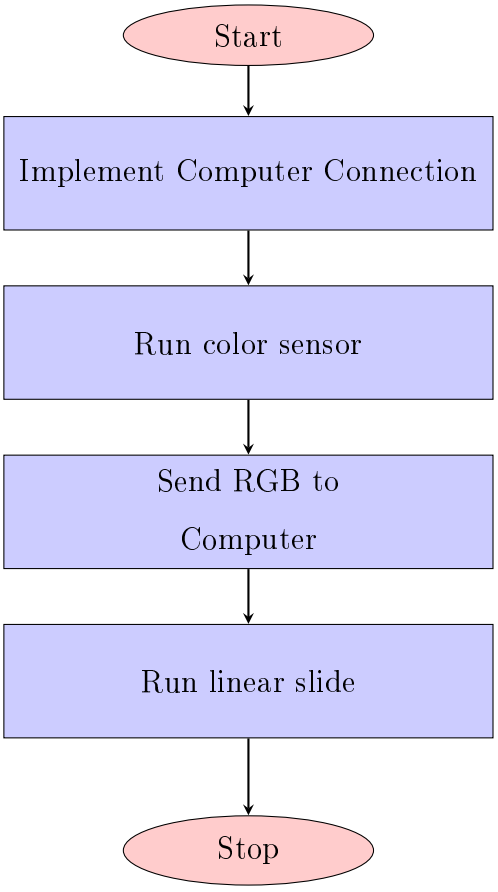
Figure 3.16 Atago manual refractometer

is equal to 1, thus $W_R + W_G + W_B = 1$. When these weigths are optimized for the human visiual system, their values would be $W_R = 0.299, W_G = 0.578, W_B = 0.114$ which major international standardization organizations withing TV and image/video coding are agreed [36].

4. METHOD

4.1 Microcontroller Part

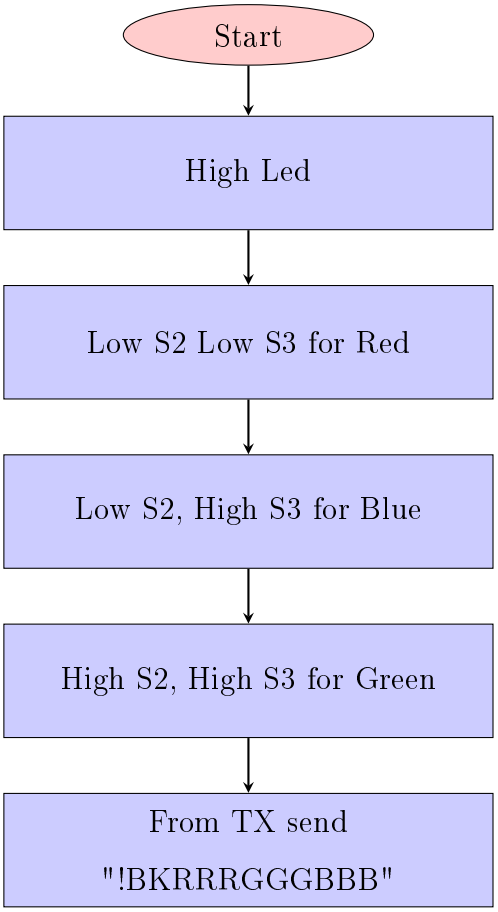
Microcontroller part consists of TCS3200-DB color sensor, the linear slide and computer communication. The process how these three system was controlled by the MCU is explained in different headings. The main algoritm of the microcontroller program can be seen below:



4.1.1 Controlling TCS3200-DB

TCS3200-DB's S2 and S3 address lines are used for obtaining red, green and blue color of the sample (see in Table 3.1). The algorithm for reading color of a sample

in Proton Basic can be seen below. This reading program was repeated 10 times for each pad.



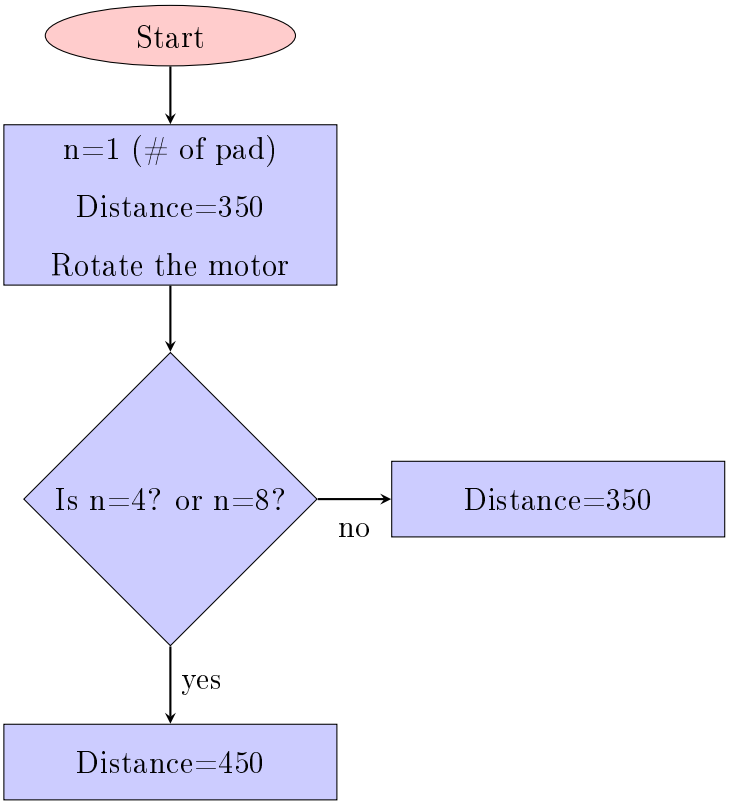
4.1.2 Controlling and Calibrating the Linear Slide

Controlling the linear slide was based on controlling the step motor. The pins of the step motor was connected to PORTB.0, PORTB.1, PORTB.2 and PORTB.3 respectively. Energizing the pins of the step motor in order ends up with 4 steps. Table 4.1 shows this process clearly.

The corresponding algorithm for controlling the step motor can be seen below:

Table 4.1
Energizing process of the step motor to end up with 4 steps

PORTB.3	PORTB.2	PORTB.1	PORTB.0
1	0	0	0
0	1	0	0
0	0	1	0
0	0	0	1



Calibration process was related to determining the number of rotation of the step motor. The slide moves 1 mm in $+x$ direction when the step motor turns counter clockwise in 360°. Since each pad should be placed under the sensor in the same direction, determining the number of rotation of the step motor was critical. The slide was expected to move the same distance which was 7.5 mm between two pads, at the same number of rotation. However, because of a production error, the number of rotations required to place each pad under the sensor was supposed to be calculated experimentally. A strip whose each reagent block has the same color was prepared. In

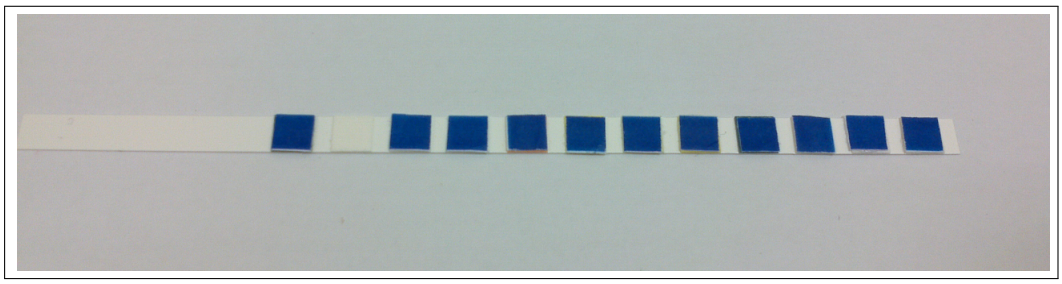


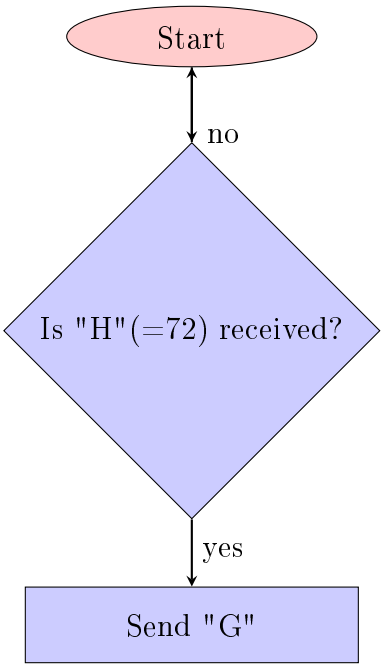
Figure 4.1 The calibration strip

other words, blue colored paper was cut according to the size of the pads and it was pasted on each block. Calibration strip can be seen in Figure 4.1.2

The calibration strip was read by the system and the color of each pad was compared using ANOVA and Repeated Measures of ANOVA method. Each pad was considered as a group having three individuals, red, green and blue while proceeding ANOVA. Thus, the number of group was 11 and sample size was 3. While proceeding Repeated Measures of ANOVA, red, green and blue values were calculated individually. This time each pad was considered as the same person undergoing the same measurement while the linear slide is moved. Hence, sample size was 11, the number of pads and the number of treatments were 10 which was the number of measurement per pad.

