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Q.C. OF BLOOD CHEMICAL ANALYSIS BY MICHAELIS MENTEN FACTOR

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Abstract: Measured data of blood chemical analysis have dispersion results in themselves, but if a statistical analysis is applied to the results, an effective factor is obtained on accuracy quality control. As a method, there is the one which confirms the existence of abnormal reaction products by Michalies-Menten factor [1]. This factor expresses the relation between the change of concentration of reagent and measurement data, and also the factor discriminates the good or bad of blood test reagent. And in this study, the effect of deposit days of the factor was studied. Then in this study, the reagent of Radioimmunoassay (RIA) [2] was selected as an experiment object. The RIA is a kind of blood chemical analysis.

In this paper, the accuracy of calibration curve of RIA was studied, and the good or bad of calibration curve was discriminated by using Michaelies-Menten factor. And then the discussion of time series variation data that the calibrator reagent changes in a deposit day was done.

Keywords: Radioimmunoassay ISO standard rule, WHO standard rule, Quality Control

1 INTRODUCTION

The quality control of RIA is performed by WHO standard rule. In the rule of WHO, the dispersion is treated by only Normal distribution, but the measurement data of RIA has be known often Non-normal distribution accord to our studies. Generally in the case of Non-normal distribution, non-linear analysis or non-parametric analysis needs and it correspond to B type of ISO standard rule to be advance of the quality control. In B type of ISO standard rule, tracer-ability, transfer-ability compatibility are included. B type will become important in future on the confirmation of impurity material in a reagent. The impurity material will become to calibration of reference concentration material of test reagents.

RIA is a test method of applied radio-isotope, and property of RIA is higher sensitive for low concentration. It is very important problem on the accuracy quality control. This study has an object to advance a accuracy control by a transferability, a compatibility and a trace-ability of the measurement level in with ISO13488 standard rule.

2 MEASUREMENT THEORY

On the measurement theory of RIA, a chemical reaction rate model is generally used. This model is controlled by “A low of action mass” [2] that the reaction rate is proportion to activated concentration of reaction matter in uniform system at a constant temperature.

Theory of RIA explained by a model, it is shown by formula (1). Where, [P*] is labeled antigen with radioisotope, [P] is non-labeled antigen [P]. [Q] is antibody, [PQ] is reaction compound product, [P*Q] is labeled reaction compound product, [PI] is abnormal reaction product k1 is association constant, k2 is dissociation constant and k=k1/k2 is affinity.

$$[P]+[P^*]+[Q] \underset{k2}{\overset{k1}{\rightleftharpoons}} [P^*Q]+[PQ]+[PI]. \quad (1)$$

Total antigen is Po=PQ+PQ*+PI, and the usually constant. Affinity is also said as binding factor or

reaction rate. And generally, concentration of antigen is evaluated by antigen product express half maximum reaction rate, Michaelis-Menten factor is same as reaction rate. Reaction rate is defined by ratio of association and disassociation. Which are usually proportion to active concentration under a constant temperature.

Differential expression of the product [P*Q] with time t indicates like next formula (2).

$$d[P^*Q]/dt=k_1[P^*][Q-k_2[P^*Q]. \quad (2)$$

At a equilibrium time which becomes d[P*Q]/dt=0, the binding factor k is shown as next formula (3).

$$k_1[P^*][Q]=(k_1-k_2)[P^*Q] \quad (3)$$

where total affinity k is $(k_1-k_2)/k_1$ by using the expression (3), and k is a ratio between association k1 and disassociation k2. k1 means of affinity energy. The certainty concentration of product [P*Q] is valuation from reaction rate. Michaelis-Memten factor [1] is possible to use as an index of dispersion what are calculation from Langmuir plot and Scachard plot. Langmuir plot(see Fig. 1) shows a relation between an association concentration[P*Q] and a total concentration [Po]. And Scachard plot shows a relation of between an total affinity k and an association concentration [P*Q]. .Scachard plot is possible to use as good judge of reaction curve. If. immunoassay reaction make a kind of compound product, scachard plot draws a liner line (see Fig. 2), and if immunoassay reaction make a abnormal compounds product, scachard plot draws an uneven curve (see Fig. 3). An uneven curve is analyzed with multi hyperbolic function as formula (4).

$$Y=V(1)X/[Kd1+X]+V(2)X/[Kd2+X]+\ldots \quad (4)$$

where , Kd1 and Kd2 are affinity. V(1) and V(2) are compound product concentration.

