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Biochemical semi-automated acoustics by Dipstick image processing based on arduino

Acústicas bioquímicas semi-automatizadas por processamento de imagem Dipstick com base em arduino

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ABSTRACT
Although many devices are available to read urinalysis reactive strips, potential failure, based on human interpretation, persists in routine tasks. Current study develops and evaluates the performance of an Arduino-based device for the semi-automated reading of reactive strip parameters. The glucose parameter of a commercial reactive strip model was analyzed by the system, which predicts analyte concentration by submitting the color observed in the strip to a regression model, adjusted to a database of color patterns. The system was assessed by reading of 80 strips with 16 samples of random glucose concentrations. The lowest coefficient of variation after five replicated readings was 4.5% and the highest was 16.6% (MSE=68.7 mg/dL; r=0.979). The device featured satisfactory results plus low costs. To make it useful in the laboratory routine, further experiments with other parameters and other classes of urinalysis reactive strips would be necessary.

Keywords: Automation. Arduino. Urinalysis.

RESUMO
Apesar da ampla disponibilidade de dispositivos para leitura de tiras reativas para análise de urina, falhas potenciais persistem na rotina baseada na interpretação humana. O objetivo deste estudo foi desenvolver e avaliar o desempenho de um dispositivo baseado em Arduino para a leitura semi-automática de parâmetros de fitas reativas. O parâmetro glicose de um modelo de tira reativa comercial foi analisado pelo sistema, que prevê a concentração do analito submetendo a cor observada na tira a um modelo de regressão, ajustado a um banco de dados de padrões de cores. O sistema foi avaliado através da leitura de 80 tiras com 16 amostras de concentrações aleatórias de glicose. O menor coeficiente de variação após cinco leituras replicadas foi de 4,5% e o mais alto foi de 16,6% (MSE = 68,7 mg / dL; r = 0,979). O dispositivo apresentou resultados satisfatórios mais baixos custos. Para torná-lo útil na rotina laboratorial, seriam necessárias novas experiências com outros parâmetros e outras classes de tiras reativas para análise de urina.

Palavras-chave: Automação. Arduino. Urianálise.

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INTRODUCTION

Urine is an interesting biological fluid in clinical practice. The acquisition of a urine sample is a simple and non-invasive procedure. Through modern laboratory techniques, it reveals essential information in the screening and monitoring of metabolic disorders, liver alterations, kidney and urinary tract diseases, and other systemic conditions[1–10]. Owing to these characteristics, urinalysis, covering physical, biochemical, cellular and sedimentary aspects, is well established as a routine test[4–8,10,11].

The biochemical features of urinalysis comprise the identification and quantification of a relatively large set of different substances. For its implementation in a practical and swift way, the essential characteristics of screening tests require the use of urinalysis reagent strips (URSs)[3,7,12]. A test using URSs provides in approximately two minutes an assessment of the sample´s glucose, ketone bodies, proteins, hemoglobin, nitrites, bilirubin, urobilinogen, the presence of leukocytes, pH and density[4,6].

A URS consists of a plastic rod to which small spaced pads, impregnated with colorimetric reagents, are attached. Each pad determines one of the test parameters, reacting specifically with its target analyte by a brief contact with the sample. The presence or concentration of the analytes is expressed by a change in color and variation in intensity. However, the transformation of the colors into values or classes is mandatory for results. This is achieved by comparing them with a series of references for the best combination[5,6,10]. Since the perception of colors by biological organisms is a phenomenon that is inherently subjective, inaccurate and potentially flawed, an automated interpretation is preferable to a human one[1,7,10,13].

Despite the great number of devices capable of interpreting URSs, analytical routine implementations based on non-automated methods still persist[5,8,10]. This fact, coupled to the growing popularization of computer technologies[14], has probably contributed to a relatively recent interest in the dissemination and diversification of methods for automated reading of URS. Studies using everyday hardware, such as mobile phones and personal computers with cameras, have addressed the problem in an alternative way and reported promising results[15–17].

