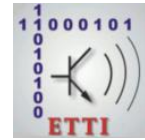




**POLITEHNICA UNIVERSITY  
OF BUCHAREST**



**Doctoral School of Electronics, Telecommunications  
and Information Technology  
Decision No. 807 from 21-02-2022**

# **Ph.D. THESIS SUMMARY**

**Ing. Manole-Ștefan NICULESCU**

---

**METODE DE ÎMBUNĂTĂȚIRE A ACURATEȚEI  
ANALIZELOR BIOCHIMICE**

**METHODS FOR IMPROVING THE ACCURACY OF  
THE BIOCHEMICAL ASSAYS**

---

## **THESIS COMMITTEE**

<b>Prof. Dr. Ing. Mihai CIUC</b> Politehnica University of Bucharest	President
<b>Prof. Dr. Ing. Rodica STRUNGARU</b> Politehnica University of Bucharest	PhD Supervisor
<b>Prof. Dr. Ing. Adriana FLORESCU</b> Politehnica University of Bucharest	PhD Supervisor
<b>Prof. Dr. Ing. Dan Alexandru STOICHESCU</b> Politehnica University of Bucharest	Referee
<b>Conf. Dr. Ing. Alin DĂNIȘOR</b> Constanta Maritime University	Referee
<b>Conf. Dr. Ing. Ovidiu URSARU</b> “Gheorghe Asachi” Technical University of Iasi	Referee

**BUCHAREST 2022**

---

# Content

1. Introduction.....	1
1.1 Presentation of the field of the doctoral thesis .....	1
1.2 Scope of the doctoral thesis.....	2
1.3 Content of the doctoral thesis.....	2
2. The Biochemical Measurement Technology .....	5
3. Three-way Automated Sampling Device Mixer for Blood-gas Analysis.....	7
4. Optical Method for Improving the Accuracy and Precision of Single-use Cuvette-based Biochemistry Analyzers.....	9
5. Biochemistry Accuracy Improvement by Using Total Dissolved Solids and Turbidimetric Measurements .....	12
6. Automated Ultrasonic Reusable Cuvettes Cleaning System .....	14
7. Optical Coagulation Analyzer Based on Image Acquisition Algorithm .....	16
8. Liquid Reagent-based Biochemistry Analyzer with Image Acquisition Algorithm.	17
9. Automated Dry Chemistry Analyzer Based on Image Acquisition.....	19
10. Reagent-free Hemoglobin Analyzer with Image Acquisition Algorithm.....	20
11. LabConcept – Telemedicine and the Laboratory Information System Platform...	22
12. Conclusions.....	23
12.1 Obtained results.....	24
12.2 Original contributions .....	26
12.3 List of original publications .....	28
12.4 Perspectives for further developments .....	29
Selection of Bibliography .....	30

# Chapter 1

## Introduction

Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity [1]. The health state is the most important indicator for every individual and a routine control must be performed in order to maintain the sanity parameters at optimal levels.

The medical diagnostic represents the proceeding that establishes the clinical state of a patient. This state can be normal or pathologic and in order to investigate, measurable results must be taken into account. The most used diagnostic technique is based on blood tests [2]. The clinical results regarding the blood testing are categorized in two classes: qualitative and quantitative. In both cases, special medical devices and equipment are used and based on the obtained results correlated with the non-measurable symptoms and the patient's entire medical history, the medical doctor can conclude and establish the appropriate diagnostic.

Both qualitative and quantitative results must be precise and accurate in order to establish a correct diagnostic. The qualitative medical tests can have only two states: negative or positive. For every specific assay exists a specific qualitative test configuration. In case of quantitative testing, the results of a blood sample are measured and the reported results consist in a value expressed in a specific unit that can frame the diagnostic depending on its severity.

The biochemical assay is a very important science because it can provide complete and consistent information about the cellular activity from the human body. The biochemical set helps in monitoring and diagnosing the cardiovascular, digestive, respiratory and excretory systems. With its help, heart, liver, kidney disease can be prevented with premature diagnostic and treatment [4].

### 1.1 Presentation of the field of the doctoral thesis

The blood based medical diagnostic is the method that measures and helps at interpretation of the blood sample's specific parameters. There are several biochemical types of blood investigations, based on the assay parameters [7]. The instruments on which the biological parameters are measured have their specific precision and accuracy, which can generate variations between the measured results and the real clinical state of the tested patient.

The field of the present doctoral thesis is situated in the medical area because it is an interesting domain and compared to the IT, where the research and development rate is exponentially growing, the medical equipment development's is slightly slower.

A proper and correct diagnostic lead to a better lifestyle and a healthy long-lasting living.

## **1.2 Scope of the doctoral thesis**

The biochemical assays are performed on dedicated instruments which can use dry test or liquid reagents in the measurement process, which can also be fully automated, semi-automated or manual.

Every analyzer has a specific accuracy and precision. It may be observed differences between the same types of instruments for the results of the same analyzed blood sample. There are situations when are registered result variations between successive measurements on the same equipment.

The problem regarding the precision and the accuracy of the biochemical assays is very common nowadays because the instruments may report false positive or false negative results due to internal issues. This may mislead the medical act and generate an improper and harmful treatment because an imprecise and accurate result is unrepeatable and unreproducible. Every biochemical assay has a normal range and if the measured results are not repeatable or reproducible, a healthy patient may be situated in the pathological range if the reported result is falsely shifted outside the normal range.

## **1.3 Content of the doctoral thesis**

The thesis consists in presenting original methods transposed into hardware and software prototypes, conceived, designed and manufactured for improving the accuracy and precision of the biochemical assays, which are tested, compared and analyzed with actual medical commercial equipment.

Chapter 2 consists in a synthesis of the approached domain, starting from the theoretical model of a biochemistry equipment, including detailed explanation about the structural and behavioral components. The spectrophotometric method starts from the Beer-Lambert law [18], which is a mathematical representation of the absorbance of the light that passes an environment containing a sample. In this case, the sample is a mixture between the blood/serum and the specific assay reagent. The structure of an automated biochemistry analyzer is further debated and the assay algorithm, including the calibration and assay processes are presented in detail in this chapter. The accuracy and precision of the measured results are established by analyzing a quality control material with known concentration, calculating the standard deviation and the coefficient of variation and the obtained results are compared with the manufacturer assigned values. After establishing the starting point of the actual biochemical technology, the following chapters present the original conceived hardware and software elements that contribute to the accuracy and precision improvement based on the observed issues from the personal extensive experience in the medical equipment area by daily practice.

Chapter 3 presents a three-way automated sample device mixer for the blood gas analysis [9], which is an automated offline device that is used to harmonize the content of

a heparinized sample syringe used in the blood gas area of the biochemical assays. In the unit's protocol, prior the analysis, the sample syringe must be properly mixed to eliminate the possibility of sintering the ions in one region from the syringe that may conduct to an artificially altered result. With the help of this device, it is eliminated the result error when blood gas samples are analyzed and the sample preprocessing procedure for the analyzer's operator.

Chapter 4 presents an optical method for improving the accuracy and the precision of the biochemical assays for the single use cuvette-based analyzers [10], which consists in an additional automated subassembly that is involved in the quartz cuvettes windows cleaning process. There are some biochemistry analyzers which have an internal manufacturing cuvette system and the fabricated cuvette molds over the reusable cuvette window. The measurement is performed through the single use cuvette and residual particles on the window cuvette generate an absorbance modification during the spectrophotometric measurement and lastly, the result is falsely altered. To prevent this situation, the cuvette windows must be periodically manually cleaned. The automatic cuvette cleaning system prototype installed before the cuvette fabrication system provides a clean cuvette window with no residual particles between each measurement cycle and saves time for the operator to clean the cuvette windows.

Chapter 5 presents a TDS and turbidity meter [11], which is a bi-purpose additional subassembly that detects the concentration of the dissolved solids in the analyzer's water intake before its water tank. It also measures the attenuation of a light beam through the water in a separate tank. An impure water generates an absorbance modification regarding the spectrophotometric measurements from the analyzer's cuvettes, where the DI water is used to clean or to prepare the specific sample-reagent mix.

Chapter 6 exposes an automatic ultrasonic cuvette cleaning system [12], which is an additional subassembly for the reusable cuvette-based biochemistry analyzers that consists in an automatic ultrasonic cleaning system involved in the cuvette washing process. In a biochemistry analyzer, the cuvette acts as a container for the sample and the reagent, where are properly mixed and incubated before the spectrophotometric measurement. The quartz cuvettes are reusable, which means that after each sample, the cuvettes are washed with DI water, eliminating the residual waste. A phenomenon of inter-sample interference was observed, which can slightly modify the precision and the accuracy of the results. To counteract and minimize this undesired carryover, on the washing station was mounted an ultrasonic transducer with its corresponding circuitry triggered by the washing's arm optical sensor, when the station is in the cleaning position.

Except the presented subassemblies, which equip the biochemistry analyzer to improve its accuracy and precision, a novel and cheap digital method was developed to measure the concentration of specific biochemical parameters. It is a multipurpose imaging device which can be used in various biochemical sciences, such as hematology, blood-gas and coagulation.

Chapter 7 presents a portable optical coagulation prototype analyzer [13] utilized for prothrombin time and INR standalone measurement that uses an USB camera as a detector. The measurement process starts right after the PT reagent is pipetted in the

preincubated sample positioned in the incubation chamber, conducting to an absorbance modification of the substance direct proportional with the clotting time. The camera captures images every second from the beginning of the measurement process. After the blood sample reacts with the PT reagent, the coagulation process is finished and is detected by the camera because the final image is compared with the initial image from the absorbance point-of-view. The number of acquired images correspond to the number of seconds until the coagulation process is finished, obtaining the PT result. Knowing the ISI of the reagent and the PT measured result, the INR can be calculated.

Chapter 8 illustrates a portable biochemistry analyzer based on image acquisition [15]. It is a biochemistry analyzer which uses liquid reagents to trigger the photometric reaction and the method is similar to the coagulation analyzer's, using the same prototype platform. The camera measures the concentration of specific biochemical parameters, using the calibration curve experimentally determined, from the reaction that takes place between the sample and the reagent after the incubation time is finished. The mean of the pixels from the acquired image is compared in accordance with the calibration line obtained in the calibration process.

Chapter 9 presents an automated dry tests portable biochemistry prototype analyzer [16] which uses the same digital camera used in the previous experiments for image acquisition of the sample that is automatically pipetted over the dry test specific for each biochemical parameter. The system is experimentally calibrated using liquid biochemistry calibrator, obtaining a calibration line used in the sample measurement process.

Chapter 10 presents a reagent-free hemoglobin prototype analyzer [14] which measures the amount of hemoglobin from a drop of capillary blood using the same hardware as the PT and INR and the liquid biochemistry analyzer. The same digital camera is used to measure the concentration of hemoglobin, except a different software approach is used. An image is captured after the cuvette containing the sample is fit in the fixed position and the concentration is measured from the dominant pixel brightness on the image's red channel. The concentration of hemoglobin is direct proportional with the light absorption that is transmitted through the sample.