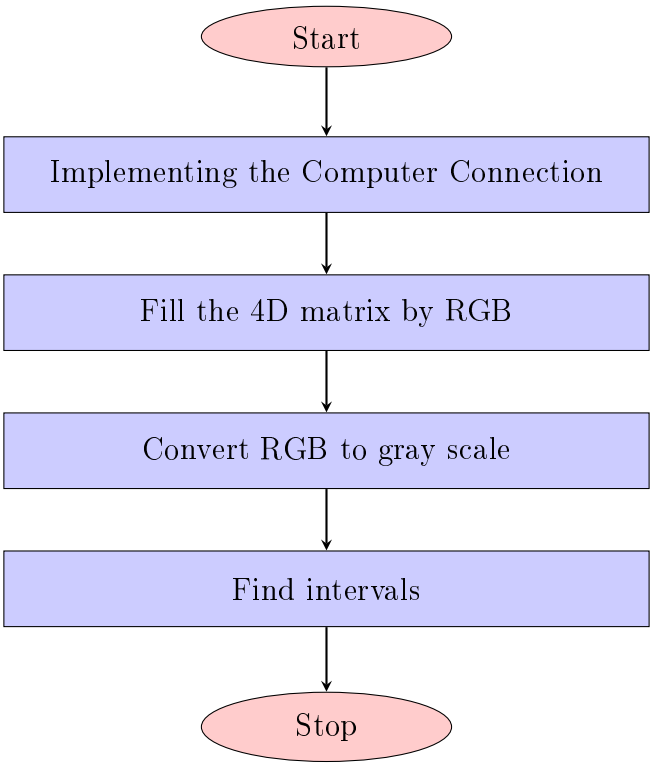
4.1.3 Implementing Computer Connection

The reading program was started after an identification process was completed. This process included that the MCU received "H" letter standing for "Hello" and the MCU replies by sending "G" standing for "Goodbye". Therefore, unless the MCU receives "H" letter, the program never starts. The flow chart representing this identification process can be seen below. Color data sent by TCS3200-DB and "H" letter was received via HSERIN command from RX pin. Similarly, "G" letter sent via HSEROUT command from TX pin. Serial baud rate is critical for preveting data loss. Both computer program and the MCU shoul have the same baud rate, which was 2400 for this system.



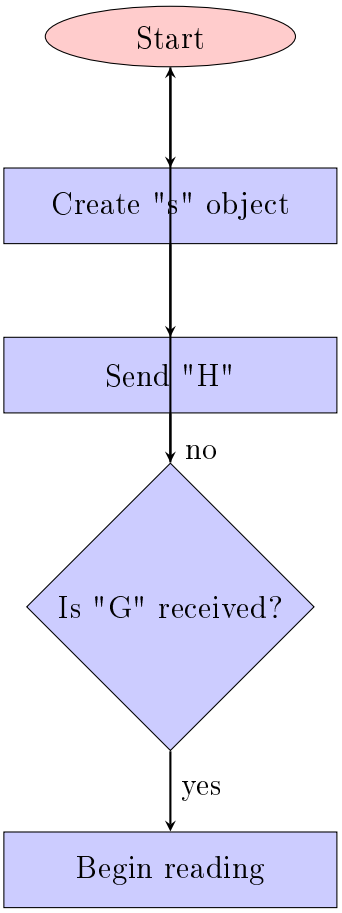
4.2 MATLAB Part

MATLAB was used for imlementing the MCU connection, hence recording RGB values and forming the gray-scale color database for each pad. Below, the general algorithm of MATLAB part can be seen:



4.2.1 Implementing The Microcontroller Connection

Instrument Control Toolbox was used for implementing the MCU connection. The algorithm in order to establish the computer connection via USB can be seen below:



"serial" command used for creating serial port object having COM port information, baud rate, terminator determinig the stop command to read data and time out which is the time passed until MATLAB stop waiting for the data. For USB connection, the serial object using COM14 port, whose serial baud rate was 2400 bps, whose terminator lines were line feed and carriage return whose ASCII values are 10 and 13 respectively and whose time out was 30 seconds was created via the command below:

$$s = serial('COM14','BaudRate',2400,'Terminator','LF','Timeout',30);$$

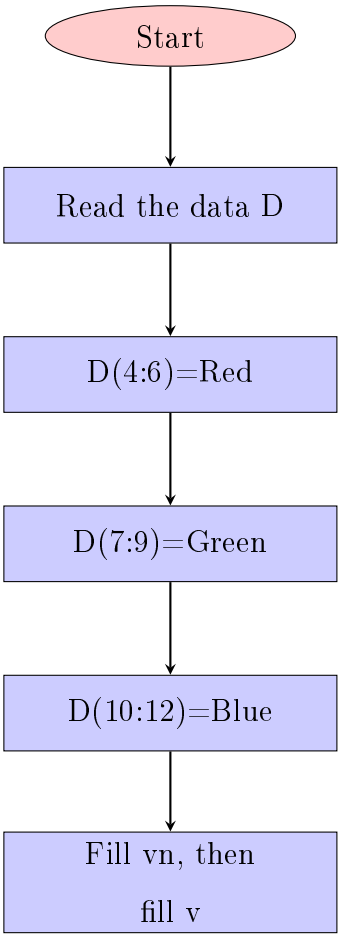
For Bluetooth connection, COM12 port was used and s object having the same properties as USB connection except COM number was created as in the code above.

$fopen(s)$ and $fclose(s)$ functions were used for begining to read data and stop reading data respectively. After $fopen(s)$ command was executed, $fscanf$ function was used for reading data from the MCU and $fprintf$ function was used for writing or

sending the data to the MCU. Both functions works with strings, in other words, the data is read in string format, whereas, the data is sent in string format.

4.2.2 Forming and Filling 4D Matrix

The 2D and 4D matrices were used to record RGB values. The algorithm for filling the matrices can be seen below:



The strip has 11 pads and each pad was read 10 times. 4D matrix was required for handling color data of all pads. The matrix formed via $v = zeros(3, 1, 10, 11)$ command formed 10 $3x1$ vector matrices for 11 pads.

Color data read via $fscanf$ function needed to be converted to number from string form in order for calculations. $str2num$ function was used for this purpose. The

read data converted string to number was in $!BKRRRGGGBBB$ form. Considering the fact that 3rd, 4th, 5th component of the data was red value, 6th, 7th, 8th components of the data was green value and 9th, 10th, 11th component of the data was blue value, 4D matrix was filled with RGB values as explained below:

The read RGB values are able to be seen as a figure via dispcolorf function written in MATLAB.

4.3 Forming The Database

Database means the color recordings of pads for different cases. The first approach for forming the database was to prepare solutions having the specific amount of bilirubin, urobilinogen, acetone or aceoticacid, glucose, albumin, blood (erythrocyte), nitrite and leukocytes. In addition, the solutions with known pH and specific gravity was aimed to be prepared. The cost table informing prices of bilirubin, urobilinogen, acetone or aceoticacid, glucose, albumin, blood (erythrocyte), nitrite and leukocytes can be see in Table 4.2.

