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IMPROVEMENT OF UNCERTINTY BY MCMC FOR BLOOD CHEMICAL ANALYSIS

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Abstract – The purpose of this research is improving so that the result of blood chemical analysis (BCA) can be assured. It can be useful for clinical diagnosis. It is also included. ISO-GUM was created as guidance for making an analysis result into an assurance performance. In supplement 1 of ISO-GUM (ISO-Guide to the express of uncertainty in measurement)[1] in detail, it recommended using Markov Chain Monte Carlo (MCMC) for "law of propagation of the uncertainty (LPU)". The main features of ISO-GUM changed the mode of expression of an analysis result into uncertainty from error, and processing of data analysis was changed into Exploratory Data Analysis (EDA) from conventional ANalysis Of VAriance (ANOVA). The reason for change is for obtaining an exact result, even if a measurement result is abnormal distribution. MCMC was taken in also in the field of BCA. In the research, it experimented for the quality assurance (QA) of calibration curve use quantitative analysis. An accuracy of calibration curve is importance situation as intermediate accuracy in a whole measurement system. The important factor is a set up reference value and a confidence interval for assurance of an analysis result. Since the satisfy conclusion was obtained, it is reported.

Keyword: MCMC, ISO-GUM, uncertainty

1. INTRODUCTION.

A purpose of this work is improving the ambiguity of the measurand in test reagents for calibration curve of BCA.

An exact of reference value is made to establish with the routine test level by ISO-GUM at this research. In that of ISO-GUM was published in 1993 by ISO and it can be respond to both internal quality control (IQC) in house and external quality control schemes (EQCS).

In that of ISO-GUM, LPU is made the analysis procedure of uncertainty with first step and then key comparison is prepared as second step. The second stage is classifying into type A and type B according to probability density distribution form of measurement result, then it are analyzed.

In type A, an uncertainty estimated by fundamental statistical analysis method of ANOVA that performs only normal probability distribution of measurement data. Tolerance limits is specified to allow reasonable leeway as error factor for accuracy of measurement result.

In type B. uncertainty estimated by other method than the statistical analysis based on specification of non-parametric test and non-linear analysis as EDA for abnormal distribution analyzed. The MCMC in Bayesian statistical model adopted one kind of EDA that are type B of ISO-GUM compliantly.

The reference value is used for the calibration curve of the quantitative analysis. Research of detection limited (DL) and minimum detectable amounts (MDA) is also adopted important for this experience undergo.

The input quantities are measuring data of $x_1,x_2,\ldots,x_n$ then it can write a functional relationship between the measurement result Y. (1) is applied to use base on a first -order Taylor series expansion[4].

$$Y=f(x_1,x_2,\ldots,x_n). \qquad (1)$$

The definition of ISO-GUM is estimated uncertainty of measurement data in three step technologies of LPU. Three steps are calculated: first is the standard uncertainty (Us), second is the combined uncertainty (Uc) and third is the

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expanded analysis (Ue). Us express as estimated standard deviation (SD) based on individual uncertainty element.

Systematically and randomly of both error elements exist in Us. Systematic uncertainty can be made into bias. Random variation is evaluated to be normally distributed with standard mean value and standard deviation.

Combined uncertainty (Uc) is combined the overall Us by root sum square (RSS) as (2). n is number of individual uncertainty element .

Expand uncertainty (Ue) is estimated by applying suitable k factor multiplier for Uc as (3), k factor is called coverage factor.[3] and computed it by Welch-Satterwaite formula based on effective free degree (EFD) to be infinite [2]. Therefore, it is considered that the value acquired by ISO-GUM is an assurance performance.

$$Uc=\sqrt{Us_1^2+Us_2^2+Us_3^2+\_\_\_+Us_n^2} \quad (2)$$

$$Ue=k*Uc \quad (3)$$

2. MEASUREMEN PRINCIOLE

In this experiment, the data carried out by the Radio-immunoassay (RIA) method in the test reagents of elastase-1 used. RIA use a labeled radioactive isotopes linked to a second antibody that is detectable marker for measurement. RIA is a kind of BCA.