The thesis continues with Chapter 11, which presents a topic about a proposal of a telemedicine software that analyzes the loaded results over a predefined period. The software can load results directly from the connected analyzers through the LIS system. In case of a quantitative HbA1c analyzer which uses lateral flow immunofluorescence test strips, the prolong incubation time may alter the precision and accuracy of the glycated hemoglobin [17], which is a very important phenomenon when measuring the HbA1c levels of patients with diabetes. An imprecise result implies an improper treatment plan which can cause severe health issues for the patient, especially when diabetes is involved. The results from home monitoring equipment are loaded into the application through LIS and any drift regarding the results is analyzed and recorded. If the results were within the normal limit until the moment of testing, most probably is a precision and accuracy issue. If not, professional consultation must be performed.

In Chapter 12 are presented the original contributions regarding the accuracy and precision improvement, which are the case study regarding the subject of the thesis,

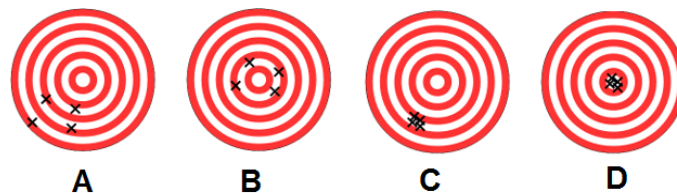
analyzing the phenomena that generate improper and inaccurate results, the description of the biochemical basics and the conceptual and hardware/software design of the presented prototypes. The prototypic ideas are compared to the existing equipment, from the theoretical point-of-view and accompanied by the experimental results in parallel with the reference available equipment. Each subsystem is explained from both hardware and software approach, followed by the end of the thesis with the final conclusions, future development ideas and the bibliographic references.

## Chapter 2

# The Biochemical Measurement Technology

The precision and accuracy in the medical engineering, especially when are involved blood assay parameters, are very important indicators in establishing and certifying the biochemistry analyzers' performance. The measurement results are influenced by temperature (the ambient temperature of the room where the equipment is installed, the sample's temperature and the instrument's internal temperature), impurities that are present in the DI water, optical obstructions in the measurement channels, robotic assemblies misalignments, improper pipetting due to hydraulic issues (lack of hydraulic liquid, improper sealed lines) and the state of the reagents, quality controls (QC) and calibrators (open well stability, expiration date, restoration issues when preparing the lyophilized serum and whole blood, improper storage conditions) [21].

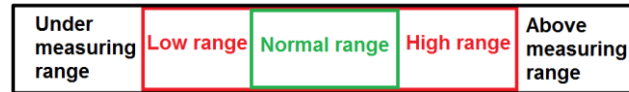
The accuracy represents how close is a measured value regarding the real value and the precision is the measurement's exactitude [22]. Generally, there are four different scenarios regarding the precision and the accuracy, which are also valid in the case of the blood parameters measurement equipment, as presented in Figure 2.1:



*Figure 2.1 The precision and the accuracy cases.*

Regarding the case of high precision and low accuracy, it must be stated that every biochemical test has its own normal reference interval. The patient ranges represent the quantification of the results where the normality or pathology is situated [23]. If the measured result is situated in the normal range, the patient is healthy and analogously, every result outside the normal range will situate the tested patient's health state in a

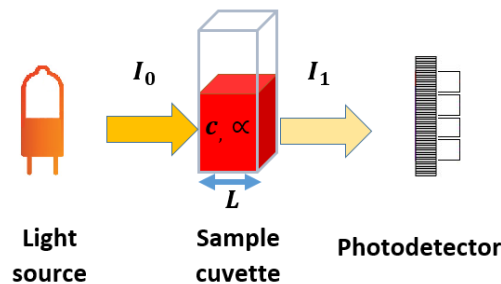
specific pathology. In the case of high precision and low accuracy, the measured results for the same sample are repeatable, but are far from the real clinical value, establishing a healthy patient in a pathological range due to the low accuracy, positioning the measured result outside the normal range, even if the real clinical values are normal. This scenario is possible for the values that are near to the extremities of the normal limit of the patient range. In Figure 2.2 are represented the general ranges of a biochemical instrument.



**Figure 2.2** The measurement domain of an analyzer.

A biochemistry analyzer has in its structure an optical subassembly that measures the absorbance of a mixture between the tested sample (serum, whole blood or plasma) and the reagent after the incubation time and the reaction reached an endpoint. This optical assembly is called a spectrophotometer and uses light to measure the concentration of a chemical parameter from a fluid by analyzing the amount of transmitted light through the cuvette that contains the respective fluid.

The transmittance is the parameter that indicates the way the incident light could be transmitted through the tested substance. As can be observed in Figure 2.4 where is presented the Beer-Lambert model, the intensity of the transmitted light is lower than the incident light because a part of it was absorbed by the substance.



**Figure 2.3** The Beer-Lambert model.

The transmittance  $T$  varies inverse proportional and in a logarithmic manner with the concentration of the tested substance, as shown in Figure 2.5, where  $I_i$  is the intensity of the incident light and  $I_t$  is the intensity of the transmitted light [31]:

$$T = I_t / I_i \quad (2.5)$$

where  $A$  represents the absorbance,  $\alpha$  is the absorbance coefficient,  $c$  is the concentration of the tested substance and  $L$  is the length of the sample path that contains the tested substance.

The logarithmic dependency of the absorbance with the concentration is:

$$A = -\lg(T) \quad (2.9)$$



If (2.9) is combined with (2.5), the absorbance expressed in accordance with the Beer-Lambert law will be obtained:

$$A = -\lg (I_t/I_i) \quad (2.10)$$

The Beer-Lambert law is the most fundamental approach regarding the measurement technologies present in the blood assay instruments and expresses the percentage of the absorbed incident light through a medium containing the tested sample in accordance with the concentration of its components and the length of the sample path.

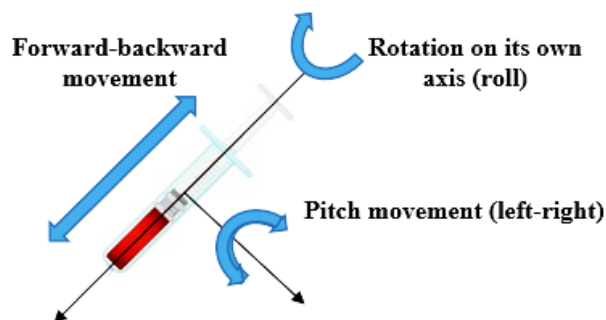
The target of this thesis is to contribute at decreasing the standard deviation and the coefficient of variation by providing new measurement methods or adjuvant subassemblies that minimizes the measurement errors and improve the accuracy and the precision of the biochemical assays.

## Chapter 3

# Three-way Automated Sampling Device Mixer for Blood-gas Analysis

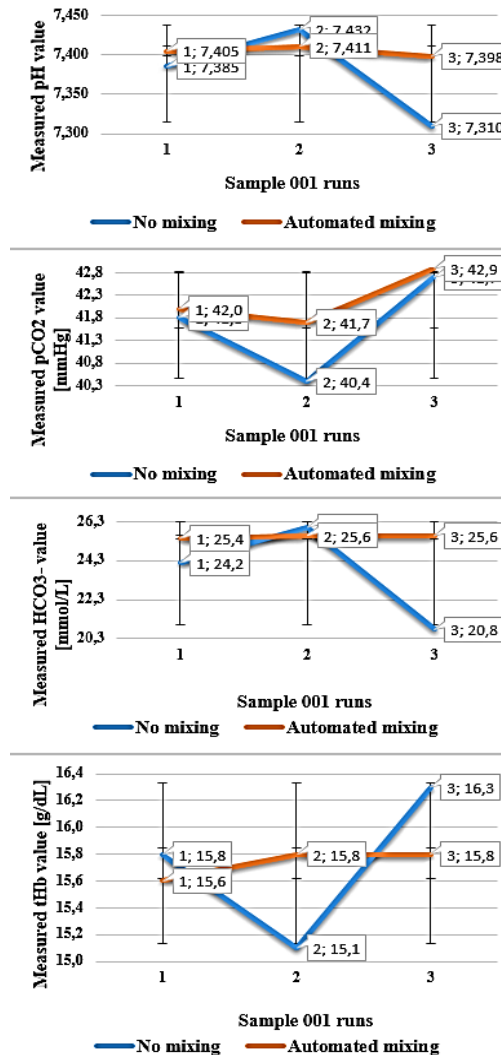
The chapter describes a novel and original three-way sample device mixer prototype is that it can be used for different type sampling devices, capillaries or syringes, where the coagulation may faster occur.

Mixing the sampling device on three axes ensures that all the ions and other blood components inside the blood device are homogeneous disposed and no particle sedimentation occur at the edge of the container, as shown in Figure 3.5. When the blood sample travels the sensor path, the same harmonized sample is analyzed by the parameter specific sensor. The same argument applies for the hemoglobin, which is more sensitive because of its components when the optical measurement in the co-oximeter is performed.



*Figure 3.1 The prototype's movement when the mixing process is engaged.*

A non-harmonized blood sample leads to erythrocyte concentration and plasma separation occurs, which is translated into an absorbance modification. The experimental results for each relevant parameter regarding the three successive samples analyzed non-mixed and also automatically mixed for 20 seconds were displayed in Figure 3.10.



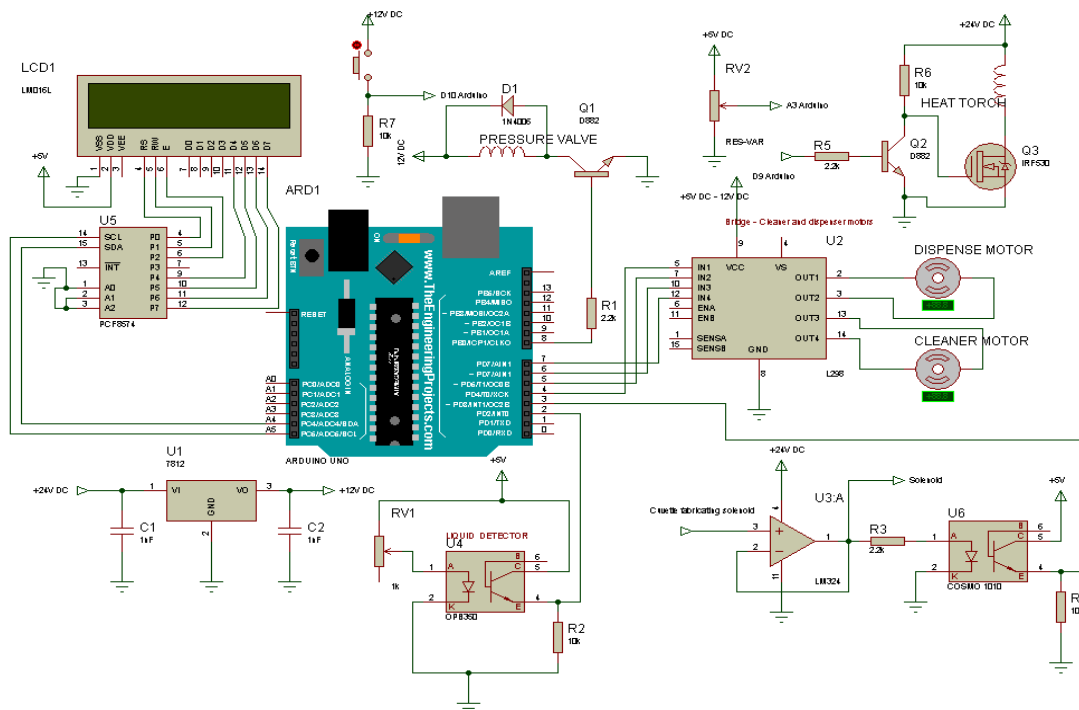
**Figure 3.2** The CV plots of the measured results for automatically mixed and unmixed blood samples.