Total cost of this approach forced the method to be changed. Instead, control solutions and patient samples were planned to be used in forming the database. Hence, this alternative method consisted of three parts. The first step was reading the strips immersed in control solutions of CombiScreen 500, taking the minimum and maximum grayscale values of each pad and forming grayscale intervals as a result. The second step was improving these intervals by samples of patients in Yeditepe Universty Hospital. The third was preparing glucose solutions including different amount of glucose and acidic or alkaline solutions with known pH.

Table 4.2
Cost account for preparing solutions

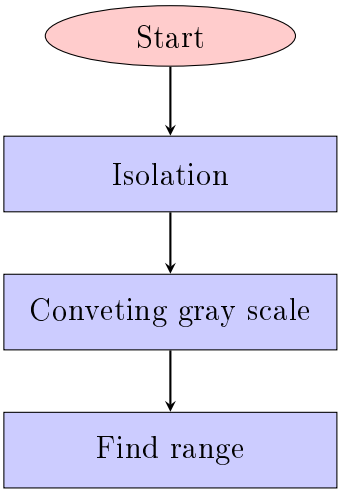
Substance	Quantity	Price
Blilirubin	100 mg	$125.00
Urobilinogen	100 mg	$150.00
Acetone	10 oz	$13.95
Acetoacetic acid	5 g	$215.00
Glucose	1 kg	42.10
Albumin	30 mg	$1,960
Erythrocytes	5 mL	$75.00
Nitrite	100 g	$11.95
Leukocytes	1 mL	$50.00
Total		$2600.9

4.3.1 Reading Control Solutions

Negative and positive control solutions were obtained from Biochemistry Lab in Yeditepe University Hospital. General procedure applied as follows:

1. Napkin is placed under the test strip.
2. Each pad of the test strip is wetted by micropipette, regarless of the volume of micropipette.
3. After completing the wetting procedure, strip was rotated 90 degrees, in order to get rid of the excess solution on strip without harming the pads. Therefore, the excess solution moves to the napkin.
4. Wetted strip is placed in the linear slide system and the reading program is started.

This procedure was repeated 5 times for both negative and positive solution. The reading program ended up with 4D matrix which includes RGB color information of positive and negative solutions. Then, all red, green and blue values of each pad were formed as a matrix and gray scale value of each reading was calculated. Maximum and minimum grayscale intervals of obtained 4D matrices were calculated by the code below:



4.3.2 Preparation of Solutions

Glucose Containing Solutions

Since glucose was available, preparation of solutions in the first approach had been able to be achived. Glucose has four positive levels in urine such as, 50 mg/dL, 150 mg/dL, 500 mg/dL and 1000 mg/dL. Because this thesis has focused on detecting negativeness and positiveness, 50 mg/dL level was considered. The procedure which was repeated three times can be seen below:

1. 100 mL of urine sample of 24 year old non diabetic female was collected.
2. 50 mg glucose was weighted in laboratory weigher and added to 100 mL urine and mixtured in magnetic stireer.

3. Urine strip is immersed in this mixture and light green color was observed.
4. The strip was read by the system.

pH Solutions with HCl and $NaOH$

Since pH is a measurable parameter, acidic and alkaline solutions can be prepared. Clinical requirements for pH are 5, 6, 7, 8 and 9. Hence, HCl and $NaOH$ solutions having 5,6 and 8,9 respectively was aimed to be prepared. The procedure for HCl solutions can be seen below:

1. %37 of HCl was used to prepare $12M$ $1L$ of stock solution.
2. $1M$ 500 mL of HCl was prepared from $12M$ solution using the equation below:

   $M1 * V1 = M2 * V2$

   $12M * V = 1M * 0.5L$

   $V = 41.67mL$

   Means that 41.67 mL was taken from $12M$ HCl solution and this volume was completed to 500 mL by adding dilute water.
3. $10^{-3}M$ 100 mL HCl solution was prepared from $1M$ solution using the equation below:

   $1M * V = 10^{-3} * 100mL$

   $V = 100$

   Means that 100was taken from $1M$ HCl solution and this volume was completed to 100 mL by adding dilute water.
4. $10^{-5}M$ 50 mL HCl solution was prepared from $10^{-3}M$ solution using the equation below:

   $10^{-3}M * V = 10^{-5} * 50mL$

   $V = 500$

Means that 500 was taken from $10^{-3}M$ HCl solution and this volume was completed to 50 mL by adding dilute water.

5. $10^{-6}M$ 50 mL HCl solution was prepared from $10^{-6}M$ solution using the equation below:

   $10^{-5}M * V = 10^{-6} * 50mL$

   $V = 5mL$

   Means that 5 mL was taken from $10^{-5}M$ HCl solution and this volume was completed to 50 mL by adding dilute water.

The procedure for $NaOH$ solutions can be seen below:

1. Since MW_{NaOH} is 40 g/mol, $1M$ 1L $NaOH$ solution contain 40g $NaOH$. Using 2g $NaOH$ and to obtain $1M$ solution:

   $V = 1L * 2g/40g = 50mL$

2. $10^{-3}M$ 250 mL $NaOH$ solution was prepared from $1M$ solution using the equation below:

   $1M * V = 10^{-3} * 250mL$

   $V = 250$

   Means that 25 was taken from $1M$ $NaOH$ solution and this volume was completed to 250 mL by adding dilute water.

3. $10^{-5}M$ 250 mL $NaOH$ solution was prepared from $10^{-3}M$ solution using the equation below:

   $10^{-3}M * V = 10^{-5} * 250mL$

   $V = 2.5mL$

   Means that 25 mL was taken from $10^{-3}M$ $NaOH$ solution and this volume was completed to 250 mL by adding dilute water.

4. $10^{-6}M$ 250 mL $NaOH$ solution was prepared from $10^{-3}M$ solution using the equation below:

   $10^{-3}M * V = 10^{-6} * 250mL$

   $V = 2.5mL$

Means that 25 mL was taken from $10^{-3}M$ $NaOH$ solution and this volume was completed to 250 mL by adding dilute water.

pH Solutions with Urine

Urine sample of 24 year old healthy female was used to prepare pH 5, 6, 7, 8, 9 samples. Urine sample was poured into five 50 mL of beakers. After measuring the initial pH, pH of each beaker was made acidic or alkaline. pH 5 solution was obtained as follows:

Table 4.3
Experimental pH 5 solution preparation

Initial pH	Added volume	Resulting pH
6.99	2 mL HCl	6.21
	2 mL HCl	5.40
	1 mL HCl	4.95
	50 μL $NaOH$	4.96
	50 μL $NaOH$	4.99
	50 μL $NaOH$	5.01

Table 4.4
Measuring pH of prepared solution

	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}	7^{th}	8^{th}	9^{th}	10^{th}
pH	5.01	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02	5.02

pH 6 solution was obtained as follows:

Table 4.5
Experimental pH 6 solution preparation

Initial pH	Added volume	Resulting pH
6.99	50 μ L HCl	6.93
	100 μL HCl	6.88
	200 μL HCl	6.80
	200 μL HCl	6.76
	200 μL HCl	6.69
	200 μL HCl	6.63
	200 μL HCl	6.57
	200 μL HCl	6.53
	200 μL HCl	6.47
	200 μL HCl	6.42
	200 μL HCl	6.38
	200 μL HCl	6.33
	200 μL HCl	6.29
	200 μL HCl	6.24
	200 μL HCl	6.18
	200 μL HCl	6.14
	200 μL HCl	6.10
	200 μL HCl	6.05
	200 μL HCl	6.01

Table 4.6
Measuring pH of prepared solution

	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}	7^{th}	8^{th}	9^{th}	10^{th}
pH	6.01	6.02	6.03	6.03	6.03	6.03	6.03	6.03	6.02	6.03

pH 7 solution was obtained as follows:

Table 4.7
Experimental pH 7 solution preparation

Initial pH	Added volume	Resulting pH
6.95	20 μL $NaOH$	6.97
	20 μL $NaOH$	6.99
	20 μL $NaOH$	7.00