The reaction principle of RIA is competitive reaction by an antigen (P) and antibody (Q). The reaction product is PQ. P* is labeled antigen with radio-isotope material of iodine-125. Reaction model of RIA is shown based on reversible reaction in. (4)

$$P+P^*+Q \underset{k_2}{\overset{k1}{\Leftrightarrow}} P^*Q+PQ+PI+P^*I \rightarrow P^*Q \quad (4)$$

Here k_1;association constant
k_2;disassociation constant
k; affinity.(Binding ratio P*Q/Po= %)
Po is total antigen Po=PQ+P*Q+PI+P*I

Po is always invariant constant of nature on “law of action mass”. In this case, measured P*Q/Po is effective binding ratio. Affinity is as same as to dynamic reaction rate. An affinity increases until the saturation of reaction in according to the reaction process time. Affinity is estimated by differential equation with time t as Eq(5)

$$D[P^*Q]/dt=k1[P^*][Q]-k2[P^*Q] \quad (5)$$

The reaction at saturation is kept on chemical equilibrium. (6) become to d[P*Q]/dt=0 and change to (7)

$$k1[P^*][Q]=(k1-k2)[P^*Q] \quad (6)$$

An affinity k is in (k1-k2)/k1……(7)

3. METHOD

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The reagent concentrations of elastase-1 prepared the six doses that are 0, 50, 150, 500, 1500 and 5000. Each concentration as calibrator set was made rationally prepared in the shape of stairs as the analytic specific reagent to creating calibration curve. The number of sample size is total 320set that is divided 3 groups (lots) that consists one group of 120 set and twice groups of 100 set. The number of one group is number of recommendation in ANOVA of IAEA. Whether a difference arises between groups at measured value compares between 3 groups. By carrying out a group, it was verified whether measurement result is influenced with the effective freedom degree

The observed data was making probability density frequency (PDF) distribution that is classified normal formula or abnormal formula when estimating uncertainty

A normal distribution is estimating the width of variation; namely, root sum square (RSS), arithmetical average (mean), standard deviation (SD), coefficient of variance (CV) distribution of the centre (medi) and etc. Reference value is estimated in quantity of fundamental statistics of ANOVA. Then it is selected central tendency Accuracy of data is obtained by uncertainty based on ISO-GUM of type A, if the frequency density distribution of data is an abnormal distribution. It will be converted into a quasi normal distribution by regression analysis and will be analyzed by statistics method.

Fig.1[2] is shown data analyzing method that was recommended by IAEA in 1977 on based ANOVA for RIA.

Fig.1 shows schematic illustration of weighting function the same as normalized distribution, it is a smooth curve that made regression analysis by least squares estimation on

based according to affinity point of response. The weight assigned to each is the reciprocal of the variance predicted point. Truncation is the same as rectangular (uniform) distribution. Weighting is a correction curve for response level. The confidence interval is setting in quasi normal distributions by t-test in Fig 1. The made calibration curve should be verify to matching condition by Michaelies Menten parameter which calculate useful both the langmuir plot and scachard plot. The confidence interval obtained for the abnormal distribution using Type A is dues not satisfy on ISO-GUM standard..

The plan of this experiment is designed the nonparametric test of 2 way layout by null- hypothesis. A null hypothesis is compared with an alternative hypothesis for problem of false. The nature dispersion is established by test of confidence interval that is in 0.05 or less. Official condition is presupposing infinite freedom degrees.

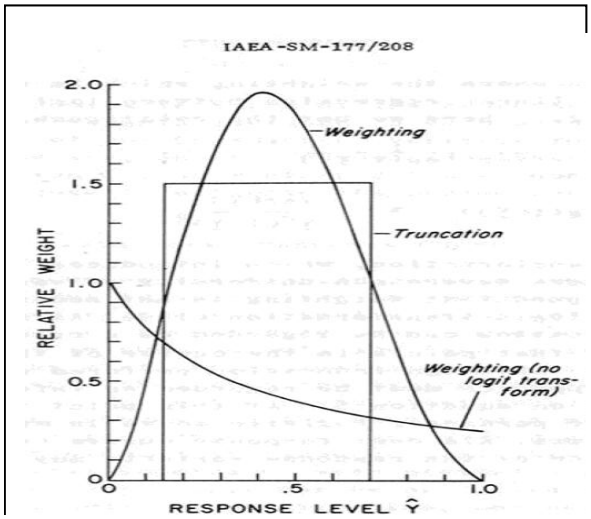

Fig.1. probability density distribution ANOVA in 1977 [3]

Fig.2[5] is recommend by National Institute of Standard and Technology (NIST) which shown the MCMC for type B of ISO-GUM. It is illustrated that the model has mutually independent inputs for calculation of expand uncertainty quantity (Ue). Fig.2 is setting three types probability density functions. these are quasi normal distribution U, t-distribution U_A and rectangular distribution U_B, and a confidence interval is set up by each distribution.