As can be observed, the coefficient of variation was approximately 1% lower in the case of the automated mixer than in the case of a non-mixed sample. The major difference regarding the coefficient of variation (CV) was observed in the case of the  $\text{HCO}_3^-$ , being an average difference between the non-mixed and automatically mixed of 6% and if a low error is introduced in the reported  $\text{pCO}_2$  and pH, it may be generated an even bigger error in the  $\text{HCO}_3^-$  calculated reported result. By mixing the sample on three axes simultaneously and also in opposite directions at the end of the movement, using accelerated own axes rotation, ensures a more performant mixing process. Another main advantage is that the three-way mixer is compatible with all types of syringes or capillaries, lowering drastically the costs with the sample devices.

# Chapter 4

## Optical Method for Improving the Accuracy and Precision of Single-use Cuvette-based Biochemistry Analyzers

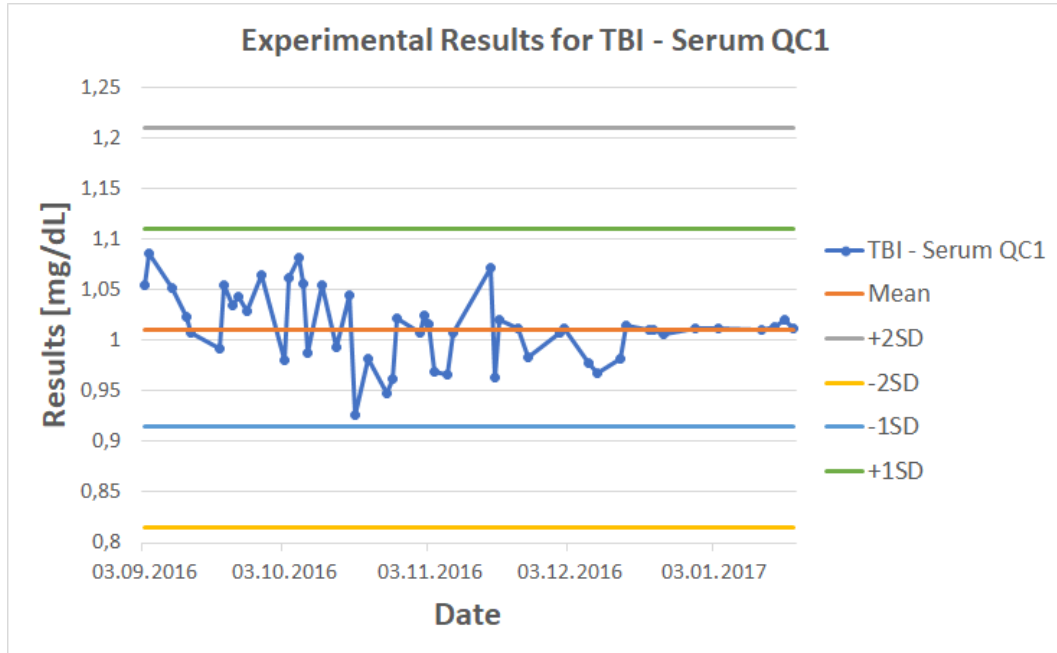
The most frequent assay condition that influences the accuracy of a biochemistry assay for a cuvette fabrication-based biochemistry analyzer is the light obstruction which is generated by dust coating on the optical mechanisms. Chapter 4 describes an automatic cleaner prototype that uses three main subassemblies: a pump valve which is connected to the analyzer's water tank, a DC motor which has a brush attached to the shaft and a heat torch that dries the remaining liquid from the brushing process, as shown in the schematic from Figure 4.10.



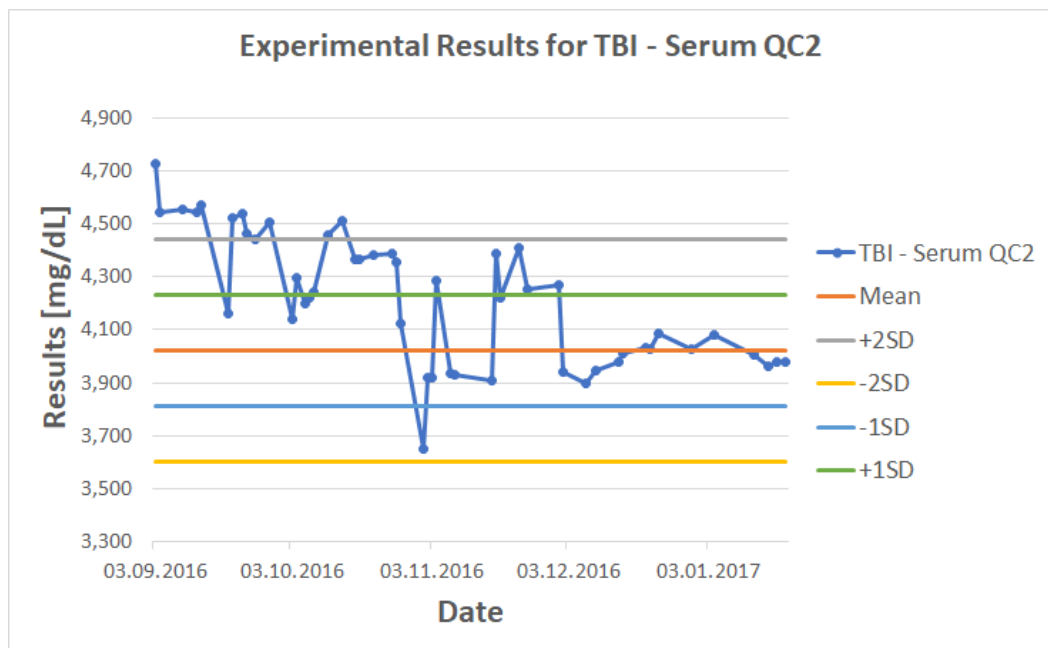
**Figure 4.1** The automatic cuvette windows cleaning prototype's schematic.

When the experiment started (3<sup>rd</sup> of September 2016), the coefficient of variation was almost the same for both QC levels in the case of the total bilirubin (TBI), 4.07% (for the first manual clean) and 3.04% (for the second manual clean) for QC level 1, 5.71% (for the first manual clean) and 5.05% (for the second manual clean) for QC level 2, and

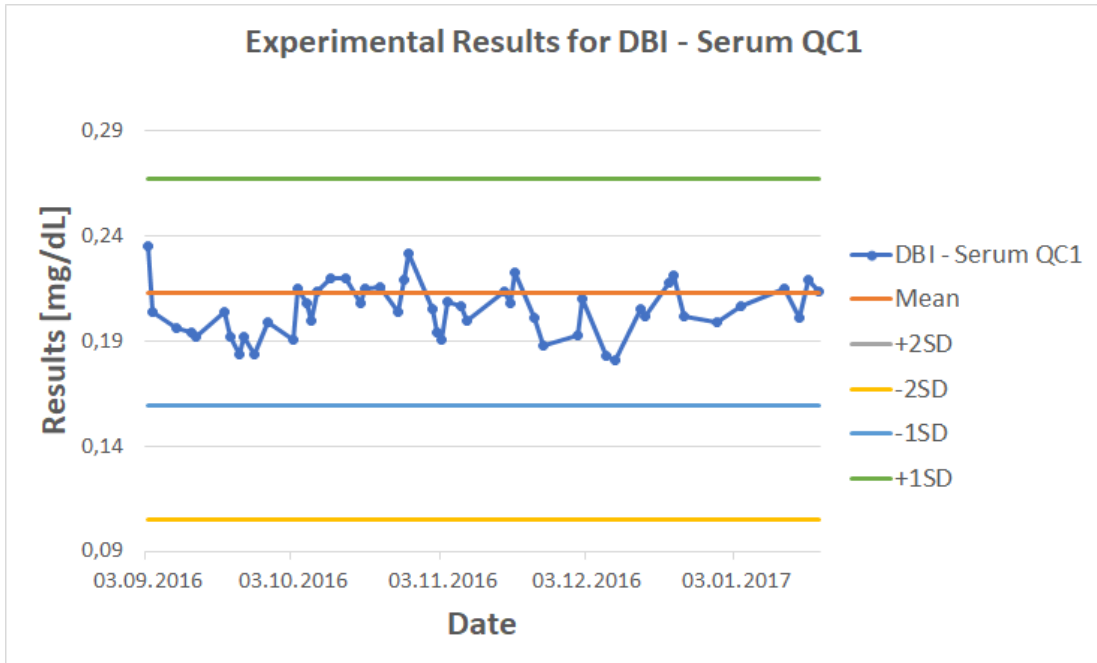
direct bilirubin (DBI), 6.41% (for the first manual clean) and 5.97% (for the second manual clean) for QC level 1, 3.11% (for the first manual clean) and 13.03% (for the second manual clean) for QC level 2. In the case of the automatic clean using the prototype, the same assay conditions tend to be maintained and this fact is demonstrated by the coefficient of variation of the measured results, which are lower than in the case of manual periodical cleaning, 0.96% for TBI for the first QC level and 1.03% for the second QC level and 3.83% for DBI for the first QC level and 1.81 for the second QC level.



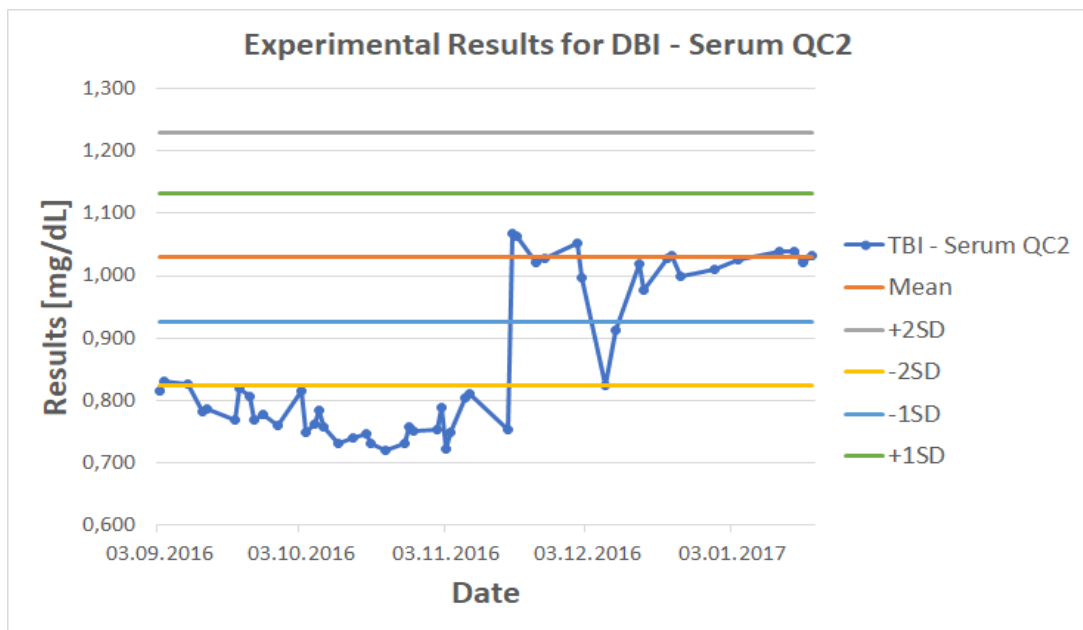
**Figure 4.2** The experimental results for TBI serum QC1.



