# A Study on Uncertainty of Measurement in the Blood Chemical Analysis

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## ABSTRACT

Measured data of blood chemical analysis (BCA) have uncertainty of measurement results [1] in themselves. Generally, this problem is treated by statistical analysis and an effort to find effective factor for the quality control (QC) is studied. Generally, WHO rule [2] is applied in the medical field. But in the rule, the uncertainty is controlled only by SD, CV and etc on the supposition, that the distribution of data is normal one. This is parametric analysis method in the statistical analysis of uncertainty data. On the other hand, recently application of ISO–GUM [3] are discussed and practiced in every QC problems. Because, the QC used ISO-GUM and ISO rule uses parametric analysis method together non-parametric analysis method (non statistical analysis), and suitable parameters are assessed. This study is a discussion to grade up the accuracy of QC of BCA. In this paper, calibration curves became important and the cause of uncertainty is cleared by the data.

**Key word.** Radioimmunoassay. ISO-GUM. WHO rule. Quality Control. Calibration curve. Test reagent. Blood chemical analysis. [1]

ISO: International organization for standardization.

GUM: guide to the expression of uncertainty measurement.

WHO: World Health Organization.

SD: standard deviation.

CV: coefficient of variance.

## 1. INTRODUCTION

Duty of a calibration curve is working in with BCA. An accuracy of calibration curve is positioning to the intermediate precision in a standard measurement system. The calibration curve is standardized by ISO rules.

In this study, the measured data of the blood test reagents of Radioimmunoassay (RIA)[4] were used. The RIA is a kind of blood chemical analysis and the theory is an immunoassay used radioisotope labeled marker.

The measured data of RIA show often an abnormal distribution included uncertainty. The uncertainty of measurement should be analyzed by non-parametric or non-liner method. In these analytical methods, the type B of ISO-GUM suits. In this paper, the parameters analyzed by type A and type B of ISO-GUM (see Fig.1) are compared and discussed. Still, the type A is same with WHO rule for QC, and accord to ISO 5725-1994 series (trueness and precision) [14] and ISO 11095–1996 (calibration curve)[20]. Type B is applied to tracerbility, transferability and compatibility.

ISO-GUM
- Type A: statistical analysis method. (Parametric method)
- Type B: another analysis method. (Non-parametric method)

Fig.1 Conception of ISO-GUM.

The special feature of non-parametric analysis suggests potential error factor in an abnormal distributed data by robustness. Generally, in standard measurement system, the matrix

reference material is used. In the routine test method, the calibrator or control serum are used as reference materials, and a calibrator has principal assigned value for reference. Therefore, the calibration curve is made of regression function with the multi calibrators.

A net substance concentration in the blood is detected from the calibration curve, that curve is made of affinity variance. Affinity of regent is variable in the deposit day. The reagents are deposited usually in the freezing stocker.

Type B contributes to an improvement of accuracy and it realizes more grad up measurement system.

## 2. MEASUREMENT THEORY OF RIA

Generally a bio chemical kinetic reaction rate model is used on the measurement theory of RIA. This model accords to "low of action mass" which a dynamic reaction rate is proportion to activated concentration of reaction matter in the uniform condition system at a constant temperature. .

The theory of RIA is indicated as an elementary process. The equation is indicated as Eq. (1). The reaction process includes the recombination. Where the symbols P* is labeled antigen, P is non-labeled antigen, Q is antibody, PQ is non-labeled reaction compound, P*Q is labeled reaction compound and PI is abnormal reaction product. Here labeled material is radioisotope I-125.

$$P + P^* \underset{k_2}{\overset{k_1}{\rightleftharpoons}} P^*Q + PQ + PI \qquad (1)$$

$k_1$: association constant

$k_2$: dissociation constant

$k = k_1/k_2$: affinity

Total antigen $P_0$ is PQ+P*Q+PI and usually it is constant. The affinity is increasing on reaction process time till the reaction saturates. A dynamic reaction rate is estimated by the differential equation with time t. The equation is indicated as Eq. (2).

