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AUTOMATED PORTABLE BIOCHEMISTRY ANALYZER BASED ON IMAGE ACQUISITION

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Key words: Biochemistry analyzer, Image acquisition, Dry chemistry, Point-of-care, Spectrophotometry.

Biochemistry is one of the most important blood sciences because it evaluates the complex of biomolecules from the living cells and the processes that take place at cellular level. A routine biochemistry analysis supplies important information about the hydro-electrolytic balance, the glycemic levels, the hepatic and renal functions. The paper presents an original automated portable biochemistry prototype analyzer which uses a real-time image acquisition algorithm instead of a reflectance photometer. The novelty consists in using an USB mini VGA camera which is triggered when the dry chemistry tests are placed in front of the camera's lens after the colorimetric reaction takes place. The presented prototype is a low-cost, reliable and performance comparable with the existing dry chemistry analyzers from the market. The main advantage represents the programming flexibility with less hardware components.

1. INTRODUCTION

Health is a very important indicator for every person because it is related with his physical, mental and social state [1]. In order to maintain the medical parameters at optimum levels, periodic assays accompanied with a professional medical interpretation must be performed.

The medical profile represents a set of biological parameters which offer information about the chemical processes and the amount of bio-components from the living organism. There are four major sciences, based on the type of the assay and the function of every biological system, regarding the clinical diagnostic: blood science, molecular diagnostic, medical imaging and functional testing [2].

Blood is the human's body non-Newtonian liquid component which flows through arteries, veins and capillaries, transporting oxygen to the cells and waste from the cells, along with the specific nutrients needed in the metabolic process [3]. It is composed of blood cells, (erythrocytes, leukocytes, platelets) and plasma, which is the liquid component that holds the blood cells in suspension, containing important dissolved proteins (albumins, globulins, fibrinogen), glucose, clotting factors, electrolytes (sodium, calcium, magnesium, chloride, potassium), hormones (amino acids, steroids, eicosanoids, peptides) and blood gases (carbon dioxide, oxygen) [4].

Biochemistry is the routine operation in the blood assay domain that analyzes the chemical processes in the living organisms: glycemic levels, the hydro-electrolytic balance, the renal and hepatic balance [5]. It is one of the most important medical science because its large scale of analyzed parameters which supply complete information about the clinical state of the tested patient. The results obtained after a biochemical profile is analyzed lead to an enhanced and correct clinical diagnostic.

The biochemistry assays are performed on dedicated medical instruments, which automatically analyze the serum or plasma, obtained from the tested blood's controlled centrifugation using liquid reagents or dry test pads [6]. There are two categories of biochemistry analyzers: the laboratory and the point-of-care instruments. Depending of the sample analysis mode, the laboratory instruments can analyze multiple samples on board fully automatic, whereas the point-of-care instruments are emergency analyzers that can analyze only one sample to reduce the result reporting time.

This paper presents an automated portable biochemistry analyzer prototype that uses an image acquisition and processing system. The prototype's performances are compared to an existing automated laboratory biochemistry analyzer. The rest of the paper is organized as it follows: in section 2 are described the measurement methods of the existing biochemistry analyzers, followed by section 3, where the automated portable biochemistry prototype is presented. The experimental results are organized in section 4 and the paper ends with the final conclusion and the acknowledgement sections.

2. THE MEASUREMENT TECHNOLOGY OF THE EXISTING BIOCHEMISTRY ANALYZERS

The biochemistry analyzers available on the market analyze the serum or plasma obtained from the centrifuged tested blood sample at a constant 2000-rpm for approximately 2 minutes. The obtained serum is pipetted and reacts with the assay specific liquid reagents or dry test pads under constant and controlled incubation temperature of 37 ºC triggering a colorimetric transformation, which is measured with a spectrophotometer [7].

2.1. THE AUTOMATED LIQUID REAGENTS BASED BIOCHEMISTRY ANALYZERS

The spectrophotometer from the liquid based reagents biochemistry analyzer is the subassembly, which measures the amount of photons, absorbed by the mixture between the sample and the liquid reagent and it is presented in Fig. 1.