**Figure 4.3** The experimental results for TBI serum QC2.



**Figure 4.4** The experimental results for DBI serum QC1.



**Figure 4.5** The experimental results for DBI serum QC2.

To ensure more accurate and precise results, if the analyzer's subsystems are in optimal conditions, an automatic cuvette window cleaning mechanism is imperative because it will be very useful not only for maintaining the same assay condition each time a sample is run, but also for helping the medical personnel to save time and work in order to clean periodically the cuvette windows of the system, eliminating the operational errors.

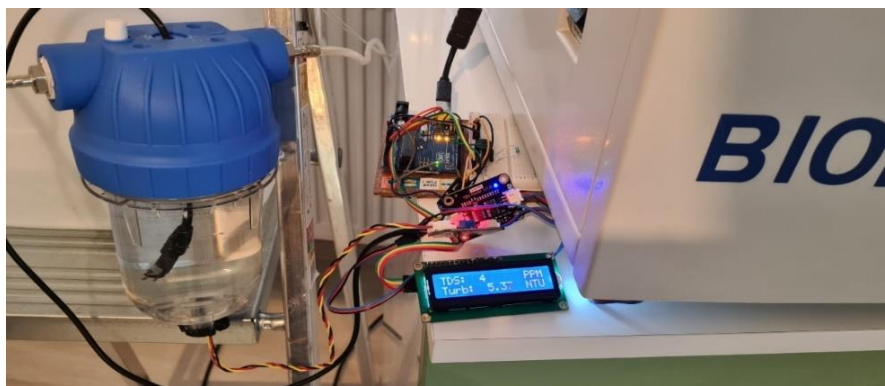
# Chapter 5

## Biochemistry Accuracy Improvement by Using Total Dissolved Solids and Turbidimetric Measurements

This chapter describes the effects of accidentally using improper deionized (DI) water at the biochemistry's analyzer supply line. The accuracy and improvement of the assayed results are improved by using a novel prototype that measures the total dissolved solids (TDS) and the turbidity of the DI water, shown in Figure 5.3.

The novelty consists in the existence of such a combined meter as an additional subassembly, connected on the analyzer's DI water intake tube, that constantly measures the quality of the supplied water. The water is used by the biochemical analyzer to rinse the needles from the robotic arms, to dilute samples and to clean the cuvettes from the residual waste.

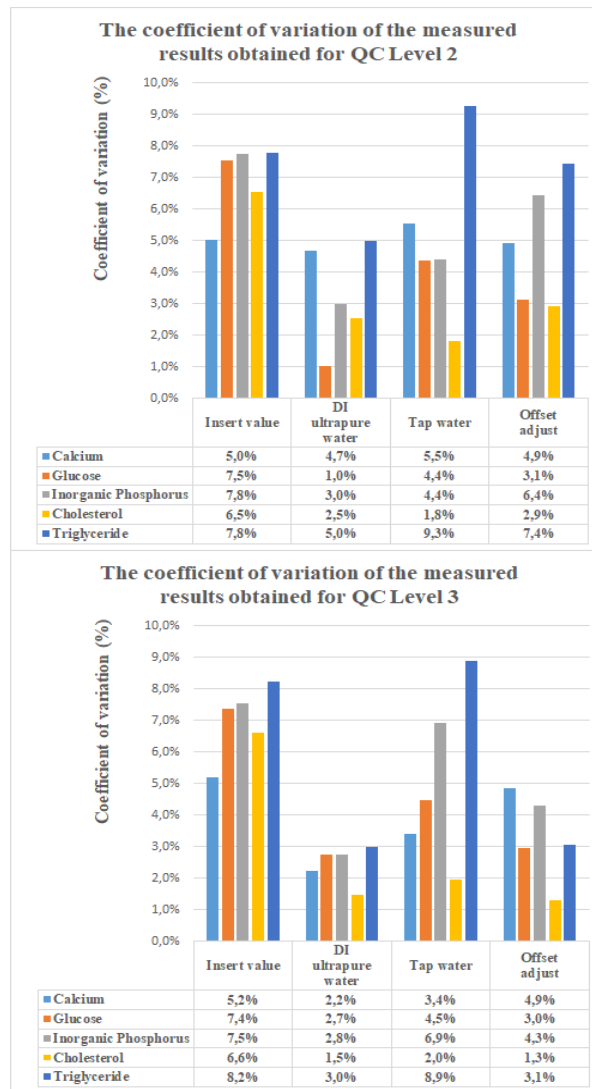
An important advantage when using the TDS and turbidity meter prototype is the continuous monitoring feature provided by the 2x16 LCD screen and the experimental adjusted alarm when the level of turbidity and TDS particles are over the normal limit.



*Figure 5.1 The TDS and turbidity meter prototype*

The experiment was performed for two types of water that were primed successively in the biochemistry analyzer. The first type is ultra-pure medical DI water with TDS of 4 ppm and 5 Nephelometric Turbidity Units (NTU) and the second one is tap water with TDS of 55 ppm and 330 NTU. There were performed three successive measurements for calcium, glucose, inorganic phosphorus, cholesterol and triglycerides for each level of QC on both types of water.

The coefficients of variations for each level of QCs were graphically plotted, as shown in Figure 5.7, to have a better visual approach regarding the precision and accuracy. As observed from the experimental results, the presence of calcium and inorganic phosphorus ions in the tap water generate a false elevated concentration of the results due to the great TDS with approximately 5% on both QC levels. In the case of cholesterol, triglycerides and glucose, the ions are not present in a large scale and the results are slightly elevated due to the turbid state of the water. The coefficient of variation when using high purity water (UPW) is under the assigned CV from the QC's insert sheet, with a significant decrease in case of glucose, cholesterol and inorganic phosphorus. The coefficient of variation when using the tap water is almost double than the case of using ultrapure DI water.



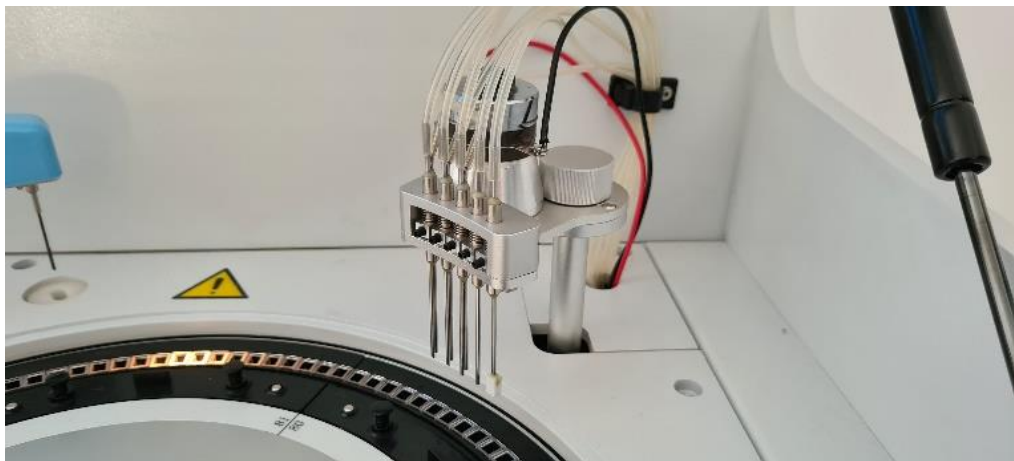
**Figure 5.2** The coefficient of variation for the measured results for QC2 and QC3.

By using this prototype in large scale, the precision and accuracy of the biochemical results are increased and provide confident results regarding the reported results because the water quality is permanently monitored and if the safety threshold is overcoming, the prototype signalizes visually and acoustically.

# Chapter 6

## Automated Ultrasonic Reusable Cuvettes Cleaning System

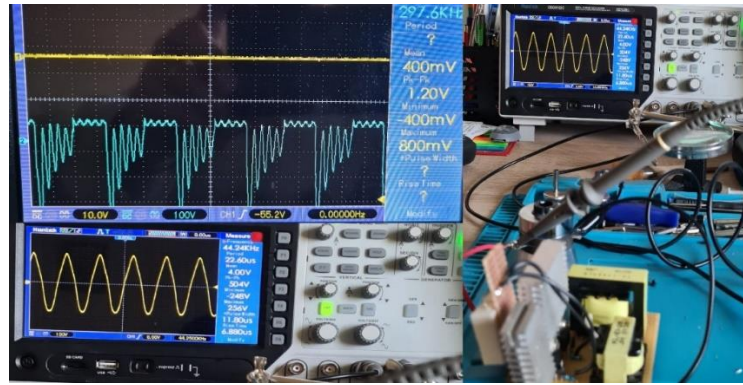
This chapter presents a novel and original prototype used for supplementary cuvette cleaning and the experimental results are compared with the manufacturer assigned values when using and not utilizing the novel subassembly. The prototype consists in an oscillator circuit that generates a 40 kHz signal applied to an ultrasonic transducer that is triggered when the wash station for the cuvettes from the laboratory biochemistry analyzer lowers in the washing position. The ultrasonic transducer is mounted on the washing arm and fixated with resin, as shown in Figure 6.1.



*Figure 6.1 The automatic ultrasonic cuvette cleaning system.*

The oscillator consists of the IR2153 self-oscillating half-bridge driver which has an RC group to control the frequency formed by 500nF capacitor along with a 10k $\Omega$  potentiometer in series with a 22k $\Omega$ . The ultrasonic transducer has a static capacitance of 4nF and must oscillate at 40 kHz, thus a 3.9mH inductor is connected in series with the transducer at the primary of the EI-28 ferrite transformer. The half bridge controls 2 IRFZ44N power transistors connected in a push-pull configuration, with supplementary protection diodes. When powered from an external laboratory power supply, there were observed high frequency and voltage computational spikes, as shown in Figure 6.5. The mean amplitude of the spikes was 160V and a snubber circuit was added. The oscillating circuit is controlled by a relay through an optocoupler connected to the washing station's arm.





**Figure 6.2** The experimental prototype under test.

The experiment, which consists in analyzing samples with and without the ultrasonic cleaner, was performed with the artificial blood bi-level quality control (QC), Randox Human Assayed Multi-Sera – Level 2 (lot 1199UN) and Level 3 (lot 949UE), for the one end-point biochemistry assays on the biochemistry analyzer. The measured results were also plotted in 2D column charts in order to perform a better visual inspection when comparing the performance of the ultrasonic cleaning system with the basic one. In Figure 6.9 are plotted the coefficients of variation for each assay performed in both cases, for every level of the quality control.