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

The end of the dynamic reaction becomes in saturation state and the reaction keeps in chemical equivalent. The Eq. (2) becomes to $[P^*]/dt=0$, and then $k_1$ and $k_2$ change to Eq. (3).

$$k1[P^*][Q] = (k1 - k2)[P^*Q] \qquad (3)$$

Here affinity k is in $(k1 - k2/k_1$

## 3. ERROR THEORY OF TIME SERIES DATA

Time series variable data (indicate by x ) of reagent is expressed by dx/dt, and the trueness is expressed by f(x,t) accord to ISO-5725-1[15] (trueness). If the data includes error elements, the data are expressed by g(x,t) accord to ISO-5725-4 [18] (practice of accuracy value), the relation of two elements is given by Eq.(4). These elements are null statistical hypothesis as an independent function.

This study has the purpose to use effectively the assignable error source in the calibrator and to improve the accuracy of a measurement system.

$$dx/dt = f(x,t) + g(x,t) \qquad (4)$$

An improvement of accuracy for QC is to minimizing g(x.t) element. The causes of assignable error are in an abnormal chemical reaction or in influence of impure matter in with chemical reaction. Then this study is adding to make best condition in the operation system for between day variance.

## 4. METHODS

A calibrator of RIA is Elastase-1 [5]. Elastase-1 is a kind of human pancreas hormone. Calibrators are composed as a set by multi concentrations. The multi concentration calibrators have the value of dose of 0, 50, 150, 500, 1500 and 5000. Sample size is 300 of calibrators. The dose means 6 kinds of principal assigned value. A principal assigned value has a referential property concentration of blood test reagent for routine test reference. Fig.2 illustrated the concept of reference transferability.

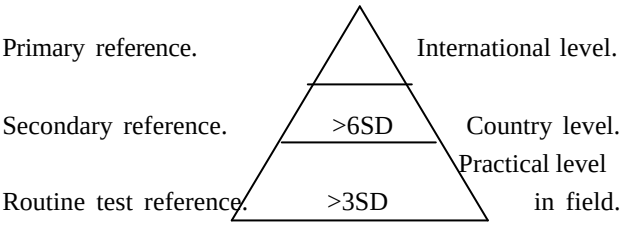


Fig.2 Concept of transferability.

In this study object, the certified reference value shall be got from abnormal distributed data. If data are an abnormal distribution, the parametric analysis method (calculation of arithmetic mean and root mean square: RMS) and the non-parametric analysis method (calculation of chaos theory and fuzzy theory) shall be used. In the Chaos theory [8], a diverging point of Figenbsum bifurcation diagram is used. In the Fuzzy theory [9], a center of gravity in with member ship function is used.

The calibration curve shall be draw within the limited accuracy interval and shall be modified for all of calibration point. When

the calibration curve was made, fit test of the curve needs and the calculation of matching parameter needs. The goodness or not of calibration curve is judged by fitting test. As matching parameter, Michaelies Menten equation is used and Michaelies Menten parameters were determined from Langmuir plot and Scachard plot. Laugmuir plot is made of nonlinear least squares regression analysis [7] by using affinity k and compound P*Q. And then Scachard plot is made of nonlinear least squares regression analysis by using association constant $k_1$ and dissociation constant $k_2$.

Michaelies Menten parameter shows matching condition of between $k_1$ and $k_2$ about all of test reagents. The outcome measure are affinity kd (d is regression coefficient) and error (residence) in regression process.

In this case, Michaelies Menten parameter is calculated by bi-hyperbolic function. Equation is indicated as formula Eq.(5) of the nonlinear least squares regression analysis.

$$f(k_1)=A_1k_2/(kd1+k_2)+A_2k_2/(kd2+k_2) \quad (5)$$

$kd_1$: affinity of zone 1. $A_1$: dose of zone 1.

$kd_2$: affinity of zone 2. $A_2$: dose of zone 2.

## 5. RESULTS

### 5.1 Reference value (trueness value)

The reference value is calculated by using a frequency distribution. Fig.3.[11] is a frequency distribution of calibrators in the case of dose (a concentration of minimum in the test reagents set of elastase-1). Fig. 4[11] is a frequency distribution of calibrator in the case of dose 5000 (a concentration of maximum in the test reagents set of elastase-1).