Arduino is a hardware/software platform that fits this profile. In fact, it is inexpensive; it has become rapidly available and successfully employed in a wide variety of automation projects. Arduino is a type of miniaturized computer in which the entire system focuses solely on the process one wants to monitor (embedded system), based on Atmel microcontrollers. Its architecture is modularized (shields) and open source, with network shields, Bluetooth, global positioning satellite, infrared, cameras, actuators, relays, pressure, temperature, humidity, acceleration sensors and others, with great versatility to the platform[14,18].

Arduino-based devices may be implemented in independent and exclusive housings designed to take into account the requirements of each application. This fact minimizes interferences, such as variations in type and intensity of lighting, positioning, and distance of the URS from the light source and color sensor, and the time and settings to capture the image. This characteristic provides an Arduino-based project at an advantage over other approaches[18,19].

Current analysis develops and evaluates the performance of an Arduino-based product for the interpretation of URSs parameters. The system was employed for the reading of glucose by a commercial URS model.

METHODOLOGY

The following steps were employed in the experiment. A device that registers the color of reactive strips was built in a controlled environment. The device, composed of 40 strips, was used to observe color change in a stratified sampling, and exposed to eight samples with spaced concentrations (standard samples). The correlation between the colors observed and the concentration in the samples was determined and a mathematical regression model was built. The model was used to predict the concentration in a new

stratified sampling, composed of 80 reactive strips, exposed to 16 samples with random concentrations (test samples).

SAMPLE PREPARATION

All samples were produced artificially by diluting glucose in a physiological saline solution. No biological material was used. After preparation, the solutions were immediately read by an automated analyzer, model Labmax Plenno (Labtest Diagnóstica S.A.) to determine and/or certificate analyte concentration.

The concentrations of the standard samples were based on the rates of the reference tables of two brands of reactive strips. After analysis in Labmax, the concentrations were 0, 20, 51, 105, 158, 262, 518 and 1036 mg/dL. The concentrations of test samples were determined at random, between the minimum and maximum rate detectable by the reactive strip used in the experiment, taking into account low, medium and high concentrations. The concentrations of test samples were 0, 38, 92, 159, 219, 300, 338, 418, 536, 588, 666, 678, 730, 808, 898 and 1022 mg/dL.

PREPARATION OF REACTIVE STRIPS

The URICOLOR check reactive strip model (Wama Diagnóstica) was used in the experiment due to its accessibility and price. Each strip was handled individually, following the manufacturer's guidelines. Color formation in the pads was stimulated by contact with the samples for approximately 3 seconds. After contact, the strips were kept horizontally, supported on an absorbent paper, for another 20 seconds. They were then immediately inserted onto a scanner tray. Each image was captured automatically by the device 33 seconds after contact with the sample because of its internal timer.

ASSEMBLY AND MOUNTING OF HARDWARE

The housing of the system was designed with SolidWorks software (Figure 1A). Whilst plastic parts were made by additive manufacturing (3D printing), the metallic ones were acquired from hardware stores. Electrical, electronic and mechanical components were acquired from shops specialized in embedded systems.

The housing´s upper region housed the central hardware, including the Arduino Mega 2560 (Figure 1B), whilst its lower region sheltered the peripheral devices, including reading head and actuators (Figures 1C and 1D). A retractable tray received and secured up to five reactive strips in pre-determined positions (Figures 1A and 1B).

The reading head is programmed to move laterally (x-axis) to select the strip (Figure 1F) and longitudinally (y-axis) to centralize the target pad (Figure 1C). Each shaft is operated independently by driver module 4988 (Figure 1E) and stepper motor. A camera module OV7670-AL422b 0.3 megapixel captured the images (Figure 1F) and four LEDs of high brightness, 30 mA white light, connected in parallel to a 3.3 V voltage source, were used for lighting (Figure 1D).

During image capture, the camera remained motionless at the center and above the target pad. Distance between the pad surface and the camera lens and the illumination was 5 and 15 mm, respectively.

