Questions of metrological support for bioanalysis. Catalytic activity of enzymes

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Abstract. The relevance of metrological support of measurements carried out in laboratory medicine is examined to increase the accuracy of analyzes results, namely, measurements of the catalytic concentration of the human body enzymes. The development of reference materials with a long shelf life is discussed. The results of the development of Russian reference materials of the catalytic concentration of α-amylase and alkaline phosphatase are discussed. Based on the measuring model (Beer-Lambert-Bouguer law), sources of uncertainty are established. The developed reference materials make it possible to measure the catalytic concentration of enzymes with high reliability, make the correct diagnoses at the early stages of the disease, and make it possible to achieve the world level of metrological support of laboratory medicine in the field of measuring enzyme parameters.

What does modern man begin with? The modern man begin with health, with intelligence, with the ability to properly organize time, with incredible efficiency and determination, as well as with a burning desire to do something for the existence of humanity every day. Of course, this is just our point of view, but this statement looks reasonable, and the probability of challenging this thesis seems small.

So, health comes first. There are individuals who were lucky enough to be born with good hereditary traits to the correct functioning of the body systems (estimated by no more than 4% of the total population of the Earth [1]), their genome allows, without worrying much about health, nevertheless, living healthy for a long time. However, there are other individuals, and there are many of them (the remaining 96%) who need to pay attention to the functioning parameters of their body - cholesterol, blood pressure, blood and urine sugar levels, enzyme activity. The accuracy of the analysis results of these parameters directly depends on the development of the metrological support of Bioanalysis, laboratory medicine.

The development of biotechnology in the real world is at an incredible pace. For accurate measurements, unprecedented opportunities of the latest discoveries and achievements of science and technology are used. The importance of studying the catalytic processes occurring in the human body was noted by the great biochemist Albert L. Lehninger ‘For the existence of life, two basic conditions must be met. First, a living organism must have the ability to reproduce itself. Secondly, the body must be able to effectively and selectively catalyze chemical reactions’[2].
How important is the study of catalytic processes, including those occurring in the body, and the fact that in the history of the Nobel Prize, which in chemistry was awarded 166 times, 73 times of them were awarded for discoveries made in bioanalysis, and for discoveries directly related to the study of catalysis and catalysts, 18 times.

Currently, millions of medical tests are performed annually in the world. Data on Nobel Prizes awarded, as well as information on the number of bioanalytical measurements carried out annually in the world, give only a rough presentation of the relevance of obtaining accurate measurements results of quantities related to bioanalysis. Accurate measurements in bioanalysis make it possible to conduct early diagnosis of diseases, which helps prevent the uncontrolled course of pathology and cure a person of a serious disease; control the quality of pharmaceuticals; provide accurate results of analyzes of the content of toxic, psychoactive, consciousness-altering agents in a patient, determine the possible presence of doping in blood, urine samples, etc.

At the moment, accurate and operational methods of analysis and measuring instruments are being developed, improved and created, which make it possible to solve these pressing tasks. Large foreign and domestic enterprises are create such measuring instruments is carry out.

Issues of ensuring metrological traceability of the chemical and biological measurements results to physical quantities primary standards, to primary reference procedures are regularly discussed and resolved at meetings of the Consultative Committee on the Quantity of Substances (CCQM) of the International Bureau of Weights and Measures (BIPM).

Twice a year, meetings of three working groups related to bioanalysis are held - the protein analysis working group (PAWG), the cell analysis working group (CAWG), and the nucleic acid analysis working group (NAWG).

Russia in this type of measurement is traditionally represented by VNIIM named aft. D I Mendeleev, and from the spring of 2015, VNIIMS joined them. VNIIMS has 5 primary measurement standards in the field of physicochemical measurements; VNIIMS is currently creating the primary reference procedure for measuring the catalytic concentration of the α-amylase.

Recall that the metrological support system for any type of measurement is based on the availability of relevant regulatory legal acts, the state primary standard or primary reference measurement procedure, the state verification scheme for transferring unit size to subordinate measuring instruments or a hierarchical scheme, reference materials and unit size transfer methods.

International symposia on reference materials BERM 14, which took place in October, 2015 in Washington, USA, and BERM 15, which took place in September, 2018 in Berlin, Germany, were devoted to the creation and use of biological reference materials and reference materials for ensuring traceability of measurements in the field of environmental protection. At BERM 14, 23 companies were represented - the world's leading manufacturers of reference materials, 99 oral presentations and 108 poster presentations were presented. Many countries are closely engaged in solving the problem of creating stable, with long shelf life reference materials of biological substances. Attracted interest a report on a very original "biological eggs".

‘Biological eggs’ (BioBall™, Biomérieux) are reference materials (SRM) enclosed in an egg-shaped shell consisting mainly of calcium (Figure 1).