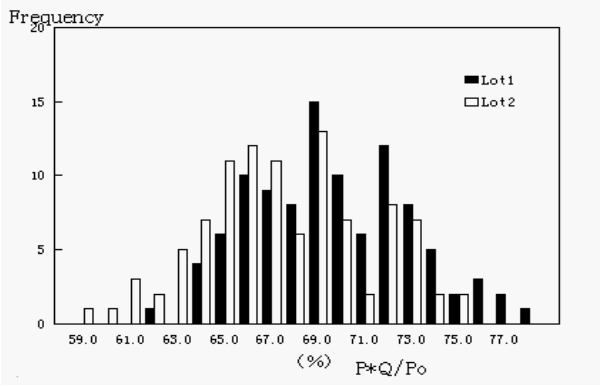


Fig.3. Frequency distribution of Elastase-1. (dose 0 of calibrator)

X-axis of Fig.3 and Fig4 indicates affinity of % unit. Y-axis of Fig.3 and Fig.4 shows frequency density. Fig.3 and Fig.4 show an abnormal distributed data and include uncertainty factor. Both graphs show distribution of divided 2 lots of sample size for reproduction test.

Other 4 kind principal assigned value calibrators showed all of nearly same as abnormal distributed data

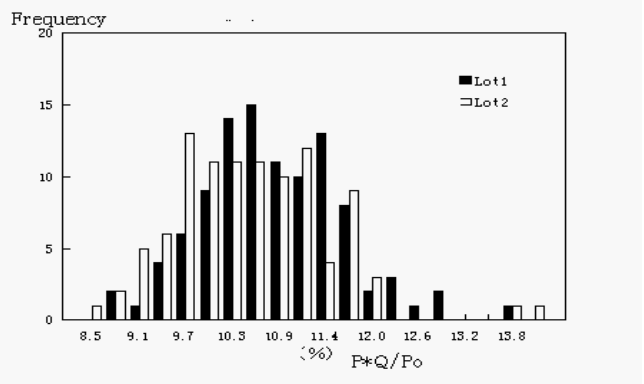


Fig.4. Frequency distribution of Elastase-1 (dose 5000 of calibrator)

Table 1 shows calculation results of affinity by the parametric analysis method and nonparametric analysis method. In the calculation methods, mean, RMS, fuzzy [9] and chaos [8] are used, and max is upper limit value and min is lower limit value. Outcome measures of net affinity are calculated from 6 doses of bar graph distributions.

Table-1. Affinity k of Elastase-1

| dose | mean | RMS | fuzzy | chaos | max | min |
|---|---|---|---|---|---|---|
| 0 | 63.8 | 68.27 | 68.35 | 71.4 | 78.2 | 46.7 |
| 50 | 61.9 | 61.95 | 61.85 | 65.4 | 71.8 | 41.1 |
| 150 | 52.2 | 52.09 | 52.09 | 54.3 | 60.0 | 34.4 |
| 500 | 34.7 | 34.69 | 34.72 | 37.4 | 43.1 | 21.7 |
| 1500 | 20.8 | 20.68 | 20.68 | 23.2 | 28.9 | 12.4 |
| 5000 | 10.5 | 10.38 | 10.41 | 17.1 | 17.1 | 6.9 |

### 5.2 Time series measurement data

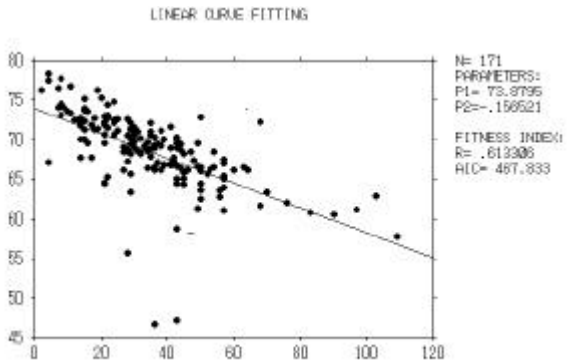


Fig.5 A time series of scatter graph is between days variance for deposit (dose 0 of calibrator)

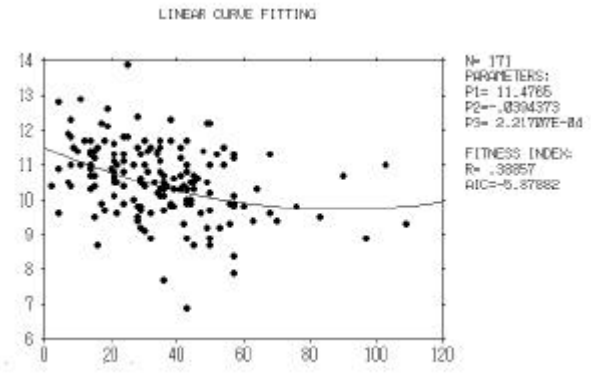


Fig.6 A time series of scatter graph is between days variance for deposit.（dose 5000 of calibrator）