A halogen lamp is used as a light source and the light beam is directed straight in a collimator, which guides it in a prism. The guided beam is split into several component wavelengths and the wavelength selector allows to pass only the specific wavelength for each assay parameter. The incident light (I0) passes across the known length (L) sample cuvette, which contains the mix between the sample and the reagent. Depending of the concentration (c) and the substance's molar absorptivity coefficient (α), a specific amount of photons are absorbed by the mixture and the transmitted light (I1) is measured by the detector [8].

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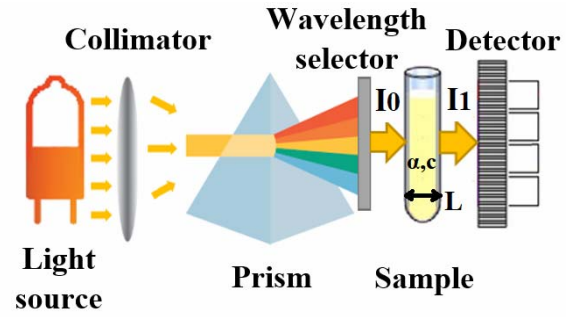


Fig. 1 – The spectrophotometer's basic structure.

The absorbance can be defined as a logarithmic ratio between the incident and the transmitted light [9]:

$$A = -\log(I_1 / I_0). \quad (1)$$

The absorbance can be also expressed using the cuvette's length, the concentration c and the substance's molar absorptivity coefficient α using the Beer-Lambert law:

$$A = \alpha \cdot c \cdot L. \quad (2)$$

To calculate the concentration of the substance, (1) and (2) can be combined resulting:

$$c = [-\log(I_1/I_0)]/(\alpha \cdot L). \quad (3)$$

The disadvantage regarding the laboratory biochemistry equipment consists in the minimum required sample volume, which is about 0.5 milliliters due to the limitation of the depth slide of the sampling robotic arm [10]. This may be an inconvenience when analyzing samples collected from pediatric or neonatal patients due to the difficulty of providing the minimum required blood volume for analysis.

2.2. THE AUTOMATED DRY BIOCHEMISTRY ANALYZERS

The dry biochemistry analyzers are mainly point-of-care instruments, which use color sensitive parameter layers embedded on a test pad. A colorimetric reaction is triggered after the serum or plasma from the blood is added on the test pad after it is incubated at a constant temperature of 37° C. The main advantage is that no distilled water or waste plumbing is needed when samples are analyzed because only the plasma or serum and the test pads are involved in the colorimetric reaction [11]. The assay parameter's concentration is measured using reflectance photometry, where it is quantified the light that is reflected by the colored test pad [12]. The physical structure of a test pad is presented in Fig. 2.

The serum or plasma is pipetted over the test pad and incubated at a constant temperature and at the end of the incubation time, the light source emits a beam over the pad. The reflected light is measured by a photodetector, which translates the amount of reflected light into concentration. The reflectance photometer is presented in Fig. 3.

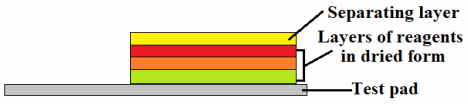


Fig. 2 – The physical structure of a dry chemistry test pad.

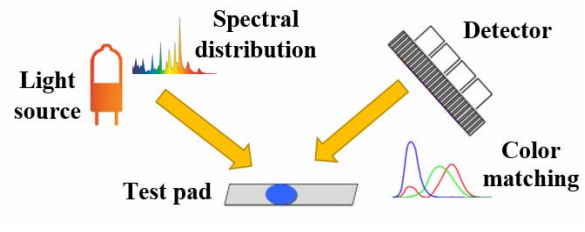


Fig. 3 – The structure of a reflectance photometer.

The reflectance photometer measures the spectral distribution of the reflected light from the test pad and the spectral distribution of the illumination, spectral reflectance and the color matching function are computed in order to obtain the assay parameter concentration values. The spectral distribution and the color matching values are stored in the analyzer's software after the calibration was performed and they are used when measuring the reflectance spectrum in order to obtain XYZ tri-stimulus values, where $S(\lambda)$ represents the illumination spectral distribution value at wavelength λ, $\bar{x}(\lambda), \bar{y}(\lambda), z(\lambda)$ are the color matching function values in the XYZ color system, $R(\lambda)$ is the sample spectral reflectance, $\Delta\lambda$ is the wavelength interval and K is a normalizing factor for the tri-stimulus [13]:

$$K = 100/\sum_{\lambda=380nm}^{780nm}[S(\lambda)\cdot\bar{y}(\lambda)\cdot\lambda], \quad (4)$$

$$X = K\cdot\sum_{\lambda=380nm}^{780nm}[S(\lambda)\cdot\bar{x}(\lambda)\cdot R(\lambda)\cdot\lambda], \quad (5)$$

$$Y = K\cdot\sum_{\lambda=380nm}^{780nm}[S(\lambda)\cdot\bar{y}(\lambda)\cdot R(\lambda)\cdot\lambda], \quad (6)$$

$$Z = K\cdot\sum_{\lambda=380nm}^{780nm}[S(\lambda)\cdot\bar{z}(\lambda)\cdot R(\lambda)\cdot\lambda]. \quad (7)$$

The main advantage of this method of analysis consists in an increased accuracy and precision because of the use of disposable test pads, sample cuvettes and tips after each assay is performed, compared to the liquid based laboratory analyzers, where the reaction cuvettes are washed and dried after each measured blood sample. If the reaction cuvettes are not properly washed and dried, residual contamination from previous samples may occur and this phenomenon is translated into a false absorbance increase regarding the next analyzed sample.

3. THE PORTABLE BIOCHEMISTRY PROTOTYPE BASED ON IMAGE ACQUISITION

The automated portable biochemistry prototype presented in this paper uses a hybrid measurement technology, combining the principles of the basic spectrophotometer and the reflectance photometer. The novelty consists in using an USB mini VGA camera as a detector positioned on the top of the test pad table.

The experiment was performed using dry chemistry tests of triglycerides (TG), inorganic phosphorus (IP), urea

(UREA), glucose (GLU) and cholesterol (CHOL) test pads.

The assays are performed sample by sample using artificial bi-level quality control (QC) serum, Randox Human Assayed Multi-Sera – Level 2 (lot 1199UN) and Level 3 (lot 949UE).

3.1. THE MEASURING PRINCIPLE OF THE DRY BIOCHEMISTRY PROTOTYPE

The dry chemistry portable prototype uses dry chemistry tests which have the reagents embedded in the test pad. After the required amount of serum (6 microliters) is dispensed over the test pad, the test is incubated for 7 minutes in order to trigger the colorimetric reaction. The amount of 6 microliters is a test pad specific volume because a larger sample volume will excessively wet the test pad and a lower volume will not trigger the colorimetric reaction as it should.

In Fig. 4 are presented three glucose tests, where the lower test is new, the middle test was used for glucose calibration with Randox Calibrator 2350 Level 2 with a glucose concentration of 6.35 mmol/L and the upper test was used for calibration using Randox Calibrator 2351 Level 3 with a concentration of 15 mmol/L.

Fig. 4 – The glucose dry chemistry experimental test pads.

The image acquisition method uses a mini VGA USB camera, which is mounted above the test after the incubation, is finished. It captures an overhaul image of the test inside the prototype and by using a Matlab Graphical User Interface (GUI) experimental designed software, the image is processed in order to obtain a concentration value as the result of the assay. In Fig, 5 is shown a captured image of a urea test pad.

The entire process is fully automatic. The prototype can analyze only single test pads. On the market are also available multi-parameter test pads, but in order for the analyzer to be compatible with the multi test panel, the length of the prototype's test table must be increased.

Fig. 5 – Image acquisition for an urea test pad.

The prototype is composed of three motors: one motor is connected to the sliding test table, the second motor is a stepper motor with a linear slider guide for up/down movement and the third motor is used for aspirating the serum from the sample cup and pipetting the sample over the test pad. Under the test table is mounted a flexible resistive film used for the test incubation at a constant temperature of 37°C. The temperature controller circuit consists in a 10 kΩ NTC thermistor installed on the resistive film and connected to the Arudino's analog pin acting as temperature probe and a relay which controls the flexible resistive film's electrical intake [14]. The collected temperature depends of the thermistor's resistive value and expressed using the Steinhart-Hart equation. The entire prototype is presented in Fig. 6.

