POSSIBILITIES OF A QUICK ORIENTATIVE VERIFICATION OF THE ACCURACY OF THE ABSORBANCE MEASUREMENT RESULTS ON ROUTINE DIAGNOSTIC INSTRUMENTS

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Abstract
Laboratory diagnostics must use equipment that allows to obtain results with acceptable reliability in a short time and for more samples. The laboratory should at least ascertain the accuracy of the instrument with respect to samples of different composition. In our work we evaluated the comparison of the accuracy of absorbance determination on two instruments. We found that with repeated determination of absorbance (n = 11) at 590 nm and 600 nm for the lowest sample dilution coefficient of variation, VK = 43.41 for analyser A and VK = 113.8 for analyser B whereby B did not reach statistical significance of the difference from the blank. Our results suggest that validating these parameters is an important prerequisite for proper laboratory work.

Key words: Laboratory diagnostics. Accuracy. Absorbance.

1 Introduction

Routine laboratory diagnostic methods in the healthcare field are primarily designed to examine larger numbers of samples over a given time period. Therefore, they are always a compromise between price and quality. Definitive methods cannot be used in routine diagnostics as they are time consuming, instrumental and costly expensive. They are therefore used in quality management processes in routine laboratory diagnostics such as the definition of control and calibration materials and external quality management [1]. Routine diagnostic methods must be affordable and, at the same time, capable of providing the result of the examination as quickly as possible for the decision-making algorithms of diagnostic-therapeutic processes [2]. From this information, it appears that while routine diagnostic methods do not show absolute accuracy, they should be at a level that provides reliable results of the parameter being investigated. In contrast to laboratory analysis of inorganic materials, intraindividual and interindividual variability is increasing in the case of human diagnostics, which is several tens of percent for some parameters, expressed by variation coefficient.

2 Instrument accuracy verifying

Nowadays, in clinical-diagnostic practice, it is customary to accept manufacturer´s data regarding the accuracy, traceability and robustness of the methods used [3]. Often, a clinical laboratory is unable to test the parameters due to their high time and cost intensity. The reason is the fact, that the laboratory does not investigate one parameter, but several hundred different parameters. In addition, laboratory must take into account the different reference biological limits that may be given by the age of the individuals, their gender or even ethnic specificity [4]. Therefore, defining the characteristics of the diagnostic is the task of manufacturer.

Nevertheless, the laboratory worker may, to some extent, carry out an orientative examination to give him a basic overview of the possibilities of the instruments as well as of the weaknesses of the diagnostic methods used. The worker knows that he must take into account the Lambert-Beer law and to define the area of linearity between the measured signal and the amount of tested analyte. However, in many cases, especially in microbiology, this is a qualitative assay, requiring only a single-signal indication (e.g., absorbance of 580-600 nm) to be obtained for substitution into the formula without the need for a calibration curve. The hidden risk of such cases lies in the variability of the laboratory equipment that can significantly affect the results. The mentioned risk is not only related to linearity, but mainly to the so-called “gray zone” risk given by the variance of the average value of measurements. If the result of the laboratory examination is located in such a zone, its value in relation to the diagnosis is actually equal to zero. Therefore in these cases, it is appropriate for the laboratory to perform an orientative verification of the instrument parameters [5-7].

3 Aim

In our work, we focused on simple orientative verifying the accuracy of routine analyzers with an emphasis on taking into account their material-methodological variability and the implications arising from it.

4 Matherial and methods

A deidentified sample of two identical 15 ml aliquots was provided to our workplace by a partner microbiological workplace that cultived microorganisms with their subsequent staining according to the valid standard operational procedures. We have prepared a series of nine sample tubes diluted in a ratio: 6/0; 5/1; 4/2; 3/3; 2/4; 1/5; 5.5/0.5; 5.9/0.1 and finally 0/6 (clean diluent, blank; Fig. 1). The total volume was 6 ml, diluted with saline buffer. On this line of tubes, measurements were made on device A, for each sample with 11 repetitions at a wavelength of 590 nm. Subsequently, 150 µl was transferred from the tubes to the microplate, with n = 11 repetitions for each dilution and n = 8 for the blank. The absorbance values of the solutions in the microtiter plate were determined on the device B at a wavelength of 600 nm (Fig. 2). Subsequently we processed the basic statistical characteristic of the data determined by the arithmetic mean, standard deviation, median, minimum, maximum and coefficient of variation.