It is prepared in order for the input ports of ywo reserves to make it correspond to triangular distribution, distribution of U type, etc.

Each distribution is useful a Fast Fourier Transformation (FFT) to operation for an abnormal distribution. And Inverse Fast Fourier Transform (IFFT) is made the convolution of Gaussian distribution f(U).

The cumulative distribution frequency (CDF) made it distinguish whether nonlinear analysis is needed. The carried put of CDF is into key comparator and take out by standard uncertainty (Us) for final output. CDF can be verity the characteristic of PDF. It is judged by verification whether it is the necessary by nonlinear analyasis/

In the MCMC analysis, carry out quantity U is estimates two coverage positions in normalized distribution, these are setting a upper limited position and a lower limited position as the final assurance interval.

Where relevant, a similar approach to goal- setting can be used for Total Analytical Error (bias +imprecision)

The standard deviation of rectangular distribution is calculated to multiplicative by square root 3,

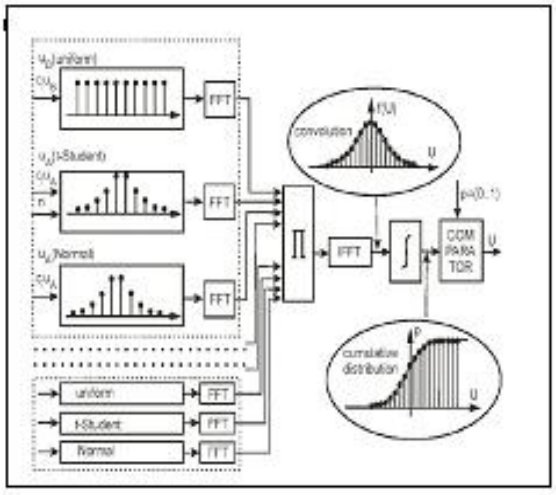

Fig. 2. MCMC of EDA in 2002[2]

In the type B of ISO-GUM, here, chaos method and fuzziness method were used for other method as softcomputing technology, The chaos value computed with difference equation of f(x)=ax(1-x), and the fuzzy value is computed as centre of gravity of 20 steps member function.

4. RESULT

Fig 3 is quoted the pareto graph that is pile up the quasi normal distribution curve (solid curve B) on the measurand frequency distribution (bar graph A). The quasi normal distribution is created by regression analysis from measurand frequency distribution. Ordinate is the account

of frequency of total 320 sample sets. Abscissa is affinity (%) that is divided into 20 steps between maximum affinity and minimum affinity. The characteristic of PDF can be find out the feature by CDF

Probability density distribution of Fig.3 is shown typical out of the data that is reagent of 0 dose. in six dosage of calibrators. All of measurement result (including the five kind of other dose) showed an abnormal distribution similarity (see Fig 4)

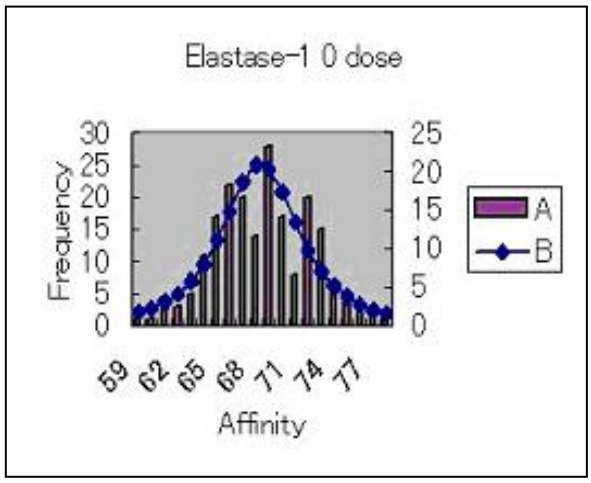

Fig 3. Probability density distribution of 0 dose

Fig 4 is quoted an annexation graph that shows six probability functions forms about the measurand of affinity to six kinds of dosage regents sets.