**Figure 6.3** The coefficients of variation for the analyzed QC samples with and without the prototype.

As can be observed, the coefficient of variation for the measurements using the prototype was at least with 0.5% lower than in the case of the basic cleaning for all the analyzed parameters. In the case of the inorganic phosphorus, the accuracy and the precision were improved even with approximately 3% regarding its CV. The average value of the difference between the CV of the basic clean and the ultrasonic clean for all the measured results for QC level 2 is 1.5% and for QC level 3 is 1.6%, which means that the accuracy and the precision of the biochemical assays performed on the reusable cuvette-based biochemistry laboratory analyzer was improved with approximately 1.5% by using the automatic ultrasonic cuvette cleaning system compared to its standard cleaning system.

# Chapter 7

## Optical Coagulation Analyzer Based on Image Acquisition Algorithm

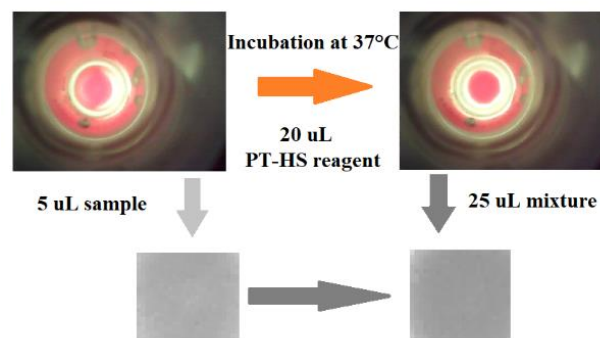
The most common point-of-care analyzers from the market use the following types of measurement technologies:

1) *Electrochemical method*: the biosensor consists in a test strip with two electrical planar contacts, connected to an electro-active substrate containing dried reagents. The blood sample is aspirated through capillarity and when it reaches the electro-active substrate containing the reagent, it produces a current that is further analyzed by the coagulation equipment and processed by its internal algorithm [79].

2) *Photomechanical method*: involves a test containing the activator and the blood is aspirated in the reagent channel by a mixing rotor. The timer starts when the sample is aspirated and the clot is picked up by the rotor, blocking the incident light, meaning that the sample coagulated and the timer stops [81].

In this chapter is presented an image processing portable coagulation analyzer that is based on analyzing the absorbance of the sample. Unlike the scattering technology and the photomechanical method, there is a direct link between the detected intensity and the emitted intensity of the light. For this prototype was used a 4300K planar LED as light source and abhorrent to it, a CMOS USB minicamera was installed on the top of the sample vacutainer.

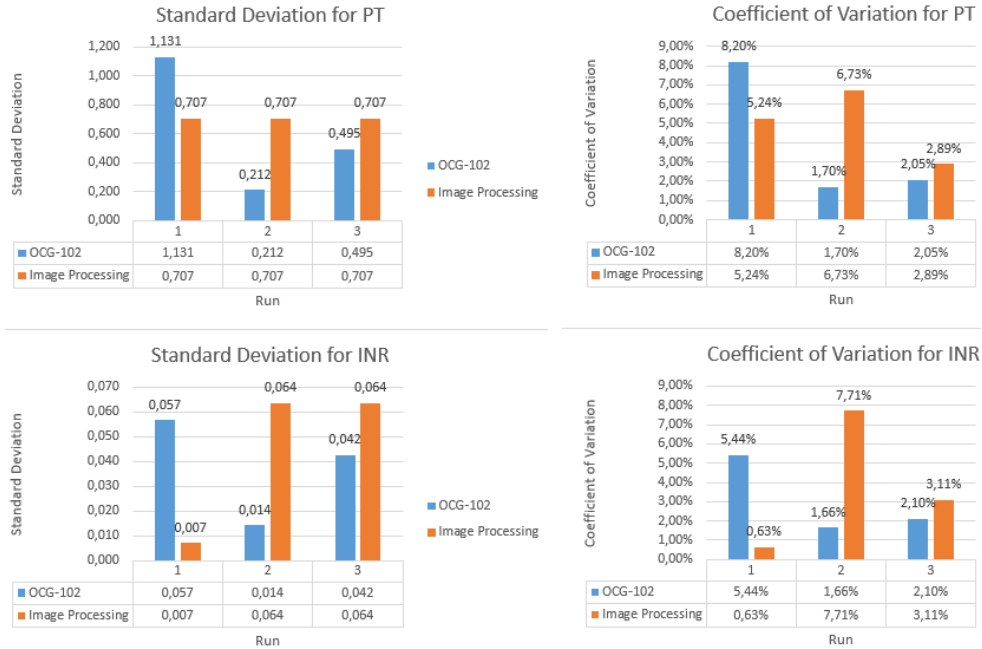
The basic coagulation process using the image processing prototype is displayed in Figure 7.9.



**Figure 7.1** The coagulation analysis using image acquisition.

The study was performed on three different patients. There were collected two sets of samples from the same patient in order to analyze and calculate the standard

deviation and the coefficient of variation between successive measurements on a photomechanical coagulation analyzer and on the prototype of the optical coagulation that uses image processing algorithm. By comparing with the reference analyzer, it is observed that the average coefficient of variation for the PT measurement is about 4.95% in case of the image acquisition prototype and 3.98% in case of the photomechanical coagulation meter.



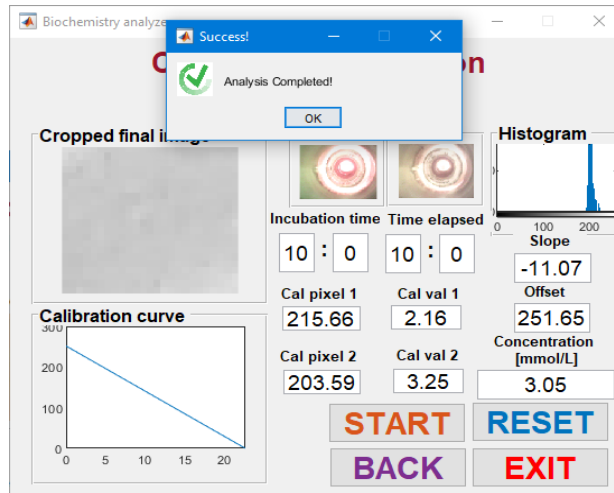
*Figure 7.2 The variation plots for PT and INR of the portable image acquisition prototype compared to the reference analyzer.*

## Chapter 8

# Liquid Reagent-based Biochemistry Analyzer with Image Acquisition Algorithm

Starting from the image acquisition method previously debated, this chapter presents another novel portable image acquisition analyzer developed to measure the concentration of the biochemistry parameters on the same prototypic platform by using different types of liquid reagents and software. The measurement's software of the biochemistry analyzer prototype is shown in Figure 8.6. The "Time elapsed" field shows the time that

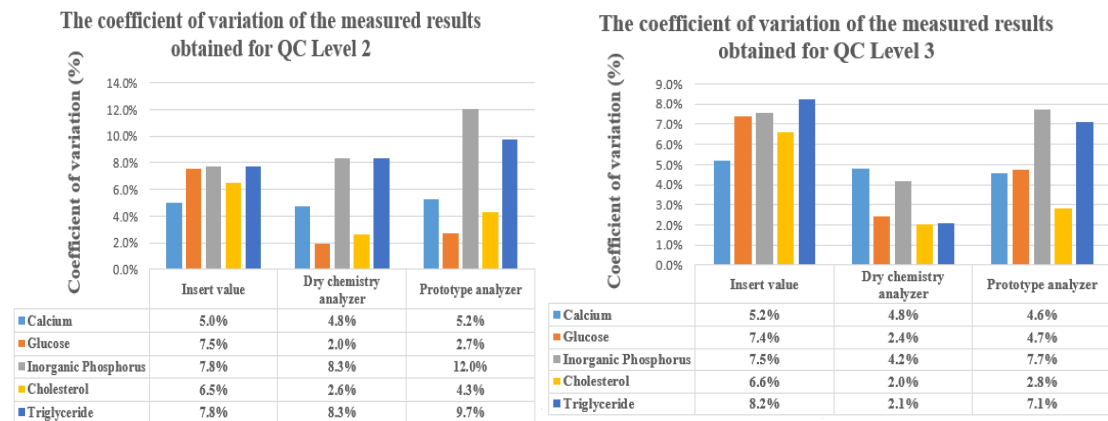
elapsed from the start of the incubation. The final image is acquired after the elapsed time is equal to the entered incubation time. When the incubation reaches 10 minutes, the final image is captured and the concentration is computed using Beer-Lambert law in accordance with the current method. The calculations were made with 2 decimals in order to increase the accuracy and the precision of the displayed results.



**Figure 8.1** The biochemical analyzer's measurement interface for Ca.

In the experiment were used two levels of artificial serum because any samples collected from patients would imply a consent from them and also a preprocessing method for obtaining serum from whole blood.

Each sample was successively run three times in order to properly compute the SD and the CV of the results obtained on both instruments.



**Figure 8.2** The coefficients of variation for the measured results on both instruments and for both QC levels.

The inorganic phosphorus and the triglycerides results are slightly higher than the results for the same parameters obtained on the dry test pads biochemistry analyzer. Comparing the CV of the measured results on the prototype analyzer with the calculated

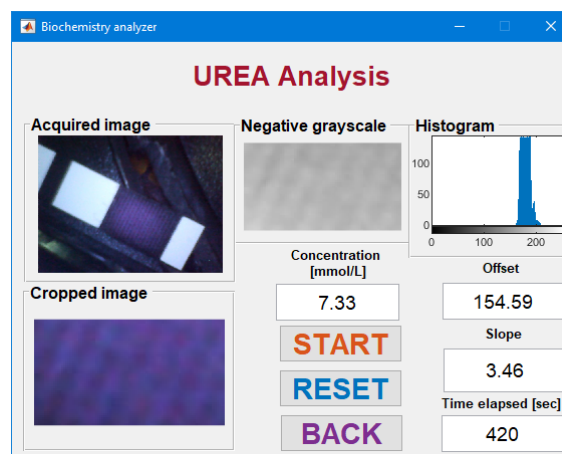
CV of the target values from the QC's insert sheet, it may be stated that only for the normal QC level 2 the results are higher with only 1.9% in case of triglycerides and with only 4.2% in case of the inorganic phosphorus. When comparing the pathological results for QC level 3, the CV of the measured results is comparable with the CV of the target results. This means that in the case of a patient with pathological state of health, the measured results are in accordance with his clinical state and not false positive when measured on the prototype analyzer.

The prototype's performance is comparable with the dry chemistry point-of-care analyzer used as reference. The system is expandable because is used the same platform, but with different software algorithm, as the coagulation analyzer, presented in the previous chapter [13].

