

Determination of Different Biological Factors on the Base of Dried Blood Spot Technology

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1 Introduction

Determination of different biological factors on the base of dried blood spot technology has a great practical importance in investigation of back lands populations, in epidemiological studies or in special people contingents monitoring, see [1], [2]. This technology presumes that blood sampling is performed by patient himself, the sample is spotted on a dry, as a rule, porous surface (filter paper, cellulose acetate membrane, etc.), and the posterior transportation to a laboratory, for example, by post in a standard or a special envelope.

Modern diagnostic equipment gives an opportunity to investigate many characteristics of dried blood spot, such as metabolites (see [3], [4], [5], [6], and [7]), hormones (see [8], [9], [10]), glycated hemoglobin (see [11]), and even some immune system parameters (see [12]). The possibility of DNA and RNA investigations in such samples is of great importance, since it gives, for example, an opportunity for mass investigations of socially important infections such as AIDS, hepatitis, etc.

In 1992, in USA a laboratory standard for dried blood spot testing (DBS) was elaborated, see [13]. In 2001, in Russia, Application Instruction on Alcor Bio Ltd Reagent Kit for immuno-enzymatic determination of thyrotropic hormone in dried blood spot of newborn was approved.

Under the dried blood spot technology using, the problem of the sample spotted volume determination remains one of the main practical questions. The existing versions of the technology may assume spotting of a known blood volume by means of some dosing device and a posterior elution, or using of special filtering device for the plasma separation under dried spot preparation, see [14].

But there are no any universal method calculating the volume of the blood spot, which does not use a dosing device. The solution of this problem gives an opportunity to increase essentially the accuracy of the results and to simplify the blood sampling procedure. There is a series of articles, where the calculations are based on the concentration of some electrolyte and on a correction of the plasma volume by the hematocrit value, see [16]. In the present work we try to elaborate a universal technology for the spotted volume calculations.

The solution of this problem is also important for such branches of Medicine as Catastrophe Medicine and Forensic Laboratory, where non-standard situations are typical, and, for example, there is no opportunity to sample patient's blood, and so it is necessary to use the remains of the patient's blood on some other objects, instead of the standard blood sample. Such a situation can appear under investigations of the victims to traffic accidents, or other catastrophes.

It is well-known that distinct biological indices (analytes) have distinct variability, see [15]. We try to use some mathematical algorithms to pick out a set of blood parameters which give an opportunity to retrieve the initial volume of the blood spotted, and use it to calculate exact concentrations of analytes interesting to a physician. For our analysis we used the database of biochemical blood parameters obtained in Russian Scientific Center of Roentgen-Radiology during 1995–2000, which includes more than 30000 of patients.

2 Mathematical Model

Let us describe the mathematical model of the problem. Let x_i , $1 \leq i \leq m$, stand for the value of the result of the laboratory analysis on the i th molecular compound content. The value x_i obtained as a result of the blood sample analysis depends on two following parameters at least: the patient p which is selected from some collection \mathbb{P} , and the volume λ of the blood sample under the analysis. Thus, the value x_i is a function of two parameters:

$$x_i = x_i(p, \lambda).$$

The problem is to find a function $f(y_1, y_2, \dots, y_m)$ whose value

$$f(x_1(p, \lambda), x_2(p, \lambda), \dots, x_m(p, \lambda))$$

is close to λ from the statistical point of view.

Notice that due to the uniform distribution of the molecules under consideration in the blood, a k -multiple extension of the volume must lead to the same increasing of all the indices x_i . In other words, we have $x_i(p, k\lambda) = k x_i(p, \lambda)$. Therefore, if f approximates the blood volume, then the following relation must be valid:

$$\begin{aligned} f(x_1(p, k\lambda), \dots, x_m(p, k\lambda)) &= f(k x_1(p, \lambda), \dots, k x_m(p, \lambda)) \approx \\ &\approx k f(x_1(p, \lambda), \dots, x_m(p, \lambda)). \end{aligned}$$

This notice is a natural motivation to look for the function f in the class of positively homogeneous functions of degree 1, i.e., we assume that the equality

$$f(k y_1, k y_2, \dots, k y_m) = k f(y_1, y_2, \dots, y_m)$$

holds for each positive k . Such functions are uniquely defined by their values at the unit sphere S^{m-1} defined by the equation $y_1^2 + \dots + y_m^2 = 1$. By g we denote the restriction of the function f onto this unit sphere.

Polynomials form the simplest but rich class of functions. Let us look for g among the functions which are the restrictions of the polynomials onto S^{m-1} . Our statistical experiments show that it is enough to consider the polynomials of degree two vanishing at the origin. In other words, we put $\rho = \sqrt{y_1^2 + \dots + y_m^2}$, and look for g in the form

$$\sum_i \alpha_i \frac{y_i}{\rho} + \sum_{i \leq j} \alpha_{ij} \frac{y_i}{\rho} \frac{y_j}{\rho},$$

so the function f is supposed to be in the class

$$f = \sum_i \alpha_i y_i + \sum_{i \leq j} \alpha_{ij} y_i y_j / \rho.$$

Thus, our problem is to find the coefficients α_i and α_{ij} such that the function obtained meets our objectives as well as possible. To formulate the latter condition mathematically, let us write down the following objective function.