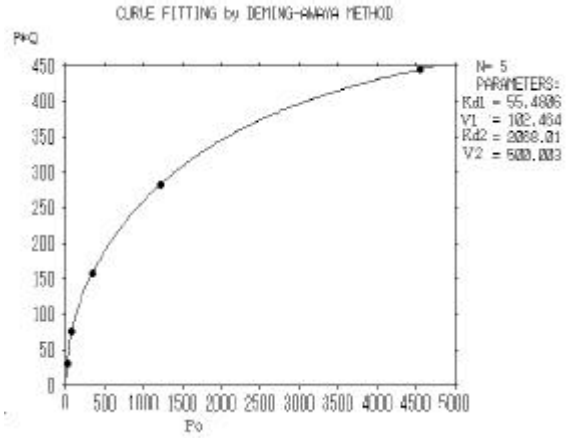

Figure 1. Langumir plot

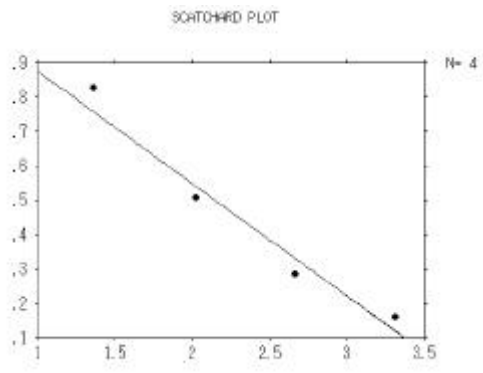

Figure 2. Linear line on scachard plot

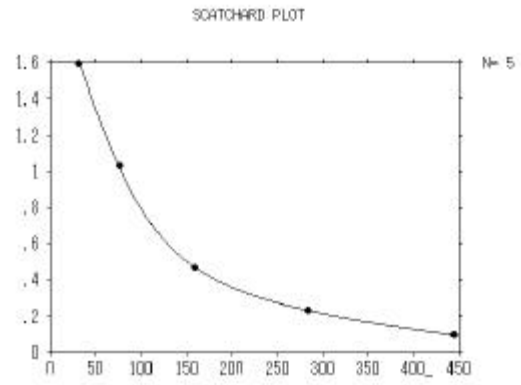

Figure 3. Uneven curve on scachard plot

An accuracy quality control must be established a measurement system as a basis. Time series data is set by measurement data dx/dt and trueness f(x,t). If data includes systematic error factor g(x,t), it is possible to express the measurement data by next formula (5).

$$dx/dt = f(x,t) + g(x.t). \quad (5)$$

These are assumed as an independent function. There are many kind error factors in biochemical fields.

The reaction ability of blood chemical analysis regent falls in nature for according progression of deposit days.

3 METHOD

In this study, calibrator reagents of 300 Elastase-1 which make the calibration curves were used. 6 kind value of each different concentration regents for routine test method were selected as the calibrators. They are principle assigned as the values of reference material. Elastase-1 is a kind of human pancreas hormone.

The example in measurement data of Elastase-1 includes uncertainty elements. Fig. 4 shows dispersion of minimum concentration 0 value by frequency distribution. Fig. 5 shows dispersion of maximum concentration 5000 values by frequency distribution. Both of figures show a Non-normal distribution.

Fig. 6 shows time series measurement data in the between days variation of Fig. 4. Fig. 7 shows in between days variation of Fig. 5. A between day variation data is taken on period test days since product assay day. Fig. 6 and Fig. 7 draw a tendency line in dispersion of the scatter charts.

Fig. 8 shows tendency of reaction ability and abnormal reaction as same data of Fig. 4 and Fig. 6. Fig. 9 shows tendency of reaction ability and abnormal reaction as same data of Fig. 5 and Fig. 7. Both of Figures make a successive connection of all measurement data points. Fig. 6 and 9 illustrates a tendency on between days variation, and the point of the abnormal reaction shows break way from tendency curve. These data analysis are able to find out a new effective factor for the accuracy quality control.