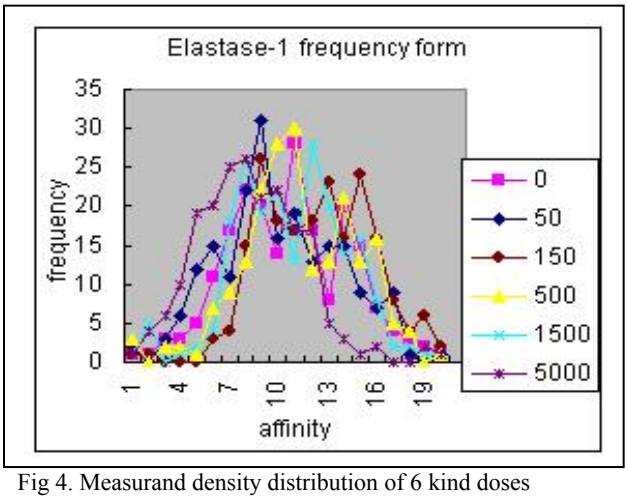

Fig 4. Measurand density distribution of 6 kind doses

Fig 4 is shown a carry out comparison examination distribution form of six dosages. It shows that frequency distribution form is nearly U-shape and not same form. Those curves cannot be computed both skewness and kurtosis. In Fig 4, Ordinate is the account of frequency of total 320 sample sets. Abscissa is affinity (%)

The quantity of fundamental statistics was calculated in with quasi normal distribution, and each distribution is required. the official test by non-parametric method

Fig 5 is shown an annexation graph that shows six dosages of quasi normal frequency distribution forms by regression analysis and cumulative distribution. Fig 5 shows each peak position differs of quasi frequency distribution of six kind dosage of affinity.

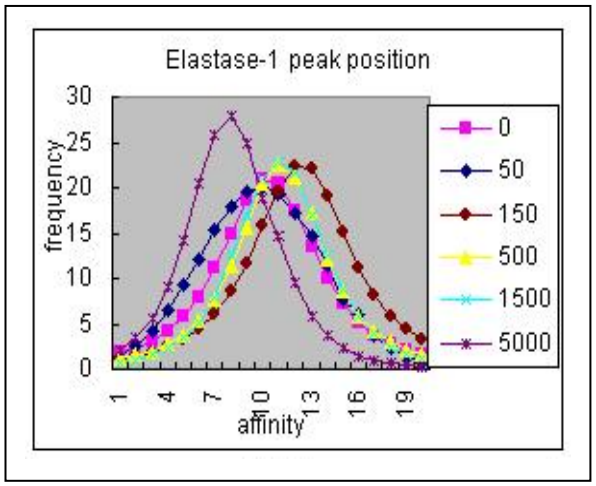

Fig.5 Quasi normal distribution of 6 kind doses

Fig.6 shows the pareto graphs that piled up the measured frequency distribution (in bar graph) on the CDF (solid curve), and data is shown the 3 groups calibrators

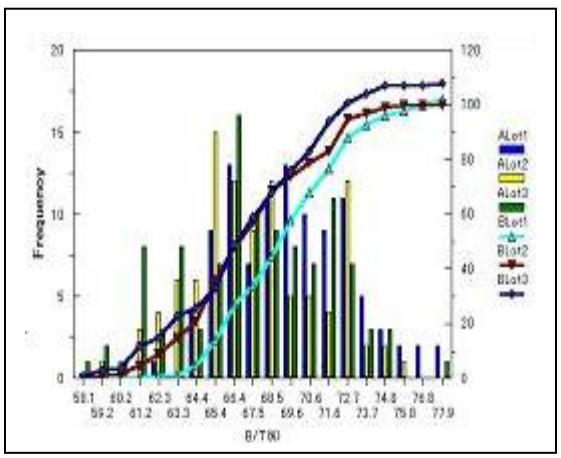

Fig.6. measured density distribution and the CDF

Fig.6 shows the frequency density distribution for calibrator of 0 dose reagent. The CDF curve showed the form of sigmoid and bending seen in some position. The Three groups of accumulation curves in Fig.6 was shown the influence by difference number of samples and each

groups.

In the Fig.6, the unit of Abscissa quotes variation of affinity (%=P*Q/P_0) that divided the affinity into 20 ranks between maximum value and minimum value. The unit of Ordinate quotes generating frequency density count.