## Chapter 9

# Automated Dry Chemistry Analyzer Based on Image Acquisition

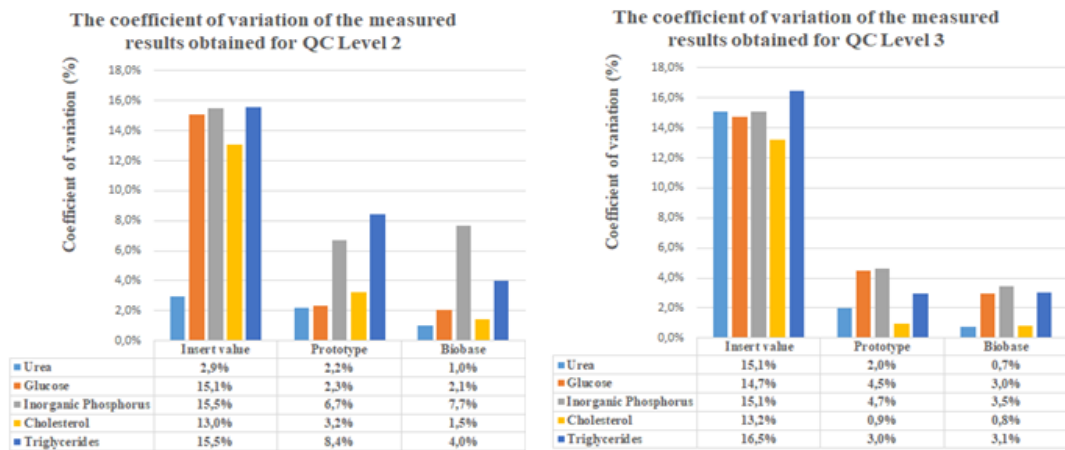
This chapter presents an dry chemistry analyzer based on the image acquisition method used in the previous chapters. The method involves a mini-VGA USB camera which captures an image of the test that is automatically positioned in front of it when the incubation is finished. The prototype uses a MATLAB Graphical User Interface (GUI) experimental designed software which processes the acquired image in order to obtain a concentration reportable result. In Figure 9.11 is shown a captured image of a urea test pad.



*Figure 9.1 The analysis software interface for urea.*

In the experiment were used the bi-level Randox Human Assayed Multi-Sera artificial quality control (QC) samples. Every sample was analyzed three times successively in order to establish the SD and the CV. The obtained results were also compared with the results obtained on the liquid-reagents based biochemistry analyzer.

The average CV of the prototype biochemistry analyzer for the tested parameters is 4.56% for QC level 1 and 3.02% for the pathological QC level 2 and in the case of the liquid-based automated biochemistry analyzer, were measured average CVs of 3.26% for the normal QC level 1 and 2.2% for QC level 2.



**Figure 9.2** The coefficient of variation of the measured results for QC Level 2 and Level 3.

The main advantage consists in using only 6µl of sample on dry chemistry tests available on the market for the measurement, meaning a very low volume of dangerous fluids involved with no post-analysis liquid waste. Thanks to the programming flexibility, the software can be adapted for other dry chemistry tests.

## Chapter 10

# Reagent-free Hemoglobin Analyzer with Image Acquisition Algorithm

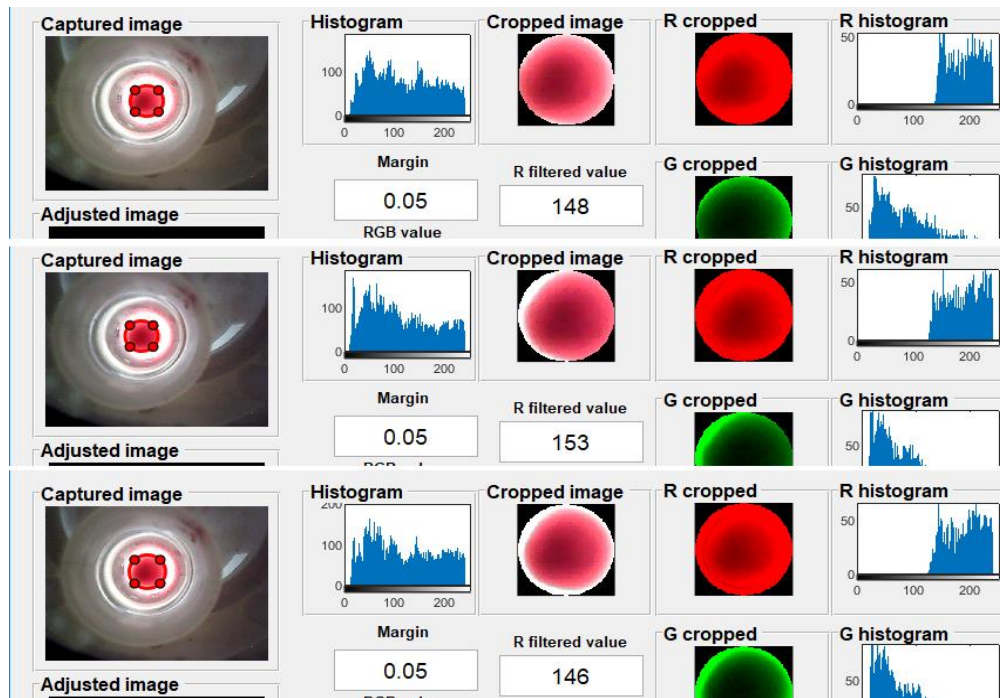
This chapter presents a novel hemoglobin and hematocrit analyzer which uses the same hardware platform and the same image acquisition method discussed in the previous chapters.

The prototype uses an USB mini camera that is situated on the top of a microtainer in order to acquire the transmitted light along the sample when the incident



light is generated by a 5500K color temperature planar LED [104] situated under the microtainer. The experimental and proposed image acquisition method is used because it is more sensitive to the chromatic modifications when a hemoglobin assay is performed. Compared to the spectrophotometric method, image acquisition supplies greater software programming flexibility and the program can be modified easily when needed. The setup is cost-effective and all the measurement technology consists in a software program.

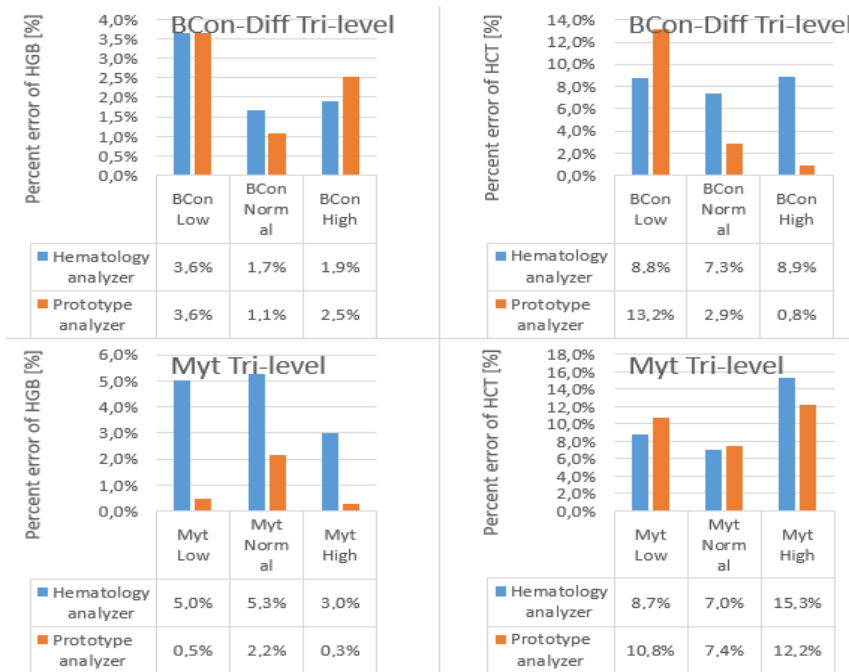
In Figure 10.6 are presented software image extracts from the measurements performed with the MYT2106 artificial control blood. As can be observed from both figures, the proposed analysis method reduces the operational errors that may occur when selecting the ROI because even if any sample free region is selected along with the sample, the obtained R values do not vary much.



**Figure 10.1** The successive measured results for MYT2106 high pathological level.

Regarding the experimental part of the study, the samples are pipetted from two types of three level artificial quality control blood, MYT2106 Tri-level and Boule Con Tri Diff. Every quality control pack has its own low, normal and high levels with assigned means and SDs.

The measured results on the prototype analyzer regarding the hemoglobin have the CV lower than the ones obtained on the 5-part hematology analyzer because the prototype was calibrated with Myt Tri-level QC from the image acquisition point-of-view and as expected, the measurement technology does not involve liquid reagents, so no light propagation phenomenon is involved, supplying accurate and precise results also in the case of analyzing the B-Con Diff-QC.



*Figure 10.2 The percent error for the quality control results of each level from the two sets performed on the hematology analyzer and the hemoglobin prototype analyzer.*

## Chapter 11

# LabConcept – Telemedicine and the Laboratory Information System Platform

This chapter presents a novel experimental telemedicine platform called LabConcept which consists in a MATLAB GUI that can scan the QR codes printed on the result bulletin of the blood assays and upload them in a locally stored database on a mobile device, for further interpretation. The QR codes are processed in MATLAB via its specific toolbox [107]. The idea of implementing printed or electronically stored on the mobile devices QR codes gains more and more popularity in everyday use. When the idea of printing the assay results QRs on the result bulletin, was not implemented the COVID-19 digital certificate, which in present strengthens even the utility of QR codes in the medical area. The software's platform organizational chart is presented in Figure 11.2.

An imprecise result implies an improper treatment plan which can cause severe health issues for the patient, especially when diabetes is involved. The results from home monitoring equipment are loaded into the application through LIS and any drift regarding



the results is analyzed and recorded. If the results were within the normal limit until the moment of testing, most probably is a precision and accuracy issue. If not, professional consultation must be performed.

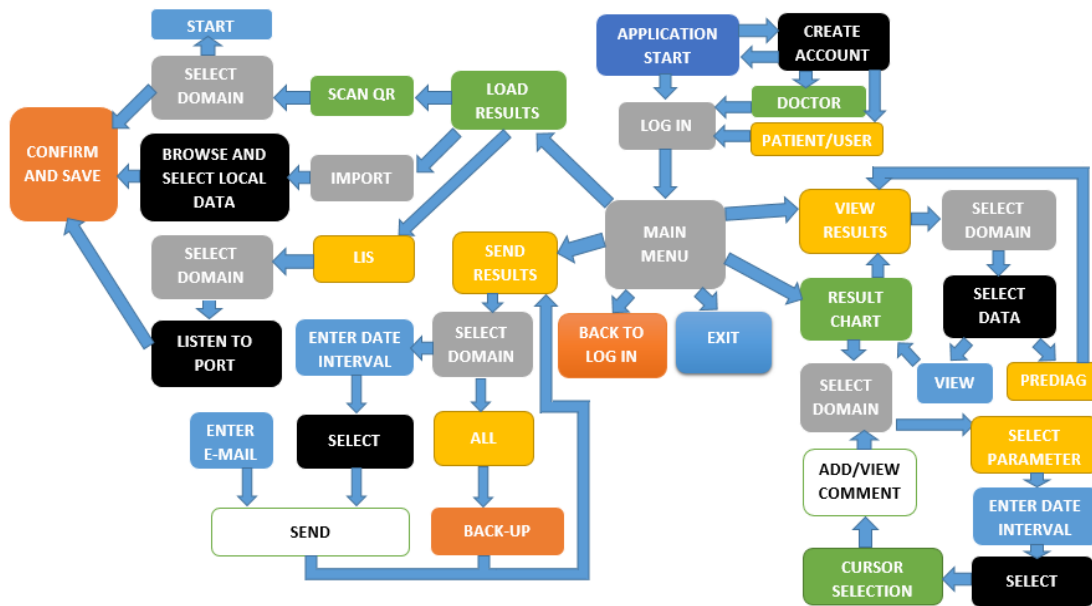


Figure 11.1 The LabConcept's organizational chart.