Table 4.8
Measuring pH of prepared solution

	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}	7^{th}	8^{th}	9^{th}	10^{th}
pH	7.00	7.01	7.02	7.03	7.02	7.02	7.03	7.03	7.04	7.03

pH 8 solution was obtained as follows:

Table 4.9
Experimental pH 8 solution preparation

Initial pH	Added volume	Resulting pH
6.92	100 μL $NaOH$	7.04
	100 μL $NaOH$	7.13
	100 μL $NaOH$	7.21
	100 μL $NaOH$	7.30
	100 μL $NaOH$	7.41
	100 μL $NaOH$	7.51
	100 μL $NaOH$	7.62
	100 μL $NaOH$	7.73
	100 μL $NaOH$	7.84
	100 μL $NaOH$	7.94
	50 μL $NaOH$	7.97
	50 μL $NaOH$	8.02

Table 4.10
Measuring pH of prepared solution

	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}	7^{th}	8^{th}	9^{th}	10^{th}
pH	8.02	8.02	8.02	8.02	8.02	8.02	8.03	8.02	8.02	8.03

pH 9 solution was obtained as follows:

Table 4.11
Experimental pH 9 solution preparation

Initial pH	Added volume	Resulting pH
6.93	200 μL $NaOH$	7.05
	200 μL $NaOH$	7.20
	200 μL $NaOH$	7.33
	200 μL $NaOH$	7.49
	200 μL $NaOH$	7.71
	200 μL $NaOH$	7.90
	200 μL $NaOH$	8.07
	200 μL $NaOH$	8.21
	200 μL $NaOH$	8.33
	200 μL $NaOH$	8.45
	200 μL $NaOH$	8.54

Table 4.12
Experimental pH 9 solution preparation

Initial pH	Added volume	Resulting pH
	200 μL $NaOH$	8.62
	200 μL $NaOH$	8.69
	200 μL $NaOH$	8.76
	200 μL $NaOH$	8.83
	200 μL $NaOH$	8.87
	200 μL $NaOH$	8.93
	100 μL $NaOH$	8.95
	100 μL $NaOH$	8.98
	50 μL $NaOH$	8.99
	50 μL $NaOH$	9.01

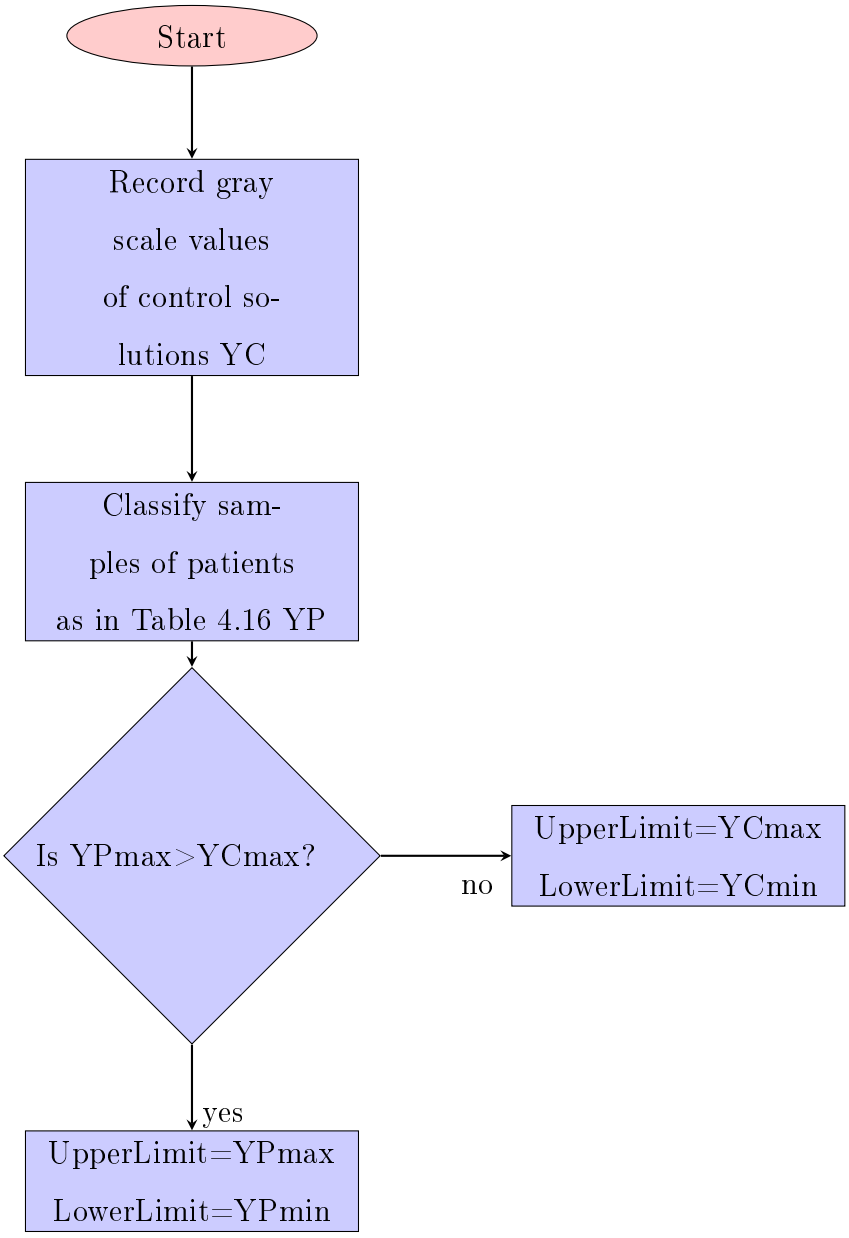
Table 4.13
Measuring pH of prepared solution

	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}	7^{th}	8^{th}	9^{th}	10^{th}
pH	9.01	9.01	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00

4.3.3 Samples of Patients

Samples of 40 patients had examined in Yeditepe University Hospital were read. The samples of 34 patients were read twice, however the samples of last 6 patients were read once due to the lack of sufficient number of test strips. The samples were classified considering the results of (seen in Table 4.14 the parameters for instance, the samples whose protein results were negative were seperated from the those whose protein results were positive. In addition, colors of the samples having positive results had been checked for the corresponding color in colorchart. Maximum and minimum grayscale intervals of such positive and negative groups were calculated. Determination of participation of samples can be seen in Table 4.16. The general flow chart of forming

database by combining control solutions, solution experiments and samples of patients can be seen below:



4.3.4 Designing the GUI

The GUI consists of three panels, namely, "Initializing", "Analysis" and "Results". "Connecting The Device via USB" and "Connecting The Device via Bluetooth" buttons in Initializing panel are responsible for making connection via USB or Bluetooth with the MCU respectively. The code illustrated in "Implementing The MCU Connection" section was written under the callback of these buttons. The callback of "Start Analyzing" button in Analysis panel includes an analysis function called GrayScaleFullAnalysis which can be run by 4D matrix. Obtained minimum and maximum gray scale values of read sample was compared to the those in database intervals and results were printed on Results panel.