The error in uncertainty element factor is calculated by generalized statistical analysis as normal distribution. If measurement data shows Non-normal distribution, then the calculation method must be used to non-statistical method of B type in ISO standard rule, and it may be any other statistical analysis method. The calibration error is taken out from frequency distribution of measurement data of each concentration. In this study, non-statistical analysis method of chaos theory and fuzzy theory are used. The analysis result make a reference value for routine test method. This results were already reported at IMEKO-XIV in Tampere, Finland [4].

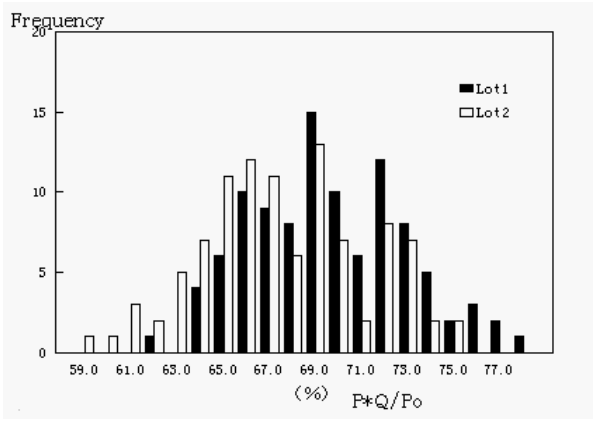

Figure 4. Elastase-1 0 value frequency distribution

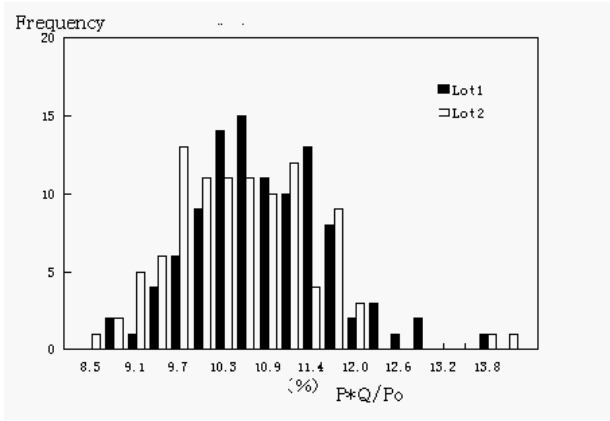

Figure 5. Elastase1 5000 value frequency distribution

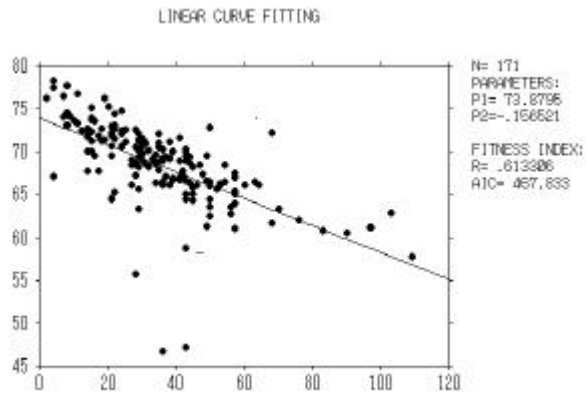

Figure 6. Elastase-1 0 value dispersion Tendency

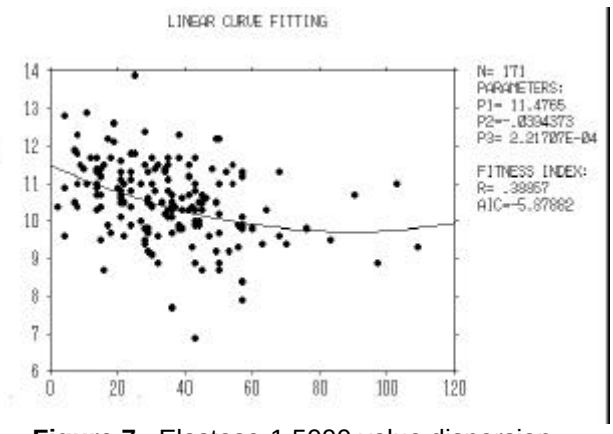

Figure 7. Elastase-1 5000 value dispersion tendency

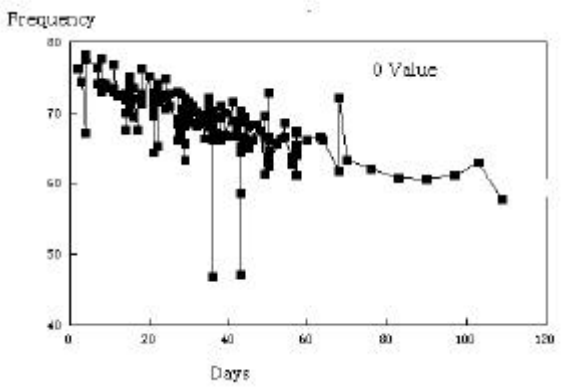

Figure 8. Elastase-1 0 value between days variance

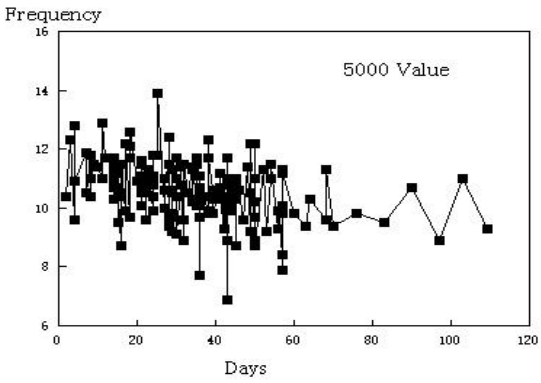

Figure 9. Elastase-1 5000 value between days variance

The between days variation in the measurement data is analyzed by time series data. In analysis method, standard deviation (SD) and coefficient of variance (CV) on every 7 days interval during till test days since product assay days are calculated. What effect exists on deposit days. On analysis results, the existence of a swing in data and a fall of reaction ability at elapse the days are found. The swing repeats stable state and unstable state region in the period of interval 28 days. The cause of swing means the occurrence of second reaction and abnormal reaction. This swing data are already reported at IMEKO-XV in Osaka, Japan [5].