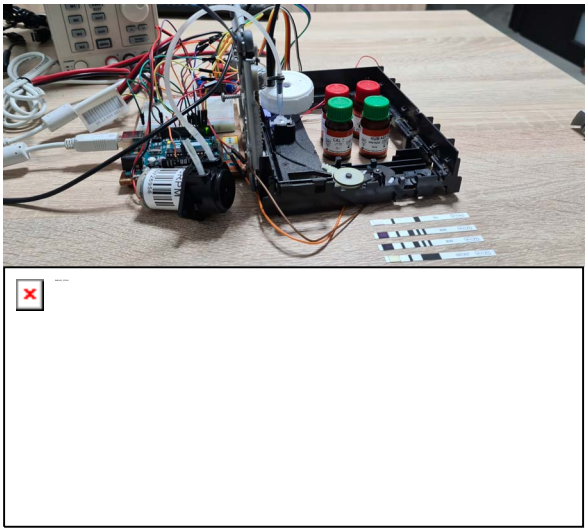


Fig. 6 – The automated dry biochemistry prototype analyzer.

The sample cup containing the centrifuged blood (in order to analyze serum) is positioned in the designated compartment from the test table. The sample tip can be easily exchanged after each sample because it is mounted and ejected manually thanks to a gasket, which encases a metal rod, acting as a tip fixative and ensuring the perfect sealing in order for the external air not to penetrate during the pipetting procedure. When the tip, sample cup and the test are properly placed in their designated compartments, the analyzer is ready to start the measurement. The entire software is simulated in Matlab using GUIs with the help of the Legacy Matlab Support for Arduino [15].

3.2. CALIBRATING THE DRY BIOCHEMISTRY PROTOTYPE

For the system's calibration was used the Randox calibration serum level 2 CAL2350 and calibration serum level 3 CAL2351, which were successively analyzed on the prototype in order to compute the calibration values.

In Fig. 7 is presented the calibration interface after the urea calibration was performed. The first step of the calibration is to enter in the software the urea's target values from both calibrators' insert sheets, followed by the analysis of the CAL2350 level 2 calibrator as a sample. After the calibration serum, the pipetting tip and the test were properly mounted, the START1 button was selected and the prototype started the automatic sampling.

The up/down stepper motor lowers into the sample cup and the sampling motor starts to aspirate from the cup a 6

microliter sample. Then, the up/down motor rises and the table motor positions the test under the pipette tip, which lowers down to pipette the aspirated volume.

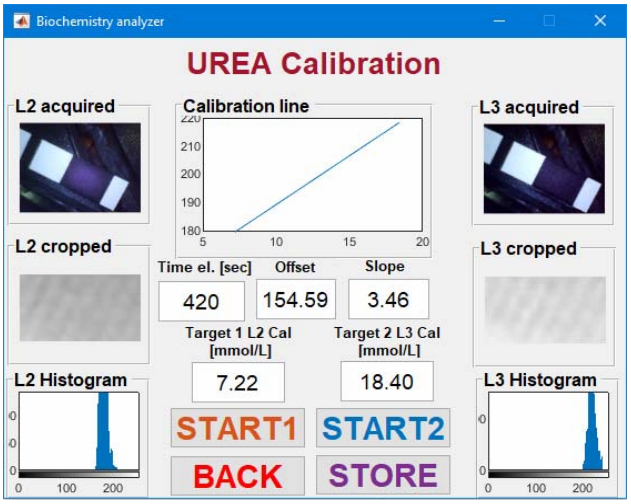


Fig. 7 – The calibration software interface for urea.

After the pipetting procedure is performed by reversing the sampling motor's direction by changing the two pair of bits corresponding to the signal inputs of the L289N dual H-bridge motor driver, the test is positioned under the mini USB VGA camera.

The test is incubated at 37º C for 7 minutes, in order for the reaction between the sample and the reagents contained in the test pad to take place and harmonize. When the incubation time is finished, the camera captures a picture of the test and crops it in a shape of an experimentally determined rectangle of 90 x 50 pixels positioned with the upper left corner in the pixel (283,294), covering the best colorimetric area [16]. The captured image is transformed into a grayscale image, followed by a procedure of a negative bias.

In the liquid reagents based biochemistry instruments, when the reagent is dispensed in the cuvette along with the sample and the mix is incubated, the reaction achieves an endpoint after a specific assay period of time.