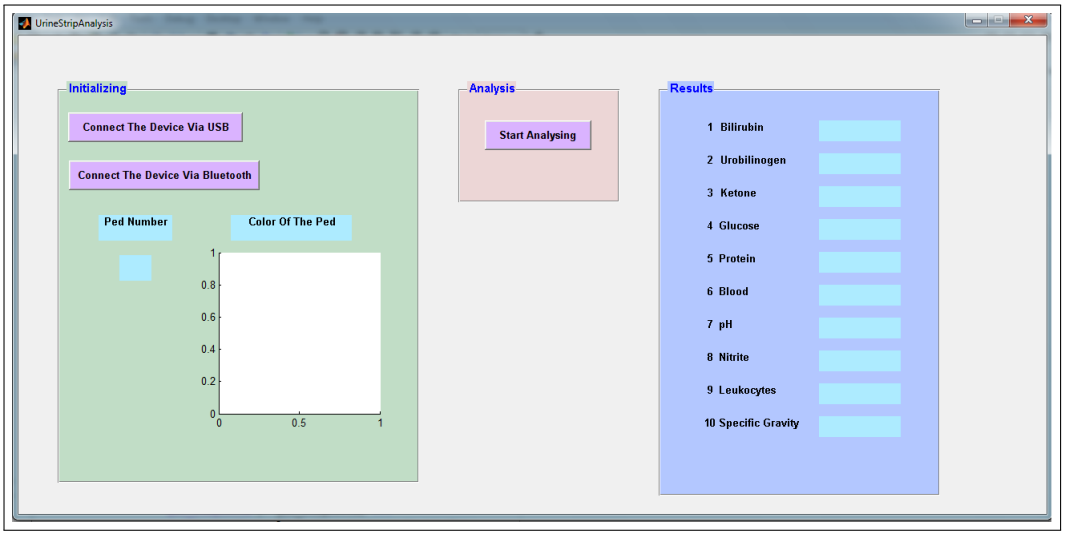
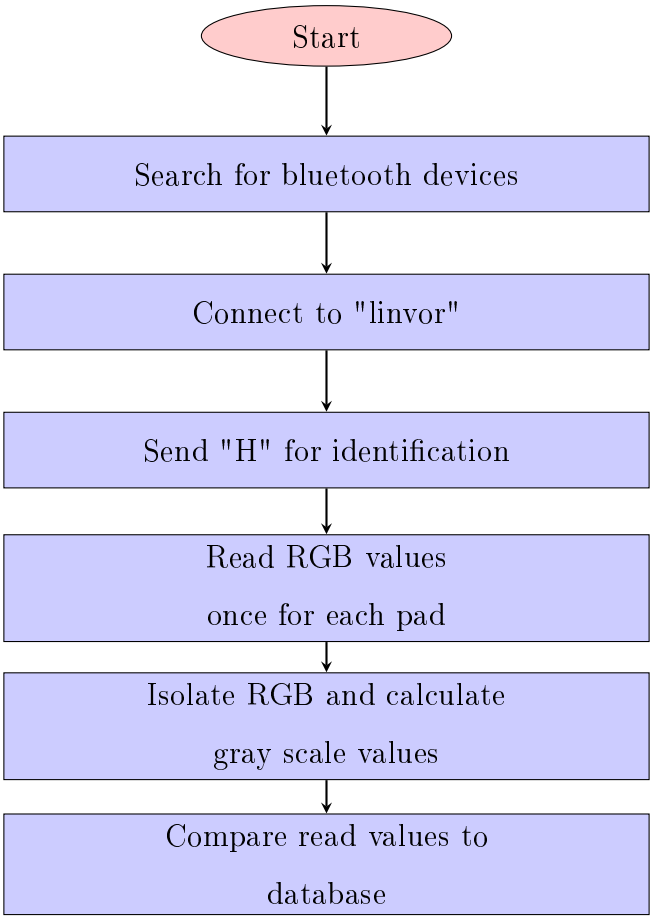


Figure 4.2 The GUI of UrineStripAnalyzer

4.4 Android Part

The algorithm of Android part is similar to that of MATLAB part. Bluetooth connection enabled data transfer between the MCU and Android. After receiving RGB values, comparison similar to MATLAB was proceeded and results were printed on a layout. Below, the general algorithm of Android can be seen:



Opening layout of UrineAnalysis is demonstrated in Figure 4.3. When the user press "Enter Urine Strip Analyzer" button, the main layout is appeared as in Figure 4.4.

Figure 4.3 Opening layout of UrineAnalysis

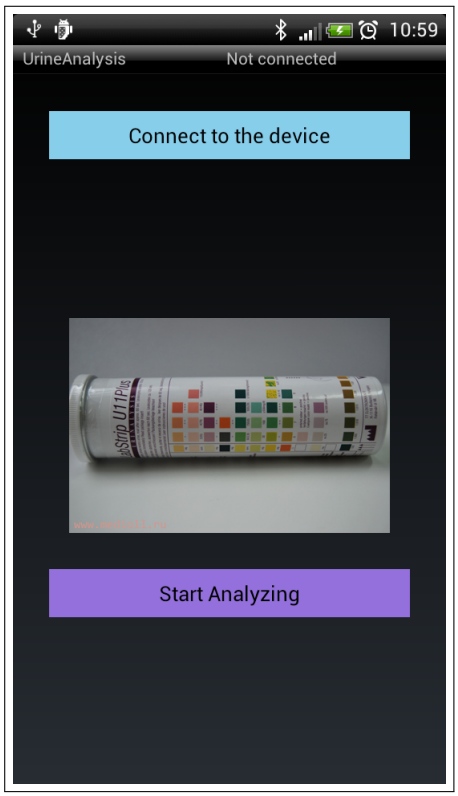


Figure 4.4 The main layout of UrineAnalysis

When the menu button on the smart phone is pressed, two selections are seen, such as, "Connect to the device" standing for connecting a bluetooth device and "Make discoverable" standing for enabling the smart phone to be discoverable for bluetooth connection. "Connect to the device button leads a sublayout containing available device in the neighbourhood as seen in Figure 4.5. The device called "linvor" is the bluetooth device of BUSA.

After connection is achieved, the user should press the "Connect to the Device" button for starting the data trade with BUSA. The user can understand that the data trade has began by "Strip is being read" notification under the "Connect to the Device" button as in Figure 4.6. In the meantime, "Connnected:linvor"information is appeared on the upperleft corner. When the reading is finished "Reading is done" notification is shown on the screen as in Figure 4.7.

Figure 4.5 Bluetooth devices in the neighbourhood

Afterwards, "Start Analyzing" button is pressed to view the results layout as in Figure 4.8.

4.5 Verification of The Specific Gravity

Results of specific gravity pads of 10 patients were compared to Atago Manual Refractometer. Before using Atago refractometer it was calibrated by dilate water. 2 drops of dilate water was placed in the measurement area and 1.000 spesific gravity was observed. After the calibration process was completed, specific gravity of samples was measured by proceeding the same procedure.

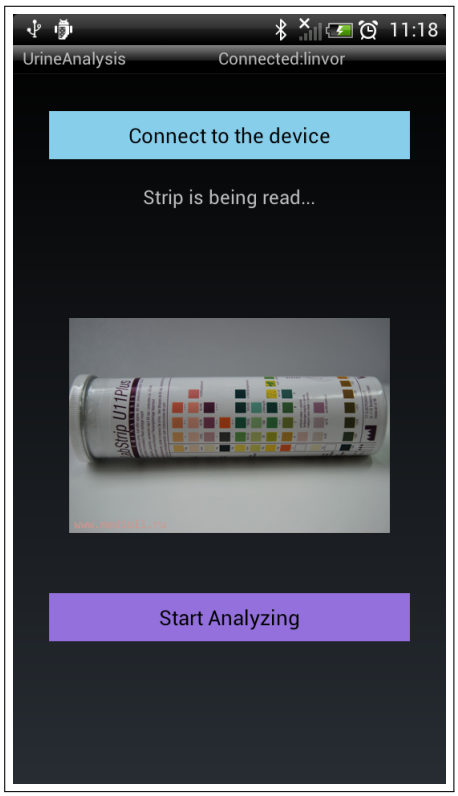


Figure 4.6 Screen view when the strip is being read

4.6 Circuit Design

Electronical circuit and its printed version was designed in Proteus's ISIS and ARES respectively. 3D visiualization of the printed circuit can be seen in Figure 4.9.