## Chapter 12

### Conclusions

The presented automated three-way mixer eliminates the probability of appearing operating errors during a blood gas assay. It saves time and it ensures the precision and accuracy of the results, increasing the quality of the medical act. It is a low-cost device that is very competitive from the mixing procedure point-of-view because the available mixer, the safePico syringe mixer, doesn't mix capillary tubes and the mixing process is device static.

The automated cuvette window cleaning system enhances the accuracy and precision of the assays performed on single-use cuvette-based biochemistry analyzers because it maintains the same assay conditions each time an assay is performed from the absorbance point-of-view by cleaning each window after each sample.

Another most frequent issue regarding the biochemistry analyzers' performance consists in the possibility of water contamination with ions or if the water is turbid. A fast and cheap way to solve this issue is to equip the analyzer at the beginning of the water supply line with a TDS and turbidity meter that constantly measures the amount of ions and the transparency of the water. An important advantage when using the TDS and

turbidity meter prototype is the continuous monitoring feature provided by the 2x16 LCD screen and the experimental adjusted alarm when the level of turbidity and TDS particles are over the normal limit.

The reusable cuvette-based analyzers have a dedicated washing station that clean the cuvettes after each sample. As mentioned before, water may affect the absorbance and also may induce carryover if not properly cleaned. To counteract this issue, the idea of utilizing an ultrasonic system mounted on the top of the analyzer's washing station improves the accuracy and the precision of the assayed parameters and also increases the cuvettes' lifespan.

Except the adjuvant subassemblies, there are presented original methods for measuring specific biochemical parameters. The coagulation PT/INR, hemoglobin, hematocrit and biochemistry parameters may be alternative measured with image acquisition hardware. On the same hardware platform, but with different software types, various parameters are quantitatively measured. The image acquisition method's performance is experimentally comparable with the reference instruments. The image acquisition method can be used also for dry chemistry tests, as presented, the accuracy and precision being improved first by assuring the same sample volume by using a peristaltic pump for pipetting to the detriment of an automated syringe to which the pressure may be lost due to the tips wear out.

The precision and the accuracy of an instrument may be monitored by a software that communicates with the analyzer, receiving the measured results via the LIS system. If an external (procedural) or internal issue occurs, there are registered result variations over a period. The need of a software that records, analyzes or transmits the results to be further interpreted by a professional doctor rises interest, especially nowadays when social distancing is mandatory and the possibility to lapse into illness is very high.

## **12.1 Obtained results**

The scope of the thesis is to contribute at the precision and accuracy improvement by analyzing the daily common issues in the medical equipment area from personal technical experience, describing the phenomena that takes place at biochemical level during a sample measurement on a blood analyzer. The original contributions extend to the development and fabrication of hardware and software elements, additional or stand-alone, used for sample concentration measurements.

The experimental results are obtained by measuring blood samples or quality control materials and comparing them to the ones obtained on a reference analyzer from the market. Each presented method is accompanied by standard deviation and coefficient of variation comparison between successive samples with or without the developed subassemblies in case of adjuvant instrument hardware and with an existing analyzer from the methodical point-of-view.

Regarding the three-way automated blood sample device mixer for blood gas, the hardware's performance was measured compared to the unmixed, freshly collected blood

samples. The obtained CV when the samples were automatically mixed was approximately 1% lower than in the case of the freshly non-mixed samples.

Regarding the automated cuvette window cleaning system, the performed assays are the TBI and DBI, which are very sensitive sub unitary and unitary results assay parameters. When the experiment started, the coefficient of variation was almost the same for both QC levels in the case of TBI, 4.07% (for the first manual clean) and 3.04% (for the second manual clean) for QC level 1, 5.71% (for the first manual clean) and 5.05% (for the second manual clean) for QC level 2 and DBI, 6.41% (for the first manual clean) and 5.97% (for the second manual clean) for QC level 1, 3.11% (for the first manual clean) and 13.03% (for the second manual clean) for QC level 2. In the case of the automatic clean using the prototype, the same assay conditions tend to be maintained and this fact is demonstrated by the coefficient of variation of the measured results, which are lower than in the case of manual periodical cleaning, 0.96% for TBI for the first QC level and 1.03% for the second QC level and 3.83% for DBI for the first QC level and 1.81 for the second QC level.

The experiments advanced further with analyzing the TDS and turbidimetric measurement for DI water supplied to the analyzer. An impure water generates false reactions between the ions contained in the water and the reagents used for assays. To overcome this situation, a permanently monitoring system is mandatory. The system monitors both the TDS and the water's turbidity, signaling the operator if the safety threshold is overstepped. For the experiment, the analyzer was successively supplied with ultrapure DI and tap water and calcium, albumin, inorganic phosphorus, cholesterol and triglycerides assays were performed. In case of the ultrapure DI water the coefficient of variation was almost half of the one obtained in case of using tap water.

Since the water supply is important regarding the analyzer's performance, the washing procedure must be properly performed with no carryover or any other residual waste after the cleaning process finishes. The presented solution is represented by mounting a 40kHz ultrasonic transducer driven when the washing arm lowers into the washing position. The coefficient of variation is lower in the case of the measurements performed with the prototype and the average difference between the CV of the measured values for the basic clean and the values for the ultrasonic clean of the assayed parameters is approximately 1.5%. It can be stated that the accuracy and the precision of the biochemical assays was enhanced with approximately 1.5% when the prototype was used, which enforces the need of equipping the analyzer with an ultrasonic cleaning system to assure more accurate and precise results.

Regarding the presented image acquisition methods, the PT/INR measurement are comparable with the results obtained on the photomechanical analyzer, but in the case of the liquid based portable biochemistry analyzer that uses image acquisition method, most parameters have the CV under the QC manufacturer assigned values, observing that the inorganic phosphorus and the triglycerides results are slightly higher than the results for the same parameters obtained on the dry test pads biochemistry analyzer. Comparing the CV of the measured results on the prototype analyzer with the CV of the target values from the QC's insert sheet, it may be stated that only for the normal QC level 2 the results were higher with only 1.9% in case of triglycerides and with only 4.2% in case of the

inorganic phosphorus. When comparing the pathological results for QC level 3, the CV of the measured results is comparable with the CV of the target results. This means that in the case of a patient with health issues, the measured results are in accordance with his clinical state and not false positive when measured on the prototype analyzer.

In case of the automated dry chemistry analyzer, the results are comparable with the ones obtained on the automated laboratory biochemistry analyzer, both instrument registering CVs under the QC assigned values.

The portable hemoglobin analyzer was calibrated with an artificial three level QC. Its precision was tested after the calibration using the same three levels of artificial control and it was observed that are obtained approximately the same results as the assigned values and any pixel variation due to subjective ROI selection or microtainer slight displacement is low and does not affect the results' precision and accuracy. When analyzing the first set of QCs, the results were comparable to the ones obtained on the reference 5-part hematology analyzer. When analyzing the QCs used for the prototype's calibration, the measured results were more precise and accurate compared to the ones obtained on the reference 5-part hematology analyzer because the analyzed QC is not instrument dedicated for the reference analyzer. As may be observed, there were also registered small variations regarding the HCT because the 5-part analyzer measured the HCT directly through potentiometry in the WBC aperture and the prototype calculates it from the measured HGB.

## **12.2 Original contributions**

The original contributions are reflected by the case study that generated the subject of the current thesis, the synthesis of the common issues and methods to counteract them, accompanied by hardware/software subassemblies and stand-alone analyzers, which were designed and fabricated from scratch for research and testing purposes.

The three-way automated sample device mixer for the blood gas analysis [9] from Chapter 3 consists in an automated offline device that is used to harmonize the content of a heparinized sample syringe used in the blood gas area, much cheaper and method-reliable than the existing safePico system from Radiometer.

The optical method for improving the accuracy and the precision of the biochemical assays for the single use cuvette-based analyzers [10] from Chapter 4 represents an automated subassembly that is involved in the quartz cuvettes windows automatic cleaning process. The biochemistry analyzers which fabricate cuvettes from a film cartridge need a periodically cuvette windows clean, a time-consuming operation from the operator's side, not to mention that residual pellicles over the windows affect the precision and the accuracy of the instrument.

The TDS and turbidity meter [11] is a bi-purpose subassembly presented in Chapter 5, which detects the concentration of the dissolved solids in the analyzer's water intake before its water tank and also measures the attenuation of a light beam through the water in a separate tank. An impure water generates an absorbance modification regarding the spectrophotometric measurements from the analyzer's

cuvettes, where the DI water is used to clean or to prepare the specific sample-reagent mix.

Automatic ultrasonic cuvette cleaning system [12] from Chapter 6 represents a subassembly for the reusable cuvette-based biochemistry analyzers which consists in an automatic ultrasonic cleaning system that is involved in the cuvette washing process. To counteract and minimize this undesired carryover, on the washing station was mounted an ultrasonic transducer with its corresponding circuitry triggered by the washing's arm optical sensor, when the station is in the cleaning position.

The portable optical coagulation analyzer [13] from Chapter 7 is a prothrombin time and INR measurement prototype which uses an USB camera as a clot formation detector and a MATLAB developed software computes the PT from the acquired images.

Reagent-free hemoglobin analyzer [14] is a portable analyzer presented in Chapter 10 which measures the amount of hemoglobin from a drop of capillary blood on the same prototype as the PT and INR. The same digital camera is used to measure the concentration of hemoglobin, except a different MATLAB developed software is used.

Portable biochemistry analyzer based on image acquisition [15], presented in Chapter 8, is a biochemistry analyzer which uses liquid reagents to trigger the photometric reaction. The method is similar with the hemoglobin and the coagulation analyzer, using the same prototype platform, but with other specialized MATLAB developed software.

Automated dry tests portable biochemistry analyzer [16] from Chapter 9 represents an automated biochemistry analyzer which uses the same digital camera for image acquisition of the sample that is automatically pipetted over the dry test specific for each biochemical parameter. The system is experimentally calibrated using liquid biochemistry calibrator, obtaining a calibration line used in the sample measurement process.

Another original contribution consists in a topic discussed and analyzed in Chapter 11 about a proposal of a telemedicine software that analyzes the loaded results over a predefined period. The software can load results directly from the connected analyzers through the LIS system. In case of a quantitative HbA1c analyzer which uses lateral flow immunofluorescence test strips, the prolong incubation time may alter the precision and accuracy of the glycated hemoglobin [17].

An imprecise result implies an improper treatment plan which can cause severe health issues for the patient, especially when diabetes is involved. The results from home monitoring equipment are loaded into the application through LIS and any drift regarding the results is analyzed and recorded. If the results were within the normal limit until the moment of testing, most probably is a precision and accuracy issue. If not, professional consultation must be performed.