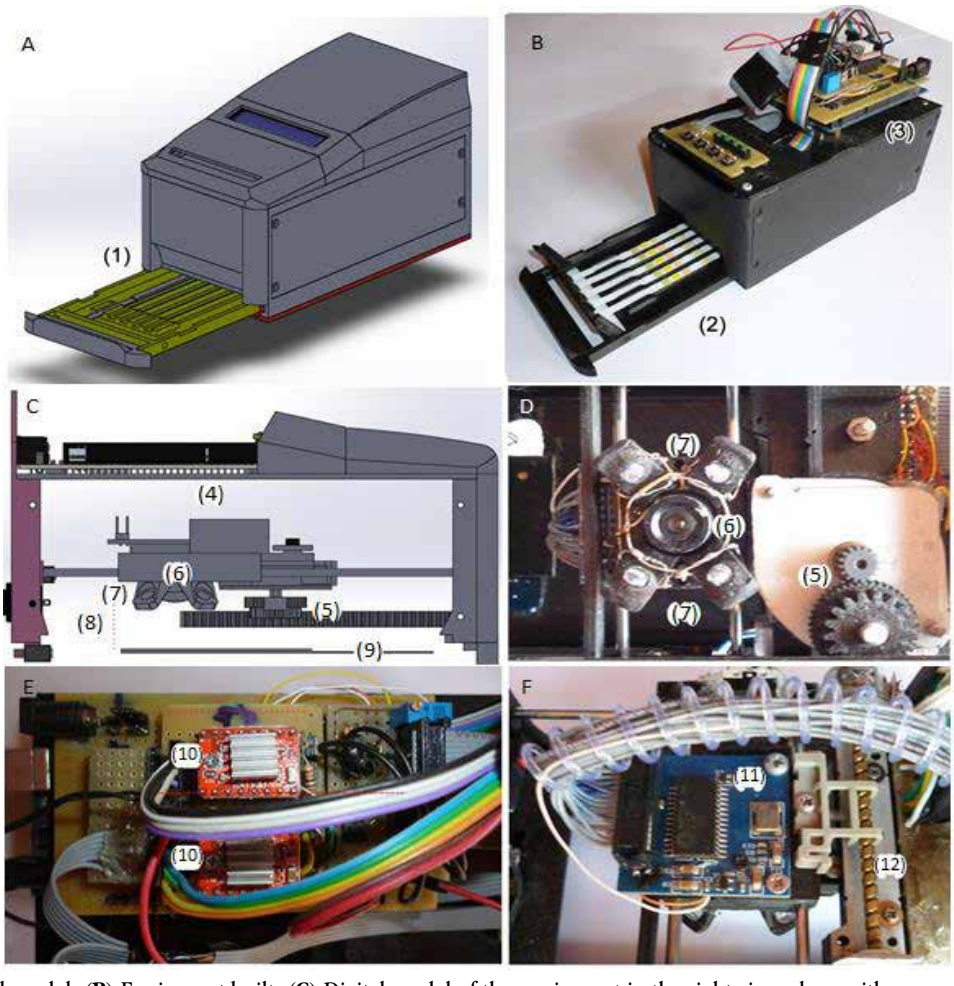

Figure 1. (**A**) Digital model; (**B**) Equipment built; (**C**) Digital model of the equipment in the right view plane with open side covers; (**D**) Actual image, lower level with tray and lower lid removed; (**E**) Top view of central hardware; (**F**) Top view of the reading head. (**1**) Extended tray; (**2**) Strips positioned in the tray; (**3**) Central Hardware exposed; (**4**) Reading head; (**5**) Gears, rack, and motor for movement on y-axis; (**6**) Camera lens; (**7**) LEDs; (**8**) Bay between the strip and the camera lens; (**9**) Reactive strip; (**10**) Driver A4988; (**11**) Module OV-7670-AL422b; (**12**) Actuator of the x-axis.