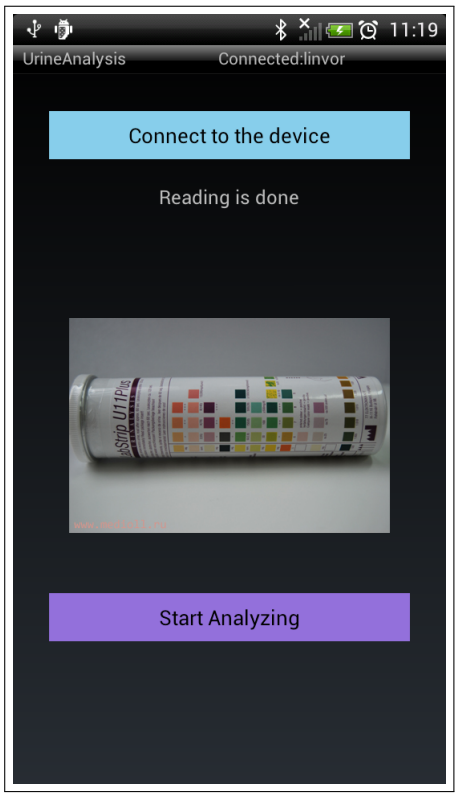


Figure 4.7 The notification occured after reading was done

Table 4.14
Results of the patients examined in Yeditepe University Hospital

No	Name	Bili	Uro	Ketone	Asc	Glu	Pro	Blood	pH	Nit	Leuko	SG
1	AD	Neg	Norm	Neg	Neg	Neg	30	300	5	Neg	Neg	1.015
2	EA	Neg	Norm	Neg	Neg	Neg	Neg	300	5	Neg	500	1.010
3	SG	Neg	Norm	Neg	20	Neg	Neg	Neg	5	Neg	25	1.020
4	ZT	Neg	Norm	10	Neg	Neg	Neg	50	5	Neg	Neg	1.020
5	NK	Neg	Norm	Neg	Neg	Neg	Neg	Neg	6	Neg	Neg	1.005
6	IU	Neg	Norm	Neg	Neg	Neg	Neg	10	5	Neg	Neg	1.020
7	FY	Neg	Norm	10	20	Neg	Neg	10	5	Neg	Neg	1.030
8	NK	Neg	Norm	Neg	Neg	Neg	Neg	Neg	5	Neg	75	1.020
9	FA	Neg	Norm	Neg	Neg	Neg	Neg	Neg	8	Neg	Neg	1.005
10	SO	Neg	Norm	Neg	Neg	Neg	Neg	Neg	5	Neg	25	1.020
11	AK	Neg	Norm	100	Neg	Neg	Neg	Neg	5	Neg	Neg	1.020
12	MG	Neg	Norm	Neg	Neg	Neg	Neg	10	5	Neg	Neg	1.020
13	GA	Neg	Norm	Neg	Neg	Neg	Neg	10	5	Neg	Neg	1.025
14	KY	Neg	Norm	Neg	Neg	Neg	100	300	5	Neg	75	1.020
15	AT	Neg	Norm	Neg	20	Neg	Neg	Neg	5	Neg	Neg	1.020
16	MB	Neg	Norm	Neg	Neg	Neg	Neg	Neg	5	Neg	Neg	1.025
17	AÇ	Neg	Norm	Neg	Neg	Neg	Neg	Neg	5	Neg	Neg	1.000
18	EC	Neg	Norm	20	Neg	Neg	Neg	Neg	5	Neg	Neg	1.020
19	DS	Neg	Norm	Neg	Neg	Neg	Neg	300	6	Neg	Neg	1.015
20	VG	Neg	Norm	10	20	Neg	30	300	5	Neg	Neg	1.030
21	AA	Neg	Norm	Neg	20	Neg	Neg	Neg	7	Neg	75	1.010
22	MK	Neg	Norm	Neg	Neg	Neg	30	300	5	Neg	500	1.005
23	ED	Neg	Norm	Neg	Neg	Neg	Neg	Neg	6	Neg	Neg	1.010
24	TÇ	Neg	Norm	Neg	Neg	Neg	Neg	Neg	5	Neg	Neg	1.010
25	OT	Neg	Norm	Neg	Neg	Neg	Neg	Neg	8	Neg	Neg	1.005

Table 4.15
Results of the patients examined in Yeditepe University Hospital - continued

No	Name	Bili	Uro	Ketone	Asc	Glu	Pro	Blood	pH	Nit	Leuko	SG
26	SO	Neg	Norm	Neg	Neg	Neg	Neg	300	5	Neg	Neg	1.005
27	NY	Neg	Norm	Neg	20	Neg	Neg	300	5	Neg	500	1.010
28	ASO	Neg	Norm	Neg	Neg	Neg	30	300	5	Neg	25	1.010
29	DB	Neg	Norm	Neg	Neg	Neg	Neg	Neg	6	Neg	Neg	1.005
30	FV	Neg	Norm	Neg	20	Neg	30	50	5	Neg	Neg	1.025
31	FV	Neg	Norm	Neg	20	Neg	Neg	300	5	Neg	500	1.005
32	MEB	Neg	Norm	Neg	Neg	Neg	Neg	Neg	5	Neg	Neg	1.025
33	CT	Neg	Norm	Neg	Neg	Neg	Neg	Neg	5	Neg	Neg	1.030
34	MH	Neg	Norm	Neg	Neg	Neg	Neg	50	5	Neg	75	1.020
35	CU	Neg	Norm	Neg	20	Neg	Neg	Neg	5	Neg	75	1.030
36	KA	Neg	Norm	Neg	Neg	Neg	Neg	Neg	7	Neg	Neg	1.005
37	HKY	Neg	Norm	Neg	Neg	Neg	Neg	Neg	5	Neg	Neg	1.025
38	NO	Neg	Norm	Neg	Neg	Neg	Neg	Neg	6	Neg	Neg	1.025
39	NI	Neg	Norm	Neg	Neg	Neg	Neg	Neg	7	Neg	Neg	1.005
40	ZG	Neg	Norm	Neg	Neg	Neg	Neg	50	5	Neg	Neg	1.020

Table 4.16
Determination of participation of patients

Parameter	Patient # for Negative	Patient # for Positive
Bilirubin	40	None
Urobiinogen	40	None
Ketone	36	4
Glucose	40	None
Protein	34	6
Blood	21	19
pH 5	29	Not applicable
pH 6	4	Not applicable
pH 7	2	Not applicable
pH 8	1	Not applicable
pH 9	None	Not applicable
Nitrite	40	None
Leukocytes	32	8
SpecGra 1.005	9	Not applicable
Specific Gravity 1.010	5	Not applicable
Specific Gravity 1.015	2	Not applicable
Specific Gravity 1.020	12	Not applicable
Specific Gravity 1.025	6	Not applicable
Specific Gravity 1.030	4	Not applicable