In this study, a swing state in the unstable condition is discussed. And in this analysis, the data of outside from the tendency is performed of break way from tendency line in the Fig.6 and Fig. 7.

4 RESULT

Scarchard plots analyze abnormal reaction which takes a break way from tendency line on Fig. 8 and Fig. 9. Both figure shows the dispersion. Scarchard plots made of all calibration reagents. Made on all scachard plots show uneven curve, so that the regression analysis used bi-hyperbolic function become effective. From analyzed result, it is possible to select the goodness calibration curve exist in all of measurement data.

Fig. 10 shows fluctuation of an affinity Kd1 and a residual by the between days variation. Fig. 11 shows fluctuation of an affinity Kd2 by the between days variation. In Fig. 10 and Fig. 11, a tendency curve is drawn.. Both of figures shows the uncertainty with elapse deposit days.

Fig. 12 and Fig. 13 shows all affinity Kd1 and Kd2 affinity data. From both of figures, the existence of Kd1 and Kd2 break way from regression analysis curve is recognized.

Fig.1 2 and Fig. 13 show the abnormal affinity of Kd1 and Kd2 in unstable region at when it is out of 28 days stable interval. And the existence is remarkable in 38-42 days, Fig. 14 and Fig. 15 shows abnormal scachad plot of Kd1 and Kd2.

Fig .14 shows higher break way of minimum point from regression analysis curve. Fig. 15 shows lower break way of minimum point from regression analysis curve.