The time series data of calibrators are variance of reaction ability in the deposit days. The between day variance data are taken on period from product assay day to blood test day. Fig.5 [12] and Fig.6 [12] show scatter diagram of time series data. In the both graphs, a trend line in scatter is down right. A trend line illustrates tendency of reaction ability and occurrence of abnormal reaction in the deposit days.

Fig.5 shows the linear drop of reaction ability in the deposit days. Calibrator of dose 0 has higher affinity thanother dose. In Fig.5 and Fig.6, X-axis of both graphs are the deposit days and Y-axis of both graphs are variance of affinity k of Elasetase-1. Fig.6 illustrates random variation of affinity, and tendency line shows the parabolic type curve. Calibrator of dose 5000 is low affinity. Namely if the dose has more lower affinity, more lager dispersion occurs.

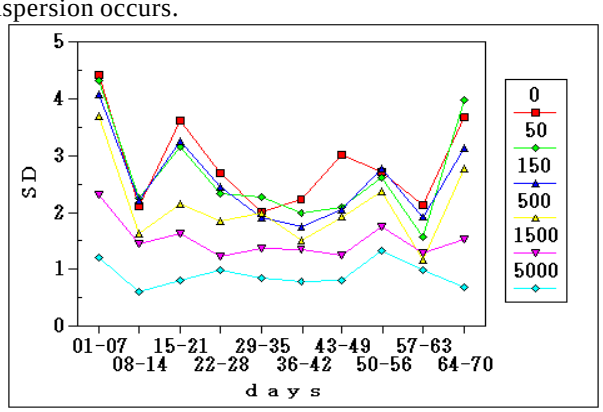


Fig.7 Variance of SD in every 7 deposit days

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Fig.7 shows variance of SD in deposit days and Y-axis is SD variance. Fig.8 shows variance of CV in deposit days. Y-axis is CV variance. X-axis is variance every 7 days.

### 5.3. Selected good calibration curve [13].

Fig.9 shows 4 calibration curves accord to table 1(mean, chaos,

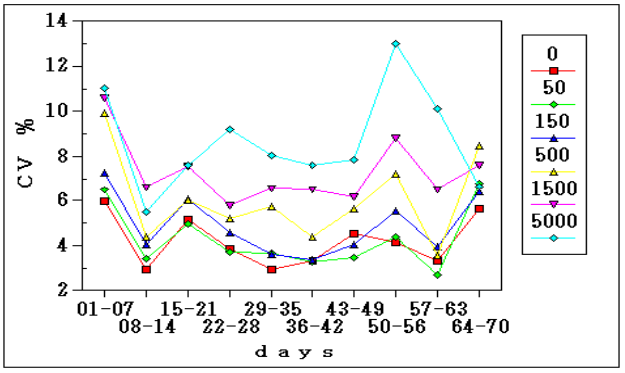


Fig.8 Variance of CV in every deposit days.

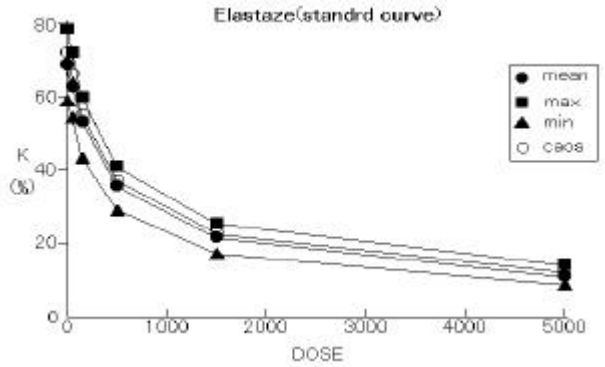


Fig.9 Calibration curves of table 1

max and min). In this case, primary outcome measure is mean value, and k values were calculated by regression function. A calibration curve shall be assessed by error character and compatibility character of Michaelies Menten parameter. Acalibration curve shall be made of the good fitting regression function. In this study, a bi-hyperbolic function Eg. (5) is used.