The endpoint is translated into an absorbance increase, meaning that the sample mix will absorb the light in a direct proportional manner with the concentration of the tested parameter. For each analyzed parameter there is a specific reagent. The reaction curve is presented in Fig. 8 [17].

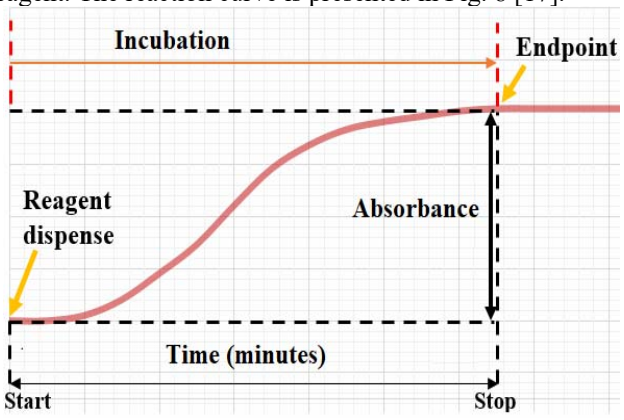


Fig. 8 – The reaction curve for an one-point end spectrophotometric assay.

This negative bias was mandatory because in the case of the dry tests, as higher the samples concentration is, the test pad darkens, meaning that for high concentrations, a high contrast must be obtained, similar to the case of the spectrophotometric assays in the liquid reagents based biochemistry. The mean of all pixels from the cropped image is calculated and memorized.

The test, the cuvette and the pipette tip are exchanged for analyzing the CAL2351 level 3 calibrator. The START2 button is selected in order to analyze the test for the second calibrator. The analysis process is similar to the previous one until the mean of all pixels in the case of the second cropped image is calculated.

Having the results for the mean (m1) of all pixels from the first captured cropped picture and the mean (m2) of all pixels from the second cropped picture and knowing the target values from the calibrators' insert sheets (t1 and t2), the calibration slope can be calculated:

$$\text{slope} = \frac{\text{m2} - \text{m1}}{\text{t2} - \text{t1}}. \tag{8}$$

As can be observed from Fig. 7, the calibration line does not start from the origin, meaning that there are also other compensation elements involved in the calibration process. The intercept must be calculated from the calibration line's equation:

$$\text{offset} = \text{m1} - \text{slope} \cdot \text{t1}. \tag{9}$$

Considering that mean represents the mean of the pixels from the sampled image during the assay process, concentration is the concentration of the substance, slope and offset, the calibration line's slope and offset, the general formula for the calibration line for all the parameters involved in the experiment is:

$$\text{mean} = \text{slope} \cdot \text{concentration} + \text{offset} \tag{10}$$

3.3. THE ASSAY ANALYSIS ON THE BIOCHEMISTRY PROTOTYPE

The sample assay process is similar to the calibration process. Instead of analyzing two succesive samples, in the case of the sample analysis process there is captured only one picture. After the aspiration, pipetation, incubation and acquisition, cropping, grayscaling, negativing and calculating its mean process the image. In Fig. 9 is presented the sample analysis software's interface.

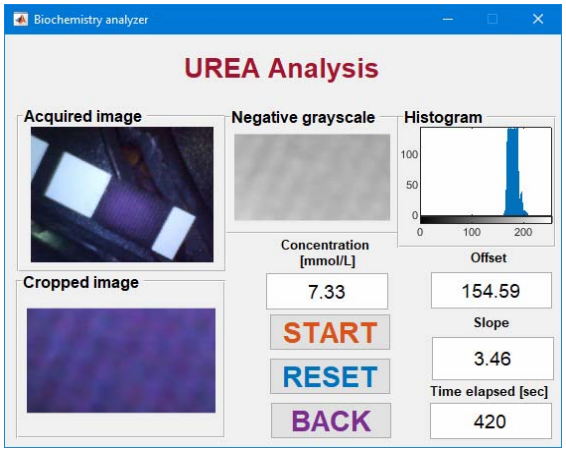


Fig. 9 – The urea's sample analysis software interface.

The slope and offset values, obtained after the

calibration, are entered in the specific fields from the analysis software. After the test, the pipette tip and the sample cup containing the serum are placed into their positions, by selecting START, the automatic analysis process is triggered. The entire process ends with the calculations of the mean of all pixels for the current captured image and the assay parameter's concentration:

$$\text{concentration} = \frac{\text{mean - offset}}{\text{slope}}. \quad (11)$$

The calculated concentration is displayed in the specific field from the software interface. In the example from Fig. 9 the mean of the total pixels is 179.95, as can be observed also from the histogram. The concentration is calculated with (11) and is obtained the value of approximately 7.33 mmol/L.