DETERMINATION OF COLOR FORMED ON THE PAD

The glucose parameter pad, as the region of interest, was employed in each captured image, while the other areas were discarded. Thereafter, a single color in RGB format (default OV7670-AL422b sensor) was obtained from this region by calculating the arithmetic average of the colors in each pixel. The RGB format is composed of three independent color channels: red, green and blue, represented by the letters R, G, and B, respectively. Each color in this format is produced/represented by a specific combination of intensities in these channels, ranging between 0 and 100% (0 to 255) (Figure 2a).

CONSTRUCTION OF THE MODEL FOR QUANTITATIVE PREDICTION

Data collected in the R, G, and B color channels were tabulated together with the rates of their corresponding concentrations and processed with Excel 2013, IBM SPSS 24, and MATLAB R2017a. The relationship between each channel and the concentration was determined by a bivariate analysis, using Pearson's correlation coefficient. The channels with the best coefficients were used for the construction of multiple linear regression and nonlinear regression models. The suitability of each model was assessed by means of the coefficient of determination (R^2). The best adjusted equations were used for the construction of the final mathematical model, employed to re-

cover the rate (quantitative) of the concentration for each sample test, by entering the data of RGB colors.

PRODUCTION OF QUALITATIVE RESULTS (CLASSIFICATION)

Quantitative results (continuous quantitative variable) measured by both systems (experimental and Labmax Plenno) were converted into qualitative (ordinal qualitative variable) by classifying them at six levels: 0 ([0, 20[), 20 ([20, 100[), 100 ([100, 250[), 250 ([250, 500[) , 500 ([500, 1000[) and 1000 (rate $>=$ 1000) mg/dL. These classes were defined according to classification established by the manufacturer of the reactive strip, while class 20 was added for possible comparisons with other reactive strip models.

EVALUATION OF PERFORMANCE

The concordance between the quantitative results produced by the experimental system and Labmax Plenno was determined by Pearson's correlation coefficient. The concordance between the results, converted into qualitative data, was determined by Spearman's correlation coefficient and by the percentage of equivalent classifications, strictly equal to and/or with a tolerance of one level of difference.

The experimental system was internally evaluated by coefficient of variation (CV) for each sample analyzed, in five replicates, and by general mean square error (MSE). The Excel program was used for plotting and analyzing the data.

RESULTS

VISUAL INSPECTION OF THE REACTIVE STRIPS AND ASPECT OF THE IMAGES

During the positioning of the strips on the equipment (properly prepared), several strips revealed sharp differences in the shades of color for the same sample. Such behavior, more evident in strips exposed to higher concentrations, was confirmed by the captured images (Figures 2b and 2c).