As we have already mentioned above, the available database gives us a table of specific values $x_{is} = x_i(p_s, \lambda_s)$. We look for the function f such that the total squared deviation from the values λ_s is as small as possible. In other words, we have to find the α_i and α_{ij} minimizing the objective function

$$\begin{aligned} h &= \sum_s (f(x_{1s}, \dots, x_{ms}) - \lambda_s)^2 = \\ &= \sum_s \left(\sum_i \alpha_i x_{is} + \sum_{i \leq j} \alpha_{ij} x_{is} \frac{x_{js}}{\sqrt{x_{1s}^2 + \dots + x_{ms}^2}} - \lambda_s \right)^2. \end{aligned}$$

Notice that h considered as a function on α_i and α_{ij} is a non-negative quadric. In general position such a quadric possesses a unique minimum which can be found as a solution of linear equations system, i.e., from the condition that the differential of h vanishes.

3 Application to the specific database

The above algorithm determining the volume of a sample for calculation of the individual values of an arbitrary analyt was examined on the database of laboratory indices. The best results were obtained, when we reconstruct the volume by means of the following analyts: TP, K, Na. The correlation coefficients for the repaired and true values were 0.95–0.97. The algorithm obtained gives an opportunity to choose distinct sets of the indices for the volume reconstruction, that makes the algorithm multipurpose, i.e. it can be used for analysis of any laboratory blood indices.

The method considered was applied to the specific database in RSCRR. This database was constructed from 35000 medical reports containing biochemical measuring data. We selected the reports containing the largest number of the biochemical data. So, we selected the set of 2637 cases with the next 17

biochemical data measured: Chol, TBil, DBil, TP, Alb, Urea, Crea, ALT, AST, Amy, ALP, K, Ca, Na, Fe, Glu, LDH.

After calculation of the coefficients α_i and α_{ij} for the function f , we find out the following result: the number of patients p_s which the inequality

$$\frac{|f(x_{1s}, \dots, x_{ms}) - \lambda_s|}{\lambda_s} > 0,05$$

holds for, does not exceed 5%. This estimate agrees with the statistical significance of the result.

References

- [1] S. P. Parker and W. D. Cubitt, "The use of the dried blood spot sample in epidemiological studies," *J. Clin. Pathol.*, **52**(9), 633–639 (1999).
- [2] V. G. Pomelova, N. S. Osin, "Outlook of Dried Blood Spot Technology Integration into Population Studies of Human Health and Environment," *Vestnik Rossiiskoi akademii meditsinskikh nauk*, No. 12, 10–16 (2007).
- [3] A. S. Abyholm, "Determination of glucose in dried filter paper blood spots," *Scand. J. Clin. Lab. Invest.* **41** (3), 269–74 (1981).
- [4] J. M. Burrin, C. P. Price, "Performance of three enzymic methods for filter paper glucose determination," *Ann. Clin. Biochem.*, **21**(5), 411–416, (1984).
- [5] D. R. Parker, A. Bargiota, F. J. Cowan, R. J. Corral, "Suspected hypoglycaemia in out patient practice: accuracy of dried blood spot analysis," *Clin. Endocrinol. (Oxf.)*, **47** (6), 679–683, (1997).
- [6] S. J. McCann, S. Gillingwater, B. G. Keevil, D. P. Cooper, M. R. Morris, "Measurement of total homocysteine in plasma and blood spots using liquid chromatography-tandem mass spectrometry: comparison with the plasma Abbott IMx method," *Ann. Clin. Biochem.*, **40**(2), 161–165 (2003).
- [7] Anjali, F. S. Geethanjali, R. S. Kumar, M. S. Seshadri, "Accuracy of filter paper method for measuring glycated hemoglobin," *J. Assoc. Physicians India*, **55**, 115–119 (2007).
- [8] K. V. Waite, G. F. Maberly, and C. J. Eastman, "Storage Conditions and Stability of Thyrotropin and Thyroid Hormones on Filter Paper," *CLINICAL CHEMISTRY*, **33** (6), 853–855 (1987).
- [9] V. G. Pomelova, S. G. Kalinenkova, "Neonatal screening on the congenital thyroid deficiency at environmentally unfavourable regions," *Problems of Endocrinology*, **46** (6), 15–19 (2002).
- [10] H. L. Levy, J. R. Simmons, R. A. MacCready, "Stability of amino acids and galactose in the newborn screening filter paper blood specimen," *J. Pediatr.*, **107**, 757–760 (1985).

- [11] Anjali, F. S. Geethanjali, R. S. Kumar, M. S. Seshadri, "Accuracy of Filter Paper Method for Measuring Glycated Hemoglobin," JAPI **55** (2007)
- [12] H. Shapiro, F. Mandy, T. Rinke de Wit, P. Sandstrom, "Dried blood spot technology for $CD4+$ T-cell counting," The Lancet **363** (9403) 164.
- [13] National Committee for Clinical Laboratory Standards, *NCCLS Approved Standard LA4-A2. Blood collection on filter paper for neonatal screening programs* (Villanova, PA:National Committee for Laboratory Standards 1992).
- [14] B. Evengard, E. Linder, P. Lundbergh, "Standardization of a filter-paper technique for blood sampling," Ann. Trop. Med. Parasitol., **82** (3), 295-303 (1988).
- [15] T. I. Lukicheva, V. V. Men'shikov, L. M. Pimenova, *Biological Variation: a single accurate measure for laboratorial analitics and diagnostic* (Moscow, Analitika, 2004 [in Russian]).
- [16] V. K. Bozhenko, A. D. Beridze, A. M. Shishkin, V. P. Guslistyi, "Use of multivariate methods in the analysis of laboratory indicators of blood", Klin Lab Diagn., no. 10, 10–11 (1997).