## 12.3 List of original publications

The presented list includes all the author's published 8 papers in scientific conferences and 2 papers in journals, one of them which was awarded by UEFISCDI – PRECISI2021 (PN-III-P1-1.1-PRECISI-2021-59746). Except the published ISI papers, the author participated also at 2 scientific events with non-published papers, but public debated accompanied by presentations of the scientific research results. The author participated at a European Project between 2019 and 2020. All the scientific papers can be found in the bibliography and the previous mentioned works have content related to the subject of the doctoral thesis.

### I. Scientific Conference Papers

1. **M. S. Niculescu**, “*Optical Method for Improving the Accuracy of Biochemical Assays*” Conference: 6th IEEE International Conference on E-Health and Bioengineering (**EHB 2017**) Location: Sinaia, ROMANIA Date: JUN 22-24, 2017, **WOS: 000445457500096**

2. **M. S. Niculescu**, “*Three Way Automated Blood-Sampling Device Mixer for Blood Gas Analysis*”, Conference: 6th International Conference on Advancements of Medicine and Health Care through Technology (**MediTech 2018**) Location: Cluj Napoca, ROMANIA Date: OCT 17-20, 2018, **WOS: 000493501100009**

3. **S. Pasca, M. S. Niculescu**, “*Portable Optical Coagulation Analyzer Based on Real-Time Image Processing Algorithm*”, Conference: 11th International Symposium on Advanced Topics in Electrical Engineering (**ATEE 2019**) Location: Bucharest, ROMANIA Date: MAR 28-30, 2019, **WOS: 000475904500056**

4. **M. S. Niculescu, A. Florescu, S. Pasca**, “*Reagent-Free Hemoglobin Portable Analyzer using Images Processing*”, Conference: 7th E-Health and Bioengineering Conference (**EHB 2019**) Location: Grigore T Popa Univ Med & Pharmacy, Iasi, ROMANIA Date: NOV 21-23, 2019, **WOS: 000558648300202**

5. **M. S. Niculescu, A. Florescu, S. Pasca**, “*Accuracy and Precision Improvement for the Biochemistry Assays by Using an Automatic Ultrasonic Cleaning System*”, 8th International Conference on E-Health and Bioengineering (**EHB 2020**) Location: ELECTR NETWORK, Date: OCT 29-30, 2020, **WOS: 000646194100085**

6. **M. S. Niculescu, A. Florescu, S. Pasca**, “*Accuracy Improvement for the Biochemistry Assays by Using Total Dissolved Solids and Turbidimetry Measurements*”, 8th International Conference on E-Health and Bioengineering (**EHB 2020**) Location: ELECTR NETWORK, Date: OCT 29-30, 2020, **WOS: 000646194100138**

7. **M. S. Niculescu, A. Florescu, S. Pasca**, “*Portable Biochemistry Analyzer Based on Image Acquisition Algorithm*”, 12th International Conference on Electronics, Computers and Artificial Intelligence (**ECAI 2020**) Location: Bucharest, ROMANIA, Date: JUN 25-27, 2020, **WOS: 000627393500061**

8. **M. S. Niculescu, A. Florescu, S. Pasca**, “*The Incubation Time Effects on the Precision and Accuracy of the Glycated Hemoglobin*”, International Conference on Advancements of Medicine and Health Care through Technology (**MEDITECH 2020**),

Location: Cluj-Napoca, ROMANIA Date: 13-15 October 2020,  
([https://link.springer.com/chapter/10.1007/978-3-030-93564-1\\_8](https://link.springer.com/chapter/10.1007/978-3-030-93564-1_8))

## **II. Scientific Journal Papers**

1. **M. S. Niculescu**, A. Florescu, S. Pasca, “*Automated Portable Biochemistry Analyzer Based on Image Acquisition*”, REVUE ROUMAINE DES SCIENCES TECHNIQUES-SERIE ELECTROTECHNIQUE ET ENERGETIQUE (**ISI Q4**, IF JCR2020=0,443), Volume: 65 Issue: 3-4 Pages: 271-276 Published: JUL-DEC 2020, ISSN: 0035-4066, **WOS: 000608261900019**

2. **M. S. Niculescu**, A. Florescu, S. Pasca, “*LabConcept – A New Mobile Healthcare Platform for Standardizing Patient Results in Telemedicine*”, APPLIED SCIENCES-BASEL (**ISI Q2**, IF JCR2020=2,679), Volume: 11 Issue: 4 Pages: 1935 Published: JUL 2020-FEB 2021, ISSN: 2076-3417, **WOS: 000632095800001**, DOI: 10.3390/app11041935

**UEFISCDI – PRECISI2021 Awarded ISI Q2 (PN-III-P1-1.1-PRECISI-2021-59746)**

## **III. Research reports**

1. Report 1 – Evaluation about sensors, devices and analysis methods used in the biochemistry analyzers
2. Report 2 – State of the art of the current thesis

## **IV. Scientific Symposiums**

1. **M. S. Niculescu**, “Noninvasive Hearing Aid System Based on Skin Transmitted Sound Waves by an Ultrasound Transducer” – SAD 2018

2. **M. S. Niculescu**, “LabConcept - A New Mobile Healthcare Platform for Standardizing Patient Results in Telemedicine” – SAD 2019

## **V. Projects**

1. Entrepreneurial Skills Development of Doctoral and Post-doctoral Students – Key to Success in Career (A-Success), contract number 51675/09.07.2019 POCU/380/6/13 – SMIS code 125125)

## **12.4 Perspectives for further developments**

As future development perspective, the presented methods may be embedded into a singular analyzer which uses image acquisition. The potential idea is to create a sample arm which transfers the sample from a microtainer to a reusable quartz cuvette installed into an obscure chamber, for the measurements not to be affected by the external light.

The obscure chamber should have on one side a camera and on the other side a 5500K dimmable LED. The sample arm should have an ultrasonic transducer shaped needle used for sample mixing after the sample transfer to the cuvette.

The cuvette should have a drain hole under it, connected to a peristaltic pump which drains the waste liquid from the cuvette after the assay is finished and the washing cycle is performed.

The obscure chamber should have a resistive layer inside in order to incubate the sample along with the reagent. Above the cuvette must be a static dispense needle that is connected to a rotary valve that selects different types of liquid reagents, air and water.

The main advantage of the presented idea of future development is that a multiparameter analyzer may be fabricated using the presented methods in the thesis.

## Selection of Bibliography

[1] World Health Organization, *Constitution of the World Health Organization – Basic Documents*, Forty-fifth Ed., p. 1, New York, U.S.A., 2006.

[2] Iannone, *E.Labs on Chips: Principle, Design and Technology*, CRC Press, p. 1093, Boca Raton, Florida, U.S.A., 2016.

[7] J. Rosenthal, M. E. Rafelson, *Clinical Physiology and Biochemistry*, Karger, Vol. 1, p. 284, Chicago, 1983.

[9] **M. S. Niculescu**, *Three Way Automated Blood-Sampling Device Mixer for Blood Gas Analysis*, Conference: 6th International Conference on Advancements of Medicine and Health Care through Technology (MediTech), Cluj Napoca, Romania, 2018.

[10] **M. S. Niculescu**, *Optical Method for Improving the Accuracy of Biochemical Assays*, Conference: 6th IEEE International Conference on E-Health and Bioengineering (EHB), pp. 381-385, Sinaia, Romania, 2017.

[11] **M. S. Niculescu**, A. Florescu, S. Pasca, *Accuracy Improvement for the Biochemistry Assays by Using Total Dissolved Solids and Turbidimetry Measurements*, 8th IEEE International Conference on E-Health and Bioengineering (EHB), Iasi, Romania, 2020.

[12] **M. S. Niculescu**, A. Florescu, S. Pasca, *Accuracy and Precision Improvement for the Biochemistry Assays by Using an Automatic Ultrasonic Cleaning System*, 8th IEEE International Conference on E-Health and Bioengineering (EHB), Iasi, Romania, 2020.

[13] S. Pasca, **M. S. Niculescu**, *Portable Optical Coagulation Analyzer Based on Real-Time Image Processing Algorithm*, 11th International Symposium on Advanced Topics in Electrical Engineering (ATEE), Bucharest, Romania, 2019.

[14] **M. S. Niculescu**, A. Florescu, S. Pasca, *Reagent-Free Hemoglobin Portable Analyzer using Images Processing*, 7th E-Health and Bioengineering Conference (EHB), Iasi, Romania, 2019.

[15] **M. S. Niculescu**, A. Florescu, S. Pasca, *Portable Biochemistry Analyzer Based on Image Acquisition Algorithm*, 12th International Conference on Electronics, Computers and Artificial Intelligence, Bucharest, Romania, 2020.



- [16] **M. S. Niculescu**, A. Florescu, S. Pasca, *Automated Portable Biochemistry Analyzer Based on Image Acquisition*, Revue Roumaine Des Sciences Techniques-Serie Electrotechnique Et Energetique, Volume: 65, Issue: 3-4, pp. 271-276 , Bucharest, Romania, 2020.
- [17] **M. S. Niculescu**, A. Florescu, S. Pasca, *The Incubation Time Effects on the Precision and Accuracy of the Glycated Hemoglobin*, Conference: 7th International Conference on Advancements of Medicine and Health Care through Technology (MediTech), Cluj Napoca, Romania, 2020.
- [18] J. W. Robinson, *Atomic Spectroscopy*, Second Ed., Marcel Dekker Inc., p. 27, New York, U.S.A., 1996.
- [21] A. Dasgupta, J. L. Sepulveda, *Accurate Results in the Clinical Laboratory – A Guide to Error Detection and Correction*, Elsevier, pp. 21-40, Chennai, India, 2013.
- [22] O. Mali, P. Neittaanmäki, S. Repin, *Accuracy Verification Methods – Theory and Algorithms*, Computational Methods in Applied Sciences, Springer Science + Business Media Dordrecht, pp. 9, 154, Germany, 2014.
- [23] J. Baynes, M. H. Dominiczak, *Medical Biochemistry*, Elsevier Saunders, 4th Edition, p. 603, United Kingdom, 2014.
- [31] T. Jue, K. Masuda, *Application of Near Infrared Spectroscopy in Biomedicine*, Springer Science+Business Media, pp. 1-3, New York, U.S.A., 2013.
- [73] A. Neuberger and L. L. M. Van Deenen, *Blood Coagulation*, Elsevier, vol. 13, Amsterdam, pp. 46-48, Amsterdam, The Netherlands, 1986.
- [79] R. J. Narayan, *Medical Biosensors for Point of Care (POC) Applications*, Woodhead Publishing, Elsevier Ltd., p. 215, United Kingdom, 2017.
- [81] Guangzhou Wondfo Biotech Co. Ltd., *Introduction to Coagulation Meter*, p. 6, Guangzhou, China, 2018.
- [107] T. Huckel, T. Neckel, *Matlab Example Programs. Bits and Bugs – A Scientific and Historical Review of Software Failures in Computational Science*, Society for Industrial and Applied Mathematics: Philadelphia, p. 239, U.S.A., 2019.