4. EXPERIMENTAL RESULTS

The analysis process in the case of a patient sample consists in preparing the blood by centrifuging it at 2000 rpm for mainly 2-3 minutes. All the biochemistry analyzers use serum or plasma for the routine biochemical profile analysis.

Testing real patient samples in this experiment means asking for their consent for blood colletion and for using their blood samples in scientific purpose. Due to the pandemic regulation that were involved when the experiment was performed, there were used only bi-level Randox Human Assayed Multi-Sera artificial quality control (QC) samples. Every sample was analyzed three times in order to establish the standard deviation and the coeficient of variation. The obtained results were also compared with the results obtained on a liquid based biochemistry analyzer, the Biobase BK-200, from Fig. 10.

Fig. 10 – The Biobase BK-200's reagent and sample turntable.

The coefficient of variation (CV) and the standard deviation (SD) were calculated using the mean of the three measured values ($\bar{x}$):

$$\bar{x} = \sum_{i=1}^{3} \frac{x_i}{3}, \quad (12)$$

$$SD = \sqrt{\frac{\sum_{i=1}^{3} \left(x_i - \bar{x}\right)^2}{2}}, \quad (13)$$

$$CV = \frac{SD}{\bar{x}} \cdot 100. \quad (14)$$

The experimental results are contained in Table 1 for Randox QC Level 2 and in Table 2 for the QC Level 3. As a performance evaluation based on scientific established rules of the biochemistry prototype analyzer, the obtained QC results must be situated in the ±2SD interval [18]. In Fig. 11 and Fig. 12 are plotted the coefficients of variation for each assay parameter performed on the prototype chemistry analyzer and on the liquid reagents based biochemistry analyzer, for each of the two levels of QC.

Table 1

The experimental results in the case of QC Level 2 analysis

Parameter	Instrument	*1*	*2*	*3*	*2SD*	*2CV* [%]
Urea [mmol/L]	Insert value	7.48	7.48	7.48	0.22	2.9
	Prototype	7.33	7.18	7.51	0.17	2.2
	Biobase	7.38	7.50	7.36	0.08	1.0
Glucose [mmol/L]	Insert value	6.11	6.11	6.11	0.92	15.1
	Prototype	5.99	6.27	6.18	0.14	2.3
	Biobase	6.05	6.29	6.25	0.13	2.1
Inorganic Phosphorus [mmol/L]	Insert value	1.29	1.29	1.29	0.20	15.5
	Prototype	1.18	1.25	1.36	0.09	6.7
	Biobase	1.25	1.36	1.18	0.09	7.7
Cholesterol [mmol/L]	Insert value	4.14	4.14	4.14	0.54	13
	Prototype	4.31	4.28	4.07	0.13	3.2
	Biobase	4.10	4.22	4.18	0.06	1.5
Triglycerides [mmol/L]	Insert value	1.03	1.03	1.03	0.16	15.5
	Prototype	0.98	1.12	1.17	0.10	8.4
	Biobase	1.17	1.08	1.12	0.05	4.0