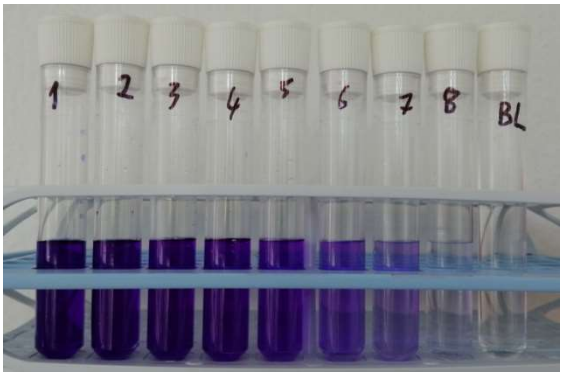


Fig. 1 *Sample preparation – device A*
Legend: Tubes 1-8 with decreasing solution ratio: samples 6/0; 5/1; 4/2; 3/3; 2/4; 1/5; 5.5/0.5; 5.9/0.1, BL- blank.

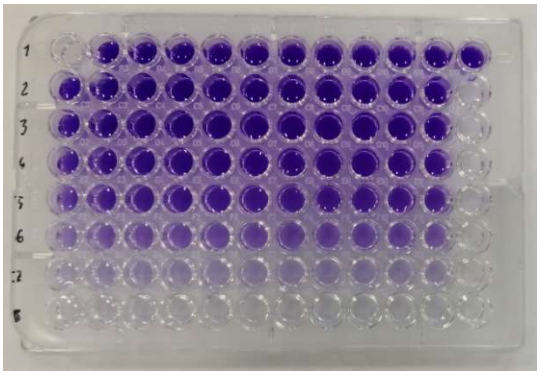


Fig. 2 *Sample preparation – device B*
Legend: Diluted sample 1-8 are loaded in the microtiter plate rows. Blank is in the first row in position 1, in other rows in 12th position.

5 Results and discussion

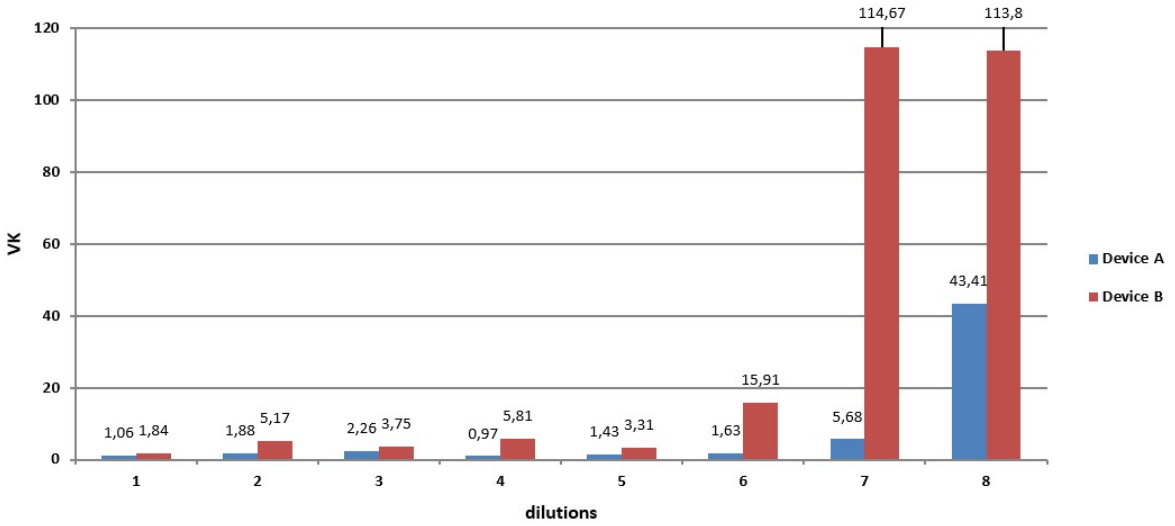
The basic statistical parameters of the data obtained by sample dilution on devices A and B are shown in Table 1. The results show that the accuracy of both instruments is the highest in the case of high values of concentration or absorbance respectively. It can be seen that in the first 5 dilutions the coefficient of variation did not reach a value of 10. In other words, the numerical value of the standard deviation once was not more than 10% of the arithmetic mean, the highest value being found on the device B for the sample no. 4 (VK = 5.81). However, with the decrease of the optical density of the dye due to the dilution increases in samples no. 6 - 8 the coefficient of variation, which increased considerably in the case of the lowest dilution on the device A to the value VK = 43.41 and on device B even VK = 113.8. The VK values are presented in Graph1. Given the sensitivity of the instruments, we verified the statistical significance of the statistical indicators of the middle of the last two dilutions No. 7 and No.8 relative to the blank values. We found that in case of dilution no. 8 analyzed on the device B, neither median nor arithmetic mean have statistically significantly higher values compared to mean blank values (p > 0.05). This means that the lowest dilution level differences cannot be reliably detected on device B. On the other hand, it should be noted that in the case of the instrument A, which had more accurate results, it was necessary to work with a higher sample volume (2 ml) than was the case with the device B (150 µl).