Fig.10 shows in the between day variation of affinity kd1 and regression error (residence). kd2 is nearly same as kd1.

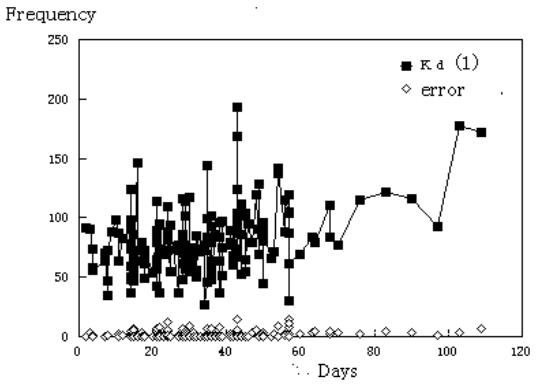


Fig.10 Variance of kd1 and error.

kd1 and error are an effective parameters for the curve fitting test, and an effective parameters is used as referred of the primary immunological endpoint.
Fig.10 shows to look for outliers point from tendency. The important outliers point is shown in the between 70 days variance from product day.

## 6. CONCLUSIONS

### 6.1. Reference value for routine test method

Result of 3 calculation values (Arithmetic mean value, fuzzy value, root mean square value) indicated nearly as same value. The values are possible to accept to reference value for the routine RIA test. Namely, these are admitted as "Calibration and Measurement Capability" of principal assigned reference. In this case RIA is a routine blood test and is useful practical level in the transferability. ISO-5725 series and ISO-3534 [14] series rule will be used to an accuracy of measurement methods and results.
Chaos value shows higher than other values. This fact shows that an influence of allosteric effect is took off by chaos theory.

### 6.2 Elastase-1 of time series data.

When main time effect becomes important, in Fig.8 and Fig.9, about 28 days cycle swing is looking and large variation of SD &CV. SD of dose change between 2-4 SD within 70 days variance. The confidence limits of SD is over 3SD on the working reference material, SD of dose 5000 changes between 0.5-1.5 SD within 70 days variance and has unless 1 SD at 7 days deposit. If SD is under 1 SD, that is except level to as ISO-3534. If SD is over 3SD, that character value clears type A of ISO-GUM (WHO rule) at only product assay day, but it is not clear type B of ISO-GUM at the blood test day. The SD variance character shall be suggested new an assignable error factor. In this study, new facts are found out.

Certify condition
- Accept Over 3SD by WHO and ISO-GUM
- Except. Under 1SD by ISO rule and ISO-GUM

Fig.11 Concept of assessed SD level

The calibrator of reactive ability is own slide down accord to the elapse days. The reactive ability has about 28 days cycle swing. Cycle swing will suggest the existence of reversible dynamic reaction to as Eq. (1). The reaction shall be selected by good condition of in the deposit term. On this fact, error source in the calibrator of reagent occurs in deposit days. This examination done on the bases of a traceability technology of ISO-GUM and ISO-5725-2 [16].

### 6.3 Acceptable good calibration curve.

When a calibration curve was made, the calibration curve must be assessed by Michaelies-Menten factors to control of qualification. Acceptable results are, 1) the fitting error of regression curve shall be unless 1, and 2) the outlier point shall be not occur from regression curve made of calibrated curve. If a calibration curve is not acceptable as nonconformity, then this calibration curve is not correlation and is not dependability. Then the compatibility is not find so that ISO-GUM is not fit. Particularly important interaction occurs in a part of high dose areas (medical treatment area). Fig.11 shows an example of calibration curves had interaction, and also shows calibration curves made of some deposit days (8, 16, 29 and 72 between days deposited).