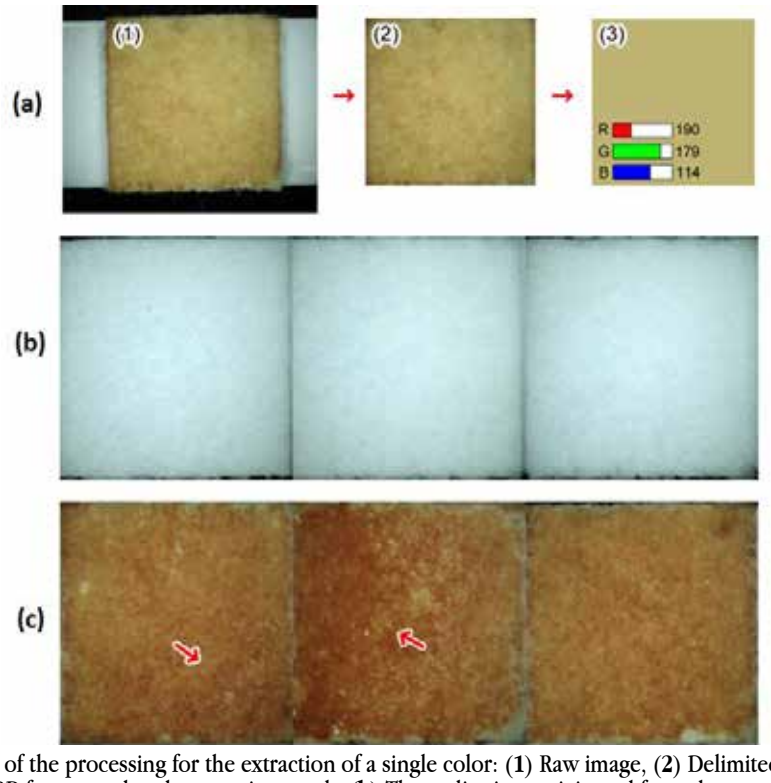

Figure 2. (**a**) Representation of the processing for the extraction of a single color: (**1**) Raw image, (**2**) Delimited and clipped region of interest, (**3**) Average color in RGB format and end processing result; (**b**) Three clippings originated from the sample with 0 mg/dL, showing homogeneous coloration internally and between peers; (**c**) Three clippings originated from the sample with 730 mg/dL, presenting peripheral regions or hemispheres in darker hues (red arrows).

CORRELATIONS, REGRESSIONS AND MODEL CONSTRUCTION

Although the bivariate analysis between R, G, and B channels and the concentration of the standard samples (N = 40) pointed to very clear relations, the analysis of the dispersion graphs revealed nonlinear distribution patterns for all channels, with better indexes for R and G. Hence, the final model was constructed by considering a combination of curves at the expense of multiple linear regressions, which are second-degree polynomial regressions for R channel and logarithmic for G channel (Figures 3a-d). The data of channel B were not taken into account (Figures 3e and 3f).

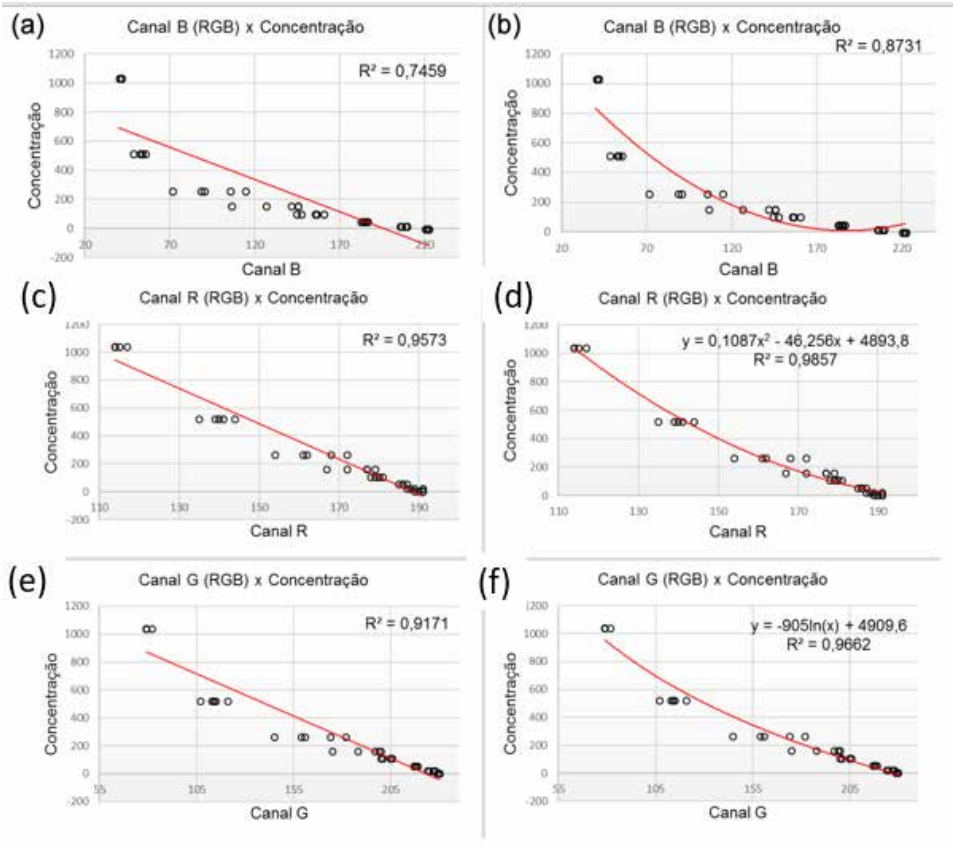

Figure 3. Scatter plots between channel B of the RGB core space and the concentration:(**a**) Linear adjustment of the R channel, (**b**) Polynomial adjustment of the R channel, (**c**) Linear adjustment of the G channel, (**d**) Logarithmic adjustment of the G channel; (**e**) Linear adjustment, (**f**) Polynomial adjustment.