Table 1 *Basic statistics of the obtained data*

Device	sample	1	2	3	4	5	6	7	8	blank
A	*n*	11	11	11	11	11	11	11	11	11
	$\bar{x}$	0.889	0.852	0.788	0.694	0.612	0.414	0.226	0.065	0.005
	sd	0.009	0.016	0.018	0.007	0.009	0.007	0.013	0.028	0.007
	med	0.890	0.850	0.800	0.690	0.610	0.410	0.220	0.070	0.000
	min	0.870	0.830	0.760	0.680	0.600	0.400	0.210	0.020	0.000
	max	0.900	0.890	0.810	0.700	0.630	0.420	0.250	0.110	0.020
	VK	1.06	1.88	2.26	0.97	1.43	1.63	5.68	43.41	126.05
B	*n*	11	11	11	11	11	11	11	11	8
	$\bar{x}$	1.346	1.183	1.003	0.783	0.558	0.249	0.310	0.054	0.045
	sd	0.025	0.061	0.038	0.045	0.018	0.040	0.355	0.061	0.057
	med	1.355	1.174	1.003	0.780	0.552	0.242	0.107	0.024	0.030
	min	1.304	1.107	0.946	0.704	0.543	0.211	0.081	0.000	0.000
	max	1.379	1.271	1.067	0.885	0.609	0.341	0.930	0.170	0.172
	VK	1.84	5.17	3.75	5.81	3.31	15.91	114.67	113.80*	126.63

Legend: *n*- number of measurements, $\bar{x}$-arithmetical mean, *sd*-standard deviation, *med*-median, *min*-minimal value, *max*-maximal value, *VK*-coefficient of variation. * - there is no statistically significant difference between the mean of the particular dilution and the blank and p>0.05.

Therefore, from a routine determination point of view, the device is more accurate, but it also more time and regency consuming. The higher required minimal working volume in the tube does not even allow in certain cases to measure on the device A the parameters of real (micro) biological samples with much smaller volume. Therefore, in such cases, device A becomes unusable.



Graph 1 *Variation coefficient of absorbance measurement on devices A and B*

6 Conclusion

The accuracy of analyzers that are used in routine laboratory diagnostics is highly relevant to its quality for diagnostic-therapeutic processes. Currently, there is a shift from more sophisticated quantitative methods to nanotechnologies that require less amounts of sample and allow higher mobility of personal (point of care methods). Nevertheless, the issue of accuracy and sensitivity of the methods still remains topical, which should in any case be verified at least in orientative way.

Acknowledgements

This work was supported by the contributions from the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Academy of Sciences (VEGA) grant 1/0431/18 „Relationship between composition, structure and properties of inorganic-organic nanocomposite layers for material protection".

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