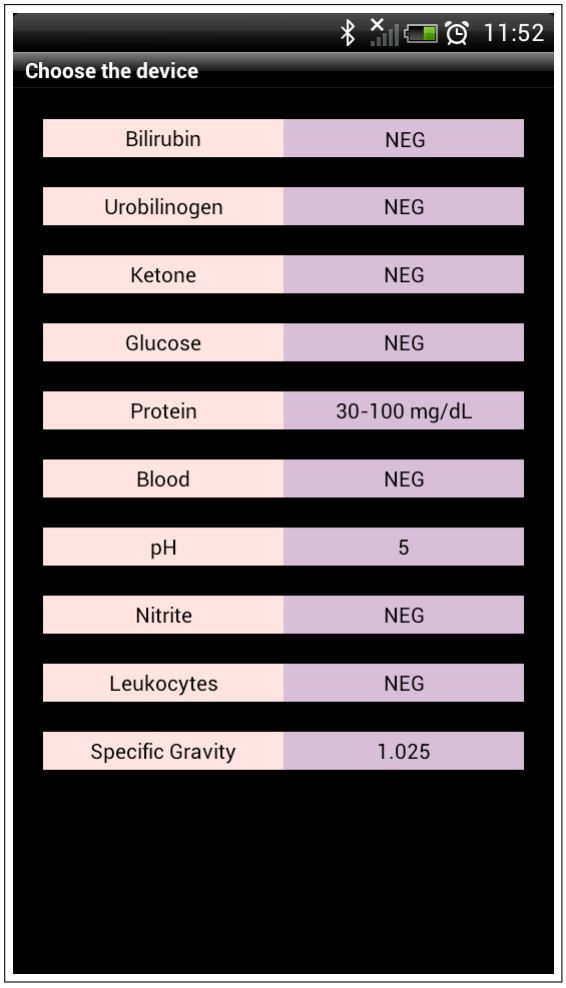


Figure 4.8 Result layout

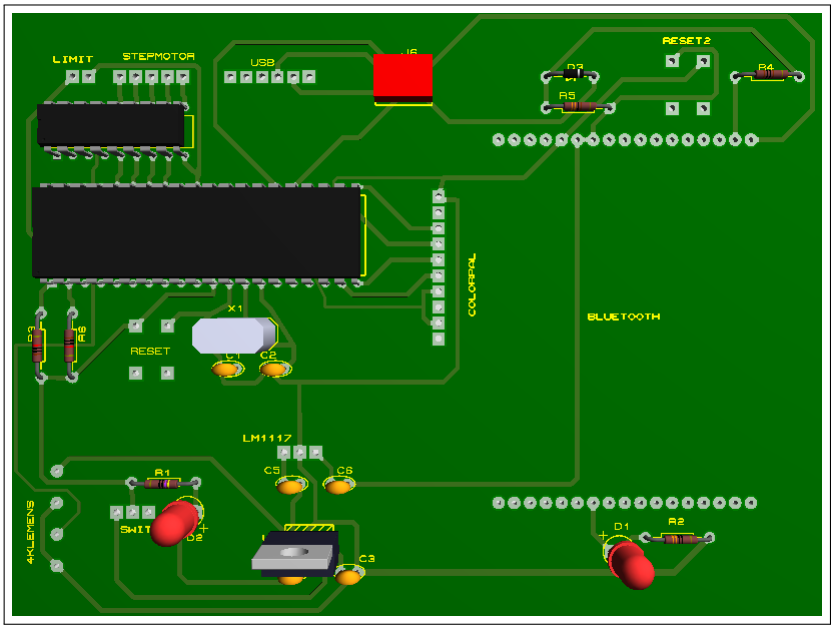


Figure 4.9 3D visiualization of the printed circuit

5. RESULTS

5.1 Calibration of The Linear Slide

Results of ANOVA method was 0.0164 and Repeated Measures of ANOVA method for red, green and blue are 0.0595, 0.0150, 0.0134 respectively where cut-off for ANOVA and Repeated Measures of ANOVA are 2.22 and 1.97 respectively for $p<0.05$.

5.2 Verification of The Specific Gravity

Specific gravity results of CombiScan 500 and Atago refractometer can be seen in Table 5.1 and Figure 5.1.

Table 5.1
CombiScan 500 vs Atago refractometer

CombiScan 500	Atago Refractometer
1.005	1.004
1.005	1.006
1.010	1.011
1.015	1.014
1.015	1.016
1.020	1.019
1.020	1.021
1.020	1.022
1.030	1.028
1.030	1.031

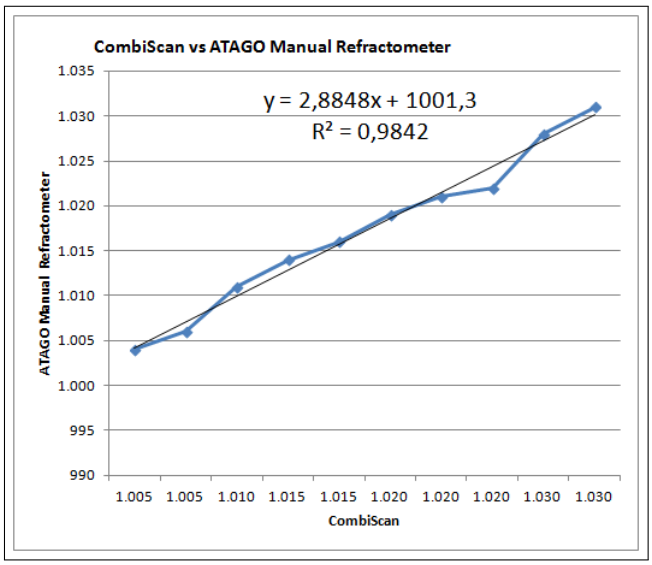


Figure 5.1 Graph of specific gravity values of CombiScan 500 vs Atago Refractometer

Spescific gravity results of Atago Refractometer and the system can be seen in Table 5.2 and Figure 5.2.

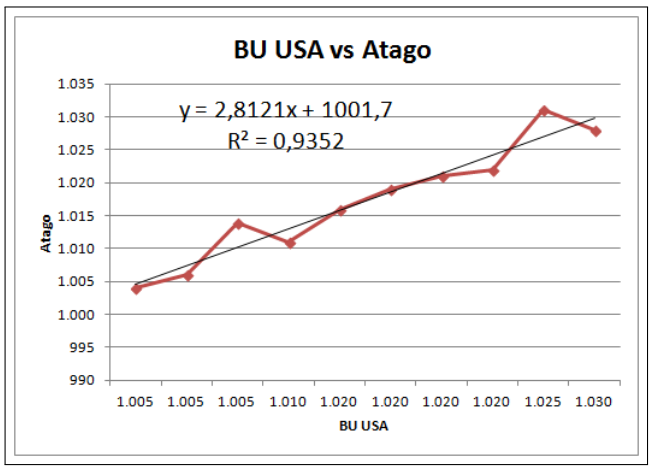


Figure 5.2 Graph of specific gravity values of Atago Refractometer and BUSA

Table 5.2
Atago refractometer vsBUSA

Atago Refractometer	BUSA
1.004	1.005
1.006	1.005
1.011	1.010
1.014	1.005
1.016	1.020
1.019	1.020
1.021	1.020
1.022	1.020
1.028	1.030
1.031	1.025

Spescific gravity results of CombiScan 500 and the system can be seen in Table 5.3 and Figure 5.3.

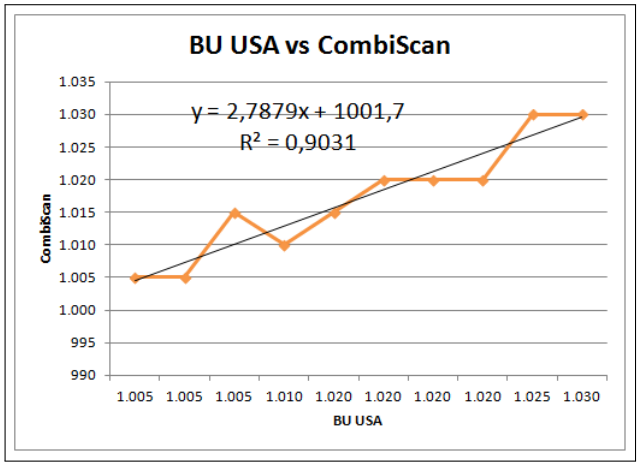


Figure 5.3 Graph of specific gravity values ofCombiScan 500 and BUSA

5.3 Intervals of Database

Upper and Lower limits of all parameter can be seen in Table 5.4.

Table 5.3
CombiScan 500 vs BUSA

CombiScan 500	BUSA
1.005	1.005
1.005	1.005
1.010	1.010
1.015	1.005
1.015	1.020
1.020	1.020
1.020	1.020
1.020	1.020
1.030	1.030
1.030	1.025

5.4 Comparing CombiScan 500 with BUSA

Results of comparison of 15 patients who were scanned in Yeditepe University Hospital to CombiScan 500 and my system is given below. Results of pH and specific gravity which Bland-Altman Method was applied are given in Table 5.5 and Table 5.6 respectively. "Not assigned" results were discarded from the analysis when specificity and sensitivity were calculated. The results of CombiScan 500 and BUSA comparison of bilirubin, urobilinogen, ketone, glucose, protein, blood, nitrite and leukoytes are given in corresponding tables.

Bilirubin

1 sample could not have been analyzed due to "Not assigned" result.

		Prediction (BUSA)	
		Positive	Negative
Exact Value (CombiScan)	Positive	0	0
	Negative	0	14

Specificity of bilirubin=number of true negatives/(number of true negatives + number of false negatives)= (14/14) = 1

Urobilinogen

		Prediction (BUSA)	
		Positive	Negative
Exact Value (CombiScan)	Positive	0	0
	Negative	0	15

Sensivity of urobilinogen=number of true positives/(number of true positives + number of false positives)= (15/15) = 1

Ketone

		Prediction (BUSA)	
		Positive	Negative
Exact Value (CombiScan)	Positive	0	1
	Negative	0	14

Specificity of ketone=number of true negatives/(number of true negatives + number of false negatives)=(14/15) = 0.933

Glucose

3 samples could not have been analyzed due to "Not assigned" result.

