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Note

Improved statistical method for the calculation of protein concentration by Laurell monorocket immunoelectrophoresis

H.P. PRINCÉ, D. BURNETT and D.B. RAMSDEN

Department of Medicine, University of Birmingham, Queen Elizabeth Hospital, Birmingham B15 2TH (Great Britain)

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'Monorocket' immunoelectrophoresis, first described by Laurell [1] is a simple, precise and sensitive means of assaying specific proteins. In this technique immunoprecipitates are formed in agarose gels in rocket-shaped peaks, the area of which is proportional to the amount of antigen applied. Because large numbers of area measurements are tedious and difficult to make without an automated planimeter, approximations such as peak height measurements or triangulation techniques are used to simplify the method. The use of peak height alone is satisfactory only over a limited antigen concentration range on any one plate and, although triangulation may increase this range it is still only an approximation to the true area. Therefore we report a programme suitable for use in a desk top computer to better describe the relationship between the antigen concentration and peak height, which is the simplest measurable and precise parameter.

METHOD

The calculator used was a Wang 600. Laurell 'monorocket' immunoelectrophoretic plates were run as described by Laurell [1] using antisera specific for a number of human serum proteins, a minimum of 4 standards per plate being used, to allow statistical calculation of the three coefficients.

The programme is a modification of one supplied by Wang for the calculation of (i) the least squares estimates $\hat{a}$ and $\hat{k}$ to the line $y = ax + k$ and (ii) the correlation coefficient r. In the new programme*, the variable x^b replaces x,

*Full details of the programme will be available on request.

and the value of b giving the highest correlation coefficient is determined by trial and error, together with the appropriate values of $\hat{a}$ and $\hat{k}$.

The formulae are:

$$r = \frac{n\Sigma x^b y - (\Sigma x^b)(\Sigma y)}{\sqrt{[n\Sigma x^{2b} - (\Sigma x^b)^2][n\Sigma y^2 - (\Sigma y)^2]}}$$

$$\hat{a} = \frac{n\Sigma x^b y - (\Sigma x^b)(\Sigma y)}{n\Sigma x^{2b} - (\Sigma x^b)^2}$$

$$\hat{k} = \frac{\Sigma y - \hat{a}\Sigma x^b}{n}$$

where n = number of points on standard curve; y = peak height; x = protein concentration.

To reduce computing time to a minimum, b is limited between 0.2 and 1 and the precision to within ± 0.0005, although values below 0.5 were exceptional (*i.e.* the curve was between a parabola and a straight line).

RESULTS

Table I shows the correlation coefficients obtained for different human serum proteins using the following three mathematical approaches:

(1) [height] = a [concentration] + k
(2) [Δ area] = a [concentration] + k
(3) [height] = a [concentration]^b + k

Approach No. 3 gave for most proteins better results than either of the other two, although the difference between 3 and 2 was often not significant because there is one extra coefficient in 3 and thus one less degree of freedom.

DISCUSSION

'Monorocket' immunoelectrophoresis is on theoretical grounds more accurate than the other simple method of specific protein assay, i.e. single radial diffusion [2]. The quantity measured, height, in monorocket immunoelectrophoresis, is usually greater than the diameter, in single radial diffusion, thus producing in the former a smaller relative error. In addition, the relative error in the latter is doubled, because antigen concentration is proportional to the square of the diameter, whereas using the approach outlined here for the monorocket technique the relative error in measuring peak height is multiplied by a factor between 1 and 2, $(1/b)$.

Peak area approximations obtained here by triangulation give correlation coefficients similar to those using the computer; however, they are manually more tedious to measure, requiring an enlarger or projector to reduce the error in width measurement, and they also need mathematical processing.

TABLE I

CORRELATION COEFFICIENTS FOR DIFFERENT HUMAN SERUM PROTEINS

Abbreviations: RBP = retinol binding protein; TBPA = thyroxine binding prealbumin; TBG = thyroxine binding globulin; PAG = pregnancy associated α_2-glycoprotein.

Serum protein	Molecular weight $\times 10^{-3}$	Number of points	Correlation Coefficients		
			(1) height (linear)	(2) area (linear)	(3) height (power)
RBP	21	5	0.9902	0.99585	0.99899
α_1-Anti-trypsin	54	5	0.9940	0.99962	0.99987
TBPA	55	6	0.9966	0.99787	0.99932
TBG	65	4	0.9940	0.99915	0.9999998
Albumin	68	5	0.9878	0.99814	0.99961
PAG	500—650	4	0.9946	0.99992	0.99994
α_2-Macro-globulin	820	5	0.9976	0.99982	0.99924
β-Lipoprotein	2400	6	0.9912	0.99883	0.99970

In conclusion, therefore, the use of this simple statistical approach increases the accuracy and reduces the number of measurements required to perform the technique.

REFERENCES

1 C.B. Laurell, Anal. Biochem., 15 (1966) 45.
2 G. Mancini, A.O. Carbonara and J.F. Heremans, Immunochemistry, 2 (1965) 235.