Table 1 fundamental statistics quantity by type A

Dose	0	50	150	500	1500	5000
Chaos	71.4	65.4	54.3	37.4	23.2	17.1
Fuzzy	**68.5**	**61.8**	**52.1**	**34.8**	**20.7**	**10.4**
mean	**68.4**	**61.8**	**52.2**	**34.7**	**20.8**	**10.5**
RSS	**68.3**	**61.9**	**52.1**	**34.7**	**20.7**	**10.4**
Medi	**68.3**	**61.7**	**53.1**	**34.8**	**20.6**	**10.4**
peak	**68.3**	**62.2**	**52.1**	**34.9**	**21.5**	**10.6**
Mode	66.1	61.2	50.2	35.1	21.7	10.5
Max	78.2	71.8	60.0	43.1	28.9	17.1
Min	46.7	41.1	34.4	21.7	12.4	6.9
F-test	2.17	2.16	2.17	2.17	2.17	2.17
t-test	2.03	2.02	2.03	2.03	2.03	2.02
EFD	0.18	0.17	0.19	0.17	0.17	0.18

Abbreviation in table

RSS: Root Sum Square

Medi: Central value in PDF

Peak: peak point in PDF

Max: Maximum value in distribution

Min: Minimum value in distribution

EFD: Effective Freedom Degree

UA: Type A uncertainty value

UB: Type B uncertainty value

UC: Combine uncertainty value

CIA Type A Confidence interval

CIB: Type B Confidence interval

CIC Combine Confidence interval

U uncertainty

Table2 Result of calculation by type B

Item	dose	0	50	150	500	1500	5000
Square dis.	central	62,45	56.45	47.2	32.4	20.65	12.0
0.95%×2	UA	8.64	8.43	7.03	5.86	4.53	2.8
Type A	CIA	53.8-71,0	48.-64.9	402-54.2	26.5-38.3	16.1-25.2	9.2-14.8
Normal dis	central	68.3	62.2	52.1	34.9	21.5	10.6
0.95%×2	UB	7.26	6.54	6.11	451	3.11	1.84
Type B	CIB	61.-75.6	55.768.7	46-58.21	30.3-49.6	18.4-24.6	8.76-12.4
Combine U	UC	11.2	10.67	9.22	7.32	5.40	3.29
Assurance	CIC	62.4-73.9	56.8-67.7	4.61-56.7	31.3-.38.6	18.824.2	9.0-12.2

In table 1, four columns of same value focused on. (Fuzzy, mean, RSS and medi) shown in the bold letter character. Standard mean value is possible to taken into must common type of reference value as central tendency by data of this experiment. That value can be setting as a reference value by Type A for IQC of in house. It is standard mean value.

Table 1 is shown what summarized the quantity of fundamental statistics (mean, RSS, Medi, peak, Max, Min F-test and t-test) and quantity of EDA (chaos value and Fuzzy value) computed based on the data of quasi normal distribution curve in Fig.3. Max is maximum value as upper limited in confidence interval. Min is minimum value as the lowest limited in confidence interval.

The assurance interval by type A was calculated by 95% level and coverage factor K=2. It is 2 sigma of a routine test level and can assurance against a QC of result.. In particular, the endpoint of 95% coverage interval that the measurand is given by the 0,025 and 0.975 fractions limited. Moreover, it also became not clear that dispersion is large on type B of ISO-GUM, so that the affinity was low. If dispersion is over the limited, the necessity of verifying from the standard deviation by t-distribution and square distribution was made.

Table 2 shows amount of confidence interval and uncertainty analyzed the t-distribution and the square distribution by MCMC. The coverage interval determined on basis of the LPU,

4. CONCLUSIOON.

4.1. Decision of Reference value for routine test level

When using a commercial test reagent with the routine test level, it can be decide upon the reference value in BCA by mean value of conventional ANOVA [6]

4.2. The assurance of final report of BCA.

When a measurement result is made from abnormal distribution, it is compensation that is required it. Center tendency and peak value of abnormal distribution differ from each other distribution, which set up a quest of each standard deviation by one or more. A report of final result of BCA will be assurance with value in range which must be narrowed quantity in the confidence interval by MCMC.

4.3. A useful ISO-GUM

The "law of propagation of uncertainty" in ISO-GUM is effective over all the measurement system. The mean value of statistic quantity can be made into the reference value generally.

Softcomputing technology useful the validity of fuzzy has proved by this research. It is good method for an uncertainty analysis. .

4.3. Regulation by law of assurance performance for patient.

The assurance of accuracy of BCA is specified with regulation of ISO/CEN 13482 & 13488 enforce in January 2006. The test result by BCA is important for getting to know a patient's condition of disease.

4.4. As for the next subject working,

The multi-variation and modeling are taken up by supplement 2 & 3 of ISO-GUM for multi test results.

4.5. Next target

The target of an accuracy improvement shall be making it an international level of 3sigma for IT era of medical information in Europe at 2010.

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