SYSTEM TEST

The quantitative prediction of concentrations in the test samples (n=80) yielded a minimum CV of 4.5%, a maximum CV of 16.6% (Table 1) and an MSE of 68.7 mg/dL. Figure 4a shows concentration-dependent MSE variation. Pearson's correlation coefficient (r) between these results and those produced by the Labmax Plenno analyzer was 0.979 ($p<0.0001$), with a coefficient of determination (R^2) of 0.959 (Figure 4b).

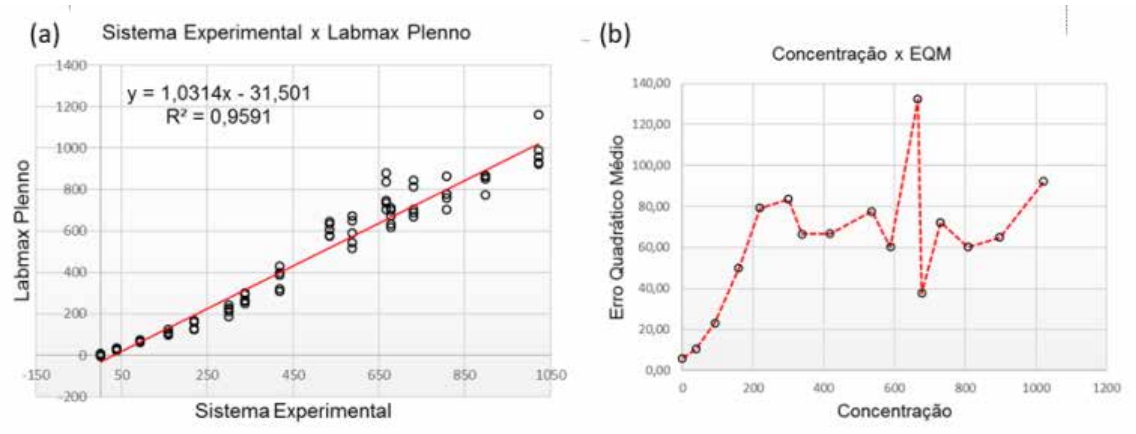

Figure 4. (**a**) Graph showing the variation of the mean square error with concentration; (**b**) Dispersion graph of the quantitative results of the experimental system and those obtained by the Labmax Plenno analyzer.

Table 1. Coefficients of variation, in percentage, by concentration ranges

		Concentration ranges (mg/dL)		
		Low	**Average**	**High**
		(0 to 332)	**(333 to 666)**	**(667 to 1050)**
CV (%)	Lowest	6.0	5.4	4.5
	Average	11.2	9.6	7.7
	Highest	16.6	13.8	10.7

Concordance of qualitative results between the systems was 86%, or rather, 69 classifications were concordant and 11 were divergent. No classification displayed a distance beyond one level. In other words, when not identical, the classifications were immediately adjacent (Table 2). Therefore, concordance reached 100% when the criterion of tolerance of one level of difference was applied. Spearman's correlation coefficient (rho) between the two systems was 0.971 ($p<0.001$).

Table 2. Cross tabulation of quantitative results between systems (in mg/dL)

Experiment	Labtest Labmax Plenno						
	0	20	100	250	500	1000	**Total**
0	**5**	0	0	0	0	0	**5**
20	0	**10**	0	0	0	0	**10**
100	0	1	**9**	0	0	0	**10**
250	0	0	6	**9**	0	0	**15**
500	0	0	0	0	**35**	0	**35**
1000	0	0	0	0	4	**1**	**5**
Total	**5**	**11**	**15**	**9**	**39**	**1**	**80**

DISCUSSION

Results in current analysis demonstrated that an Arduino-based system is sufficiently sensitive to detect and distinguish color variations in the reactive strips. The predictions generated by the implemented model correlate satisfactorily with the results produced by the reference method ($r=0.979$ and $rho=0.971$) and with statistical significance ($p<0.0001$ and $p<0.001$)[20]. Further, correlation indexes were consistent with those obtained from well-established commercial systems.