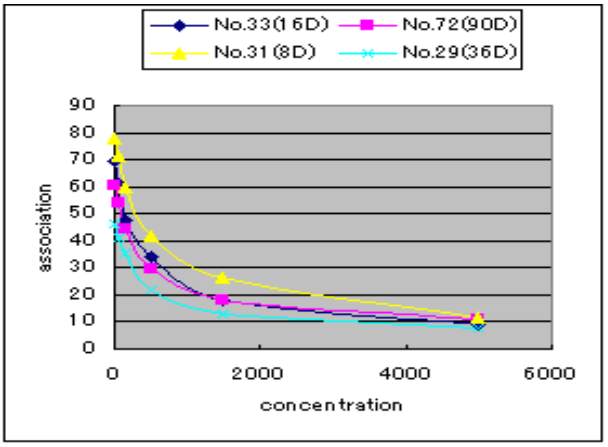


Fig.11 Example of interaction calibration curves

Fig.11 showed calibration curve of stable area and unstable area in the deposit days variance.
The QC of ISO-GUM makes effectively the estimation of latency error sources, and it improves the accuracy control of type B in ISO-GUM. ISO rule 5725-3 [17] is applied to the time series data at the medical field in the practice. And it will improve the accuracy of diagnostics acts for patients. Data of patients will indicated by a bias form of ISO-GUM for error result
The final quoted results is determined by check method as ISO 5725-5 [19] and ISO 11095 series [20] for an acceptability.

These results are important for improved intermediate measures precision of RIA total measurement system.

**6.4 Reliability**

In this study, the examination for other 4 kinds of regents are practiced ( progesterone of androgenic hormone, testosterone of female hormone, plasma rennin active of kidney hormone and thyroxin T4 of growth hormone). All data of calibrators are nearly same with elastase-1. Then all data have perfect reliability.

## 7. REFERENCE


[1] T .Kashiwaki Statistical analysis in medical. Asakuma shoten pp.1-5 1979

[2] Y. Sasaki. Accuracy manegement of WHO. New medical education text. pp.68-70

[3] H. Imai Expression of uncertainty for measurement on history and a review. Measurement and control. Vol.37. No.5 1998 pp.300-305.

[4]K.Ichihara. A accuracy quality control of radio –immunoassay Clinical pathology Vol.53.No.2 pp 98-107

[5]T.Ooyama, K.Kawamura, and H.Orido Radio- immunoassy method for measurement quantity of the pancreas elastase-1 serum. Step by step in medical. Vol.48 1990,

[6] T.Fujita. Plasma renin activity. Japan clinic. Extra issue. Vol.48. 1990.

[7]I.Sato:. Least squares method softwera KOTARO SANKAIDO pp.21-40 1996

[8] K.Sato. Fractal/Chaos 2. pp.268-271 Rassel books Ltd.1994.

[9].N.Mituya and K.Tanaka. Practical use Fuzzy book by C language pp.7-34 Rassel book Ltd. 1990

[10]Nobuyuki Amino: Theory and Application of Immunoassay. Clinical pathology Vol.53 No.2 pp 3-7

[11] Yasuo IWAKI Tadao INAMURA and Komyo KARIYA. ADVACE OF PERSION FOR RADIO ISOTOPE USED BLOOD TEST BY APPLICATION OF CHAOS THEORY. IMEKO-XIV WORLD CONGRESS. Vol. VIII pp 308-312 1997 IN FINLAND.

[12] Yasuo IWAKI Tadao INAMURA and Komyo KARIYA: Discussion of Suitable Deposit Day of the Reagent Quality Control of Reagent Chemical analysis. IMEKO-XV WORLD CONGRESS. Vol. VIII pp.333-339 1999 IN JAPAN

[13]Yasuo IWAKI Tadao INAMURA and Komyo : KARIYA:QC OF BLOOD CHEMICAL ANALISIS OF MICHAELIS-MENTEN FACTOR. IMEKO – 2000 WORLD CONGRESS 2000 IN AUSTRRIA

[14] ISO3534-1:1993 Statistical interpretation of data Part -1:Statisitical presentation of data

[15] ISO-5725-1-1994 Accuracy (trueness and precision) of measurement methods and results 1:General principle and definition

[16] ISO-5725-2-1994 Accuracy (trueness and precision) of measurement methods and results-Part2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

[17] ISO-5725-3-1994 Accuracy (trueness and precision) of measurement methods and results- Part 3: Intermediate measures of the precision of a standard measurement method.

[18] ISO-5725-4-1994 Accuracy (trueness and precision) of measurement methods and results- Part 4: Basic methods for the determination of the trueness of a standard measurement method.

[19] ISO-5725-5-1994 Accuracy (trueness and precision) of measurement methods and results- Part 5: Alternative methods for the determination of the precision of a standard measurement method.

[20] ISO-11095-1996 Linear calibration using reference material.