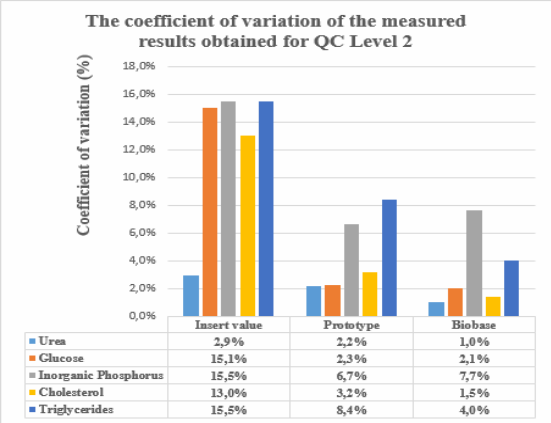
Table 2

The experimental results in the case of QC Level 3 analysis

Parameter	Instrument	*1*	*2*	*3*	*2SD*	*2CV* [%]
Urea [mmol/L]	Insert value	19.9	19.9	19.9	3.00	15.1
	Prototype	19.45	18.90	19.67	0.40	2.0
	Biobase	19.40	19.12	19.32	0.14	0.7
Glucose [mmol/L]	Insert value	15.6	15.6	15.6	2.30	14.7
	Prototype	14.65	15.42	16.10	0.73	4.5
	Biobase	16.10	16.80	15.90	0.47	3.0
Inorganic Phosphorus [mmol/L]	Insert value	2.12	2.12	2.12	0.32	15.1
	Prototype	2.24	2.36	2.47	0.12	4.7
	Biobase	2.28	2.36	2.45	0.09	3.5
Cholesterol [mmol/L]	Insert value	7.41	7.41	7.41	0.98	13.2
	Prototype	7.35	7.38	7.25	0.07	0.9
	Biobase	7.48	7.60	7.52	0.06	0.8
Triglycerides [mmol/L]	Insert value	2.91	2.91	2.91	0.48	16.5
	Prototype	2.78	2.95	2.85	0.09	3.0
	Biobase	2.95	2.87	2.78	0.09	3.1

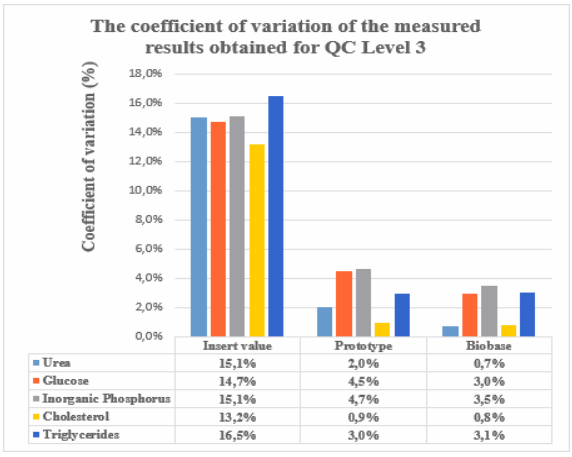
From the results obtained after this experiment, it can be stated that the prototype dry automated biochemistry analyzer has a good performance with a CV in most of the cases under the manufacturer's assigned CV from the insert sheet regarding each level of QC. If the results obtained on the prototype analyzer are comparable to the manufacturer's

assigned values and also to the obtained results of the measurements performed on a liquid reagent based commercial biochemistry analyzer, no additional clinical validation is required. This fact is empowered by framing the measured results between the ±2SD from the QC's insert sheet, according to the laboratory Westgard rules [19].



	Insert value	Prototype	Biobase
Urea	2,9%	2,2%	1,0%
Glucose	15,1%	2,3%	2,1%
Inorganic Phosphorus	15,5%	6,7%	7,7%
Cholesterol	13,0%	3,2%	1,5%
Triglycerides	15,5%	8,4%	4,0%

Fig. 11 – The coefficient of variation of the measured results for the second level of QC.



	Insert value	Prototype	Biobase
Urea	15,1%	2,0%	0,7%
Glucose	14,7%	4,5%	3,0%
Inorganic Phosphorus	15,1%	4,7%	3,5%
Cholesterol	13,2%	0,9%	0,8%
Triglycerides	16,5%	3,0%	3,1%

Fig. 12 – The coefficient of variation of the measured results for the third level of QC.

5. CONCLUSIONS

This paper presents a portable and automated biochemistry prototype analyzer with the measurement technology based on an image acquisition and processing algorithm, accompanied by a hardware setup. The presented technology is low cost compared to the existing analyzers in the market because are needed only a few hardware components for the automation and only a mini VGA USB camera for the measurement subassembly, with no additional filters at assay specific wavelengths.

The main advantage consists in using only 6 microliters 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.

Another advantage consists in the wide measurement range because there are no hardware limitations when using the USB camera, providing the possibility to measure high concentration pathological samples without any manual dilution. The test pad is placed under the USB camera in an enclosed space and illuminated by the camera's led in order to assure that no external light will affect the image acquisition's performance during the measurement.

The performance of the instrument is situated in the Westgard imposed range and its accuracy and precision are comparable to the ones of the Biobase BK-200 liquid based automated biochemistry analyzer.

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