![Figure 1. ‘Biological egg’ [3.]](image)
Due to its tightness, a constant microclimate is maintained inside this shell, which allows biological elements (DNA, proteins, and peptides) enclosed in it to maintain their properties almost unchanged. The percentage of SRM stored in BioBall that remains stable for 12 months is approaching 100% (97%). While the same biological substances, traditionally stored in glass containers, remain stable in about 70% of cases (according to Biomérieux [3]).

In Russia, primary measurement standards are created and improved, verification schemes are developed, methods are developed, but reference materials of approved types that play one of the most important roles in bioanalysis (for the purpose of transferring the size of a unit) are currently practically absent. With only an approximate assessment of the need for SRM, calculated in thousands, about 30 were registered with the Federal Information Fund for Ensuring the Uniformity of Measurements.

Our institute conducts extensive research and development of Russian reference materials used for the diagnosis of diseases, namely reference materials of catalytic activity / concentration of enzymes. The first in the ever-expanding list of sought-after enzymes were α-amylase and alkaline phosphatase, reference materials of which were created as part of the experimental design work of ‘Bioanalysis’.

Given that measurements of the catalytic concentration are carried out by spectrophotometric method, the measurement model is based on the Beer-Lambert-Bouguer law, and the equation of optical density measurements is converted to the form (1):

$$A = \varepsilon l c = \frac{\varepsilon l c_0}{D}$$

(1)

where:

- $A$ - the optical density of the solution;
- $\varepsilon$ - the coefficient of molar extinction;
- $l$ - the length of the optical path (thickness of the cell);
- $c$ - the concentration of 4-NP in the reaction solution *;
- $c_0$ - the concentration of 4-NP before dilution *;
- $D$ - the dilution factor.

* - in the case of α-amylase

Differentiating equation (1) we obtain equation (2):

$$\frac{dA}{dt} = \frac{d(\varepsilon l c_0)}{D} = \frac{\varepsilon l dc}{D \times dt}$$

(2)

The reaction rate can be calculated from equation (3):

$$r = \frac{dc}{D \times \varepsilon l \times dt} = \frac{31k}{1012 \times 0.01} \times 1000 = 3063k \mu\text{kat/L}$$

(3)

where:

- $r = \frac{dc}{dt}$ - α-amylase activity
- $D$ - dilution factor
- $\varepsilon$ - the coefficient of molar extinction;
- $l$ - the length of the optical path (thickness of the cell);
- $c$ - the concentration of 4-NP in the reaction solution;
- $k = \frac{dA}{dt}$ - the slope of the regression.
Based on the measuring model (equation 1), the following sources of uncertainty are accepted for consideration:
- dilution factor - takes into account the uncertainties of all volumetric utensils used for the preparation of solutions;
- the length of the optical path - depends on the accuracy of the measured thickness of the cuvette, is taken from the certificate for the cuvette or determined empirically;
- the slope of the direct regression - depends on two factors - the first - on the measurements of optical density (taken from the certificate for the spectrophotometer) and time, the second - on the linearity of the regression;
- correlation of the slope angle, dilution factor and optical path length;
- deviation of the measurement method;
- pH of solutions;
- solutions temperature.

The created reference materials have the characteristics given in table 1.

**Table 1. Metrological characteristics of SRM [4]**

| №  | Name of Characteristics | Dimension | Value of the characteristic | Limit deviation - extended uncertainty of catalytic concentration measurements at (k = 2) |
|----|-------------------------|-----------|------------------------------|----------------------------------------------------------------------------------|
| 1  | Catalytic concentration of the main component | kat/dm³ | 5,0·10⁻⁷ ÷ 1·10⁻⁵ | 0,5 ± 7 % |
|    | SRM α-amylase           |           |                              |                                                                                  |
| 1  | Catalytic concentration of the main component | kat/dm³ | 5,0·10⁻⁷ ÷ 1·10⁻⁵ | 0,5 ± 8 % |
|    | SRM alkaline phosphatase|           |                              |                                                                                  |

The shelf life of reference materials is 12 months.

Figure 2 shows the appearance of reference materials of α-amylase and alkaline phosphatase.

As a result, conditions have been created for import substitution and import advances, conditions have been created for Russia to enter the forefront in the manufacture of affordable high-tech,
competitive metrological support tools - reference materials for laboratory medicine; national reference materials of catalytic concentration of the analyzed enzymes of world-class were created.

The developed reference materials were created taking into account the existing world experience and the level of technological development using the results and procedures of international comparisons CCQM BIPM. The studies performed make it possible to measure the catalytic concentration of the identified analyzed substances with high reliability and to make the correct diagnoses at the early stages of the disease, and allow achieving the world level of metrological support of laboratory medicine in the field of measuring enzyme parameters.

Thus, the work on the creation of methods and means of metrological support of bioanalysis brings us closer to more accurate test results and the achievement of a healthy person.

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