Although current study assessed non-biological samples, results of URSs evaluation by the Arduino system are not insignificant, because several studies have been published in which the performance of commercial equipment was evaluated with the use of totally artificial or artificially adjusted solutions[4,21].

When quantitative results for glucose dosage by URSs, produced by commercial analyzers Uriscan Optima, Uriscan Pro II and Uriscan Super Plus, are compared to the Siemens Advia 1800 quantitative analyzer, they yielded Pearson's correlation coefficients of $r=0.733$, 0.807 and 0.712 ($p<0.001$), respectively[7].

In the qualitative mode, studies, featuring classifications that differed by up to one level of difference (more or less), are accepted as concordant. Although a complete agreement has not been reported in any of these publications[7,21,22], the application of this criterion to experiments performed in current study generated a 100% concordance level.

Uriscan devices, mentioned earlier, yielded concordances of 96.8, 98.2, and 94.7% when compared to equivalent devices from Roche[7]. Another study compared the results of the devices from Bayer Clinitek Atlas, Roche Urisys 2400, and Arkray Aution Max, and obtained

concordances of 98.3% (Atlas against Aution Max), 97.1% (Atlas against Urisys) and 97.0% (Aution against Urisys 2400)[22].

The potential superiority of the indices obtained herein, compared to other publications, should not be interpreted as evidence of better performance, but rather that the concept is valid and satisfactory. Taking into account the validity of the samples used in the experiment, real urine samples may display intense color variations owing to the presence of abnormal substances[4]. Therefore, applications in laboratory routine depend on maintaining satisfactory results even in adverse conditions. It is reasonable to assume that the commercial devices, mentioned above, also underwent rigorous evaluations.

Moreover, one must consider the fact that the quality of results in these systems is intrinsically dependent on the quality of the reactive strip used and the attention employed in handling. If color formation in the reactive strip is unstable and/or irreproducible, results by any system are compromised.

No study evaluated the URICOLOR check reactive strip using the manufacturer's apparatus. This information would be useful to determine the input responsible for the variance in the results and to establish comparisons under more similar conditions.

As shown in Figure 4a, there is a declining trend in MSE when concentration is close to 0 mg/dL and stability reaches above 200 mg/dL. This behavior may be a consequence of URS quality. It may evidence a supposed increase of instability/variation in color formation as the amplitude between the original and final color increases. The above interpretation complies with visual observation during the handling of reactive strips in which very sharp hue variations occurred for the same sample at the highest concentrations (Figure 2c).

The quantitative approach in current study does not objectively suggest its use in a laboratory routine but reports detailed information about the system's capacity, mainly to enable the assessment of other parameters of URSs or for other classes of reactive strips. To make it useful in the laboratory routine, further experiments with other parameters and other classes of urinalysis reactive strips would be necessary.

Results show that an Arduino-based system was able to read satisfactorily one of the parameters of the urinalysis reactive strip. Results also point to the benefits that may be achieved by including this versatile resource within the arsenal of biomedical research and other invaluable integrations. It would be necessary, however, to reverse the predominant idea that such resources are exclusive to technology professionals.

CONCLUSION

The system was built and operated in accordance with the expected performance and produced results compatible with commercial systems. Hardware sensitivity is sufficient for this type of application. The acquisition of hardware, software and support libraries was simple, practical and low cost. Full achievement of the goals set forth herein reaffirms the value of this remarkable platform.

However, results recommend further studies, either by extending the method to other parameters of the strip, or by evaluating the system in real urine samples, or by evaluating the applicability to other classes of reactive strips.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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