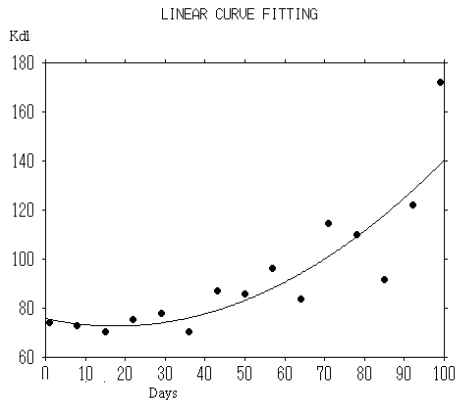

Figure 10. Variation scatter plots of an affinity Kd1

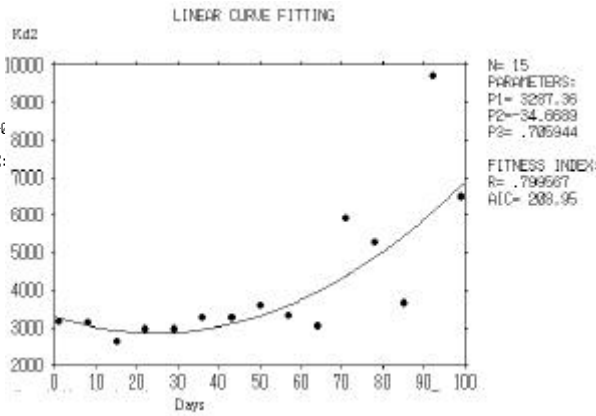

Figure 11. Variation scatter plots of an affinity Kd2

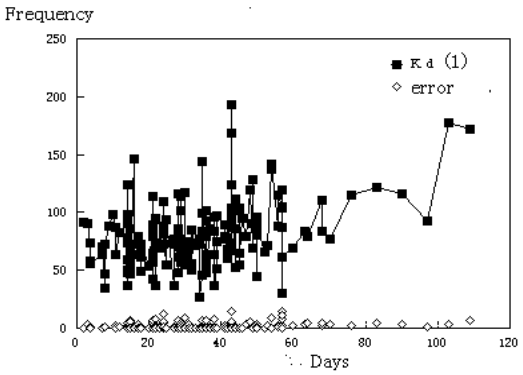

Figure 12. Plotted all affinity Kd1 and error (residence) data

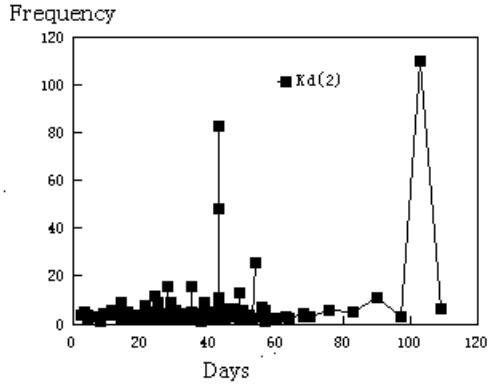

Figure 13. Plotted all affinity Kd2 data

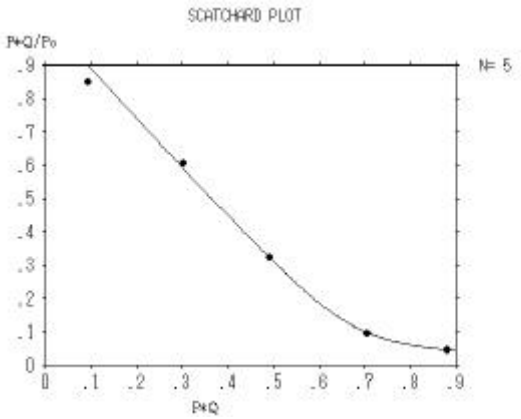

Figure 14. Abnormal scachard plot of Kd1

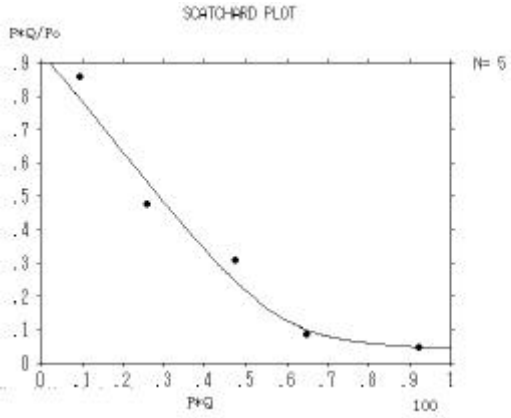

Figure 15. Abnormal scachard plot of Kd2

5 DISCUSS

Michelis Menten factor be able to select best fitting curve with regression analysis. A fitting curve is better than the residual of regression analysis is smaller. In this case. if the residual is smaller than <1, then the fitting curve is goodness affinity. But if the residual is larger than >1, then the fitting curve is not goodness affinity. This is a property to said to both of Kd1 and Kd2 same property. If a reagent includes some impurity material, the reaction ability has more break way, and a scachard plot shows uneven curve. If break way from regression curve exist, the fitting curve of scachard plot becomes abnormal, and it a more large break way from fitting curve , then a scachad plot is brake down. And it mean not justice calibration curve. Although if that calibration curve is made by inner limited normal interval, consequently, the periodical swing and the fall down affinity occur. It is very important problem the accuracy quality control on the view point of application of B type of ISO 13488 standard rule.

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