		Prediction (BUSA)	
		Positive	Negative
Exact Value (CombiScan)	Positive	1	0
	Negative	0	11

Sensivity of glucose=number of true positives/(number of true positives + number of false positives)=(1/1) = 1

Specificity of glucose=number of true negatives/(number of true negatives + number of false negatives)=(11/11) = 1

Protein

		Prediction (BUSA)	
		Positive	Negative
Exact Value (CombiScan)	Positive	1	0
	Negative	4	10

Sensivity of protein=number of true positives/(number of true positives + number of false positives)=(1/5) = 0.2

Specificity of protein=number of true negatives/(number of true negatives + number of false negatives)=(10/10) = 1

Blood

1 sample could not have been analyzed due to "Not assigned" result.

		Prediction (BUSA)	
		Positive	Negative
Exact Value (CombiScan)	Positive	3	0
	Negative	0	11

Sensivity of blood=number of true positives/(number of true positives + number of false positives)=(3/3) = 1

Specificity of blood=number of true negatives/(number of true negatives + number of false negatives)=(11/11) = 1

pH

Results of pH comparison can be seen in Table 5.5. Mean and standard deviation of differences belonging Bland Altman can be seen in below:

$Mean_{difference} = 0.133$

$StandardDeviation_{difference} = 0.5164$

Nitrite

		Prediction (BUSA)	
		Positive	Negative
Exact Value (CombiScan)	Positive	0	0
	Negative	0	15

Specificity of nitrite=number of true negatives/(number of true negatives + number of false negatives)= (15/15) = 1

Leukocytes

		Prediction (BUSA)	
		Positive	Negative
Exact Value (CombiScan)	Positive	0	0
	Negative	0	15

Specificity of ketone=number of true negatives/(number of true negatives + number of false negatives)= (15/15) = 1

Specific Gravity

Results of specific gravity comparison can be seen in Table 5.6. Mean and standard deviation of differences belonging Bland Altman can be seen in below:

$Mean_{difference} = 0.0017$

$StandardDeviation_{difference} = 0.0059$

Table 5.4
Upper and Lower Limits of Parameters

Parameter	Lower Limit	Upper Limit
Bilirubin	Neg=217.9880 Pos=174.7830	Neg=251.580 Pos=203.8370
Urobilinogen	Neg=246.7750 Pos=154.210	Neg=251.580 Pos=168.4230
Ketone	Neg=247.9320 Pos=212.1760	Neg251.580 Pos=240.3980
Glucose	Neg=211,6330 Pos=38.6210	Neg=225.1970 Pos=182.4610
Protein	Neg=219.1620 Pos=188.7070	Neg=242.6880 Pos=219.0310
Blood	Neg=188.8710 Pos=39.1590	Neg=236.0080 Pos=228.360
pH5	144.5720	168.7190
pH6	158.8880	181.7470
pH7	115.6280	160.0070
pH8	43.0820	142.6860
pH9	42.0110	88.4660
Nitrite	Neg=247.4760 Pos=198.4980	Neg=251.2380 Pos=204.5790
Leukocyte	Neg=230.5040 Pos=85.0040	Neg=250.8960 Pos=228.270
Specific Gravity 1.005	41.1550	97.6640
Specific Gravity 1.010	59.3860	83.6880
Specific Gravity 1.015	68.2190	86.7580
Specific Gravity 1.020	76.5250	114.2120
Specific Gravity 1.025	92.9350	128.3330
Specific Gravity 1.030	120.3310	150.6410

Table 5.5
Results of pH Comparison

CombiScan 500	BUSA
7	5
6	6
5	5
6	6
6	6
7	7
6	6
5	5
5	5
5	5
8	8
5	5
5	5
6	6
6	6

Table 5.6
Results of Specific Gravity Comparison

CombiScan 500	BUSA
1.025	1.030
1.000	1.005
1.000	1.005
1.000	1.005
1.020	1.020
1.005	1.005
1.025	1.025
1.020	1.020
1.005	1.005
1.025	1.020
1.030	1.025
1.010	1.005
1.030	1.025
1.015	1.005
1.020	1.005

6. DISCUSSION

In this thesis, developing an alternarnative device towards refractometry working with a color sensor was achieved. That BUSA was able to work with both USB and bluetooth communication cause be controlled by both computer and smart phone having Android operation system. More importantly, Android control indicates that any medical device having bluetooth connection can be run by a smart phone, which enables a faster data trade in medicine among physicians and surgeons.

That F values of ANOVA and Repeated Measures of ANOVA were lower than the cut off value indicates that each pad of calibration strip was treated as the same component by the linear slide. In other words, step number required for each pad was determined exactly, yet BUSA might have been wrong 5% of time.

Considering the relations between three comparisons of specific gravity whose R^2 values larger than 0.9, it was obvious that three system was linear according to each other. In other words, the one can rely on the measurement of specific gravity by Atago, CombiScan and BUSA.

Since the results of positive control solutions belonged to the highest positive value, they would not have been a significant limit for positiveness, therefore positive results belonging to lower values - samples of patients - should have been used.

Bilirubin measurement is reliable with 1 specificity. Bilirubin positiveness is rare in urine analysis since jaundice is a rarely seen disease. Regarding the results of bilirubin comparison, bilirubin negativeness was quite similar to those of CombiScan, on the contrary, the response for bilirubin positiveness was observed as "not assigned" because there was not any patient whose bilirubin result was positive among 40 patients while formin the database.

Urobilinogen measurement is reliable with 1 specificity. Similar to bilirubin, urobilinogen is also rare in urine analysis and it is related to jaundice. Considering the results of urobilinogen comparison, urobilinogen negativeness was rather parallel to those of CombiScan. Nevertheless, if a patient whose urobilinogen result was negative had been analyzed, the system would have come up with "not assigned" result since there was not any patient whose urobilinogen result was positive among 40 patients while formin the database.

Ketone measurement is reliable with 0.9333 specificity. It can be concluded that the number of samples with ketone negativeness was high enough to form a database because BUSA would have been able to detect 14 negative samples. However, the reason why BUSA could not have detected 1 positive sample is that the number of samples with ketone positiveness was only 1.

Glucose measurement is reliable with 1 sensivity and 1 specificity. The problem in glucose measurement was that BUSA could not have detected 1 glucose positive samples, instead it responded as "Not Assigned". When the color of glucose pad of this sample was observed, it had been noticed that the color was far yellow to be positive. In addition, since it was not positive either, it was normal for BUSA responding "Not Assigned".

Sensivity and specificity of protein measurement is 0.2 and 1 respectively, which is not a quate reliable data. BUSA treated negative samples as positive samples. The colors of protein pad of wrong positive samples were dark yellow which was close to the positive threshold level.

Blood measurement is realible with with 1 sensivity and 1 specificity. All positive samples were measured as positive by BUSA. Only 1 negative sample was treated as "Not Assigned".

pH and specific gravity measurement are realiable because of small mean and standard deviation. Even regardless of mean and standard deviation values, Table 5.5

and Table 5.6 show the obvious similarity between CombiScan and BUSA.

Nitrite measurement is rather reliable in negative cases regarding comparison table. Similarly, leukocytes measurement is quite reliable in negative cases considering comparison table. It is hard to predict the BUSA's behaviour about positive samples since there is not any of them.

Designing a home based urine strip analyzer is critical due to patients who are too old to leave their homes or bedridden. Smart phones are widely used, therefor, once a patient obtains urine strips, Android application enables BUSA to run and analyze the strips. On the other hand, linear slide mechanism of BUSA may not always be accurate considering the movement of strips towards under the color sensor. Secondly, Android application takes one reading per each pad, which might results in false positive or false negative results. Regarding redesigning BUSA, cameras having high sensivity and resolution placed on the top of each pad might eliminate th problems related to movement of linear slide. In addition to this, scan time will be reduced since each pad will be analzyed at the same time. Alternatively, if cameras of smart phones will be qualified enough to recognize the colors using the flash regardless of ambient light, the whole analysis will be conducted using only the smart phone and application.

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