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ORIGINAL ARTICLE

Estimation of MU for Glucose in Human Serum Using Bottom-up Approach

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SUMMARY

Background: **Glucose is an important material in human metabolism. The establishment of glucose reference measurement procedures and to study the uncertainties of measurement is of great significance. Linear fitting and dilution of reference material were used in the measurement of glucose concentration and they are common operations in daily work. Investigation of the measurement uncertainty of these operations will be of important significance to clinical laboratory medicine. However, in the field of laboratory medicine, related research was rarely reported.**

Methods: **The spectrometric quantification of glucose is an application of the enzymatic reference method with hexokinase. The sources of uncertainty in the measurement process were analyzed. The measurement uncertainties in the study were evaluated according to GUF method and the method introduced by Quantifying Uncertainty in Analytical Measurement (QUAM) was also applied in the evaluation of the measurement of linear fitting.**

Results: **The standard curve was built successfully according to the measurement procedure recommended by the CDC and the linear equation was y = 0.000807x + 0.001213 (R^2 = 0.999179). The measurement uncertainty of glucose in the sample was 0.450,408 mmol/L.**

Conclusions: **The method for the determination of serum glucose concentration by hexokinase in our laboratory has been successfully established. The measurement uncertainty was consistent with the GUF method and the method introduced by Quantifying Uncertainty in Analytical Measurement (QUAM) in the process of linear fitting when the glucose concentration was measured by the reference method (hexokinase method).**

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KEY WORDS

measurement uncertainty, GUF, residual method, QUAM, linear fitting

INTRODUCTION

Glucose is an important material in human metabolism, it is one of the main sources of energy. Plasma glucose concentration must be maintained at a certain level to maintain the needs of the various organs and tissues in the body [1,2]. Detection of glucose metabolism is of the important values for clinical diagnosis. Therefore, the establishment of a glucose reference measurement procedure and to study the uncertainty of measurement is of great significance [3,4]. Accurate measurement results are of great significance in the process of clinical diagnosis and treatment. So, the research of measurement uncertainty is necessary.

Measurement uncertainty (MU) is defined as "the non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used". In recent years, with the development in the field of laboratory medicine, the research on uncertainty of measurement has been paid more and more attention [5,6]. In ISO 15189, the clear requirements were emphasized [7]. The standardization and consistency of test results have become the general trend, which is inseparable from the investigation of measurement uncertainty. The presupposition of this is that the results of the measurement can be traced to the international units (SI units). The research of measurement uncertainty could effectively ensure the reliability of traceability.

The concept of measurement uncertainty is foremost introduced by NIST in 1963. It has been widely used in the field of chemical, engineering, and other industries. However, the research in the field of laboratory medicine started late, but it has been widely investigated. In recent years, the GUM uncertainty framework method (GUF) and Monte Carlo method (MCM) have made great progress in the research of evaluation of measurement uncertainty [8-10]. It was pointed out explicitly in ISO 15189 that "the laboratory should be determined for each measurement procedures uncertainty" [7]. At present, the method of linear fitting is widely used in clinical measurement and reference measurement, especially in the aspect of clinical biochemistry. Therefore, the investigation of the measurement uncertainty of linear fitting will be of important significance to clinical testing. However, in the field of laboratory medicine, related research is rarely reported.

Furthermore, most of the results of measurement uncertainty were obtained on the basis of considering all the components in the measuring process, instead of considering the measurement model and the calculation formula. In this way there may be some double counting and missing of the problem. We need a more reasonable way to solve the problem. Therefore, we investigated the measurement uncertainty of the dissolution of reference material and linear fitting.

MATERIALS AND METHODS

Materials

Instruments

(1) Agilent Cary 4000 Ultraviolet/visible spectrophotometer
(2) HART SCIENTIFIC 1521thermometer
(3) HI 98183 pH detector
(4) BT25S electronic scales
(5) Eppendorf reference pipettor
(6) water bath

All the instruments used in our experiments were verified by Nantong Verification and Test Institute. Standard substances for glucose and reagents (from NIST).

Reagents

$ZnSO_4 \cdot 7H_20$, $Ba(OH)_2 \cdot 8H_2O$, C_6H_5COOH, Tris-HCl, Tris-Base, $(CH_3COO)_2Mg \cdot 4H_20$, $C_{10}H_{14}N_5Na_2O_{I3}P_3 \cdot 3H_2O$, β-$NAD^+$, HK and G6PDH (from SIGMA).

Methods

Reference Procedure of the Measurement of Glucose in Serum

The spectrometric quantification of glucose is an application of the enzymatic reference method with hexokinase. We built the procedure of measuring the concentration of glucose in serum according to the measurement procedure recommended by the CDC [11]. As a Reference Laboratory recognized by JCTLM, all devices used in our experiments were calibrated.

The concentration of glucose was calculated according to formula (1), where c was the concentration of glucose in the sample to be tested, A was the absorbance value of the sample, a was the intercept of the standard curve and b was the slope of standard curve.

$$c = \frac{A - a}{b} \quad (1)$$

To evaluate the measurement uncertainty of the test results, the GUF method was applied. All the facts in the testing process should be considered in the evaluation process. The mathematical model applied in the evaluation process was expressed by formula (2).

$$c = \frac{A - a}{b} \times f(\boldsymbol{glucose\ solution}) \quad (2)$$

The preparation of the glucose solution was according to weight method. The weight of the glucose standard substance was recorded as mGlu. The weight of the empty volumetric flask was recorded as M1. The weight of the volumetric flask with dissolved glucose standard substance was recorded as M2. The volume of the volumetric flask was calibrated before the experiment and the volume was recorded as V50mL. The calibrated concentration and density were calculated according to formula (3) and (4).

$$c = m_{Glu} \times Glu\ putity/(M_2 - M_1) \quad (3)$$

$p = (M_2 - M_1)/V_{50mL}$ (4)

The glucose solution was diluted to different concentrations to prepare our working solutions according to Table 1. This process was still according to weight method. The volume of the volumetric flasks used was calibrated before the experiment and the calibrated volume was shown in Table 2. The weight of glucose solution added into the volumetric flask was recorded as m. The volume of the volumetric flask was recorded as V_{10mL}. The calibrated concentration of working solution was calculated according to formula (5).

$c_{working\ solution} = m\ x\ c/(p\ x\ V_{10mL})$ (5)

Evaluation of Measurement Uncertainty

According to GUF method, we evaluated the measurement uncertainty in the study using measurement model (2). The method introduced by Quantifying Uncertainty in Analytical Measurement (QUAM) was also employed in the evaluation of the measurement of linear fitting [12]. The factors considered in the evaluation process were the concentration of the glucose solution, the absorbance of the samples, and the fitting of standard curve (intercept and slope).

RESULTS

Concentration and Density of the Glucose Solution

In our experiment, the weight of empty volumetric flask was 33.405 g and the weight of the volumetric flask with dissolved glucose standard substance was 83.565 g. The volume of the volumetric flask used (number CKS-211) was calibrated and the calibrated volume was 50.1936269 ± 0.005209 mL. In the experiment, the weight of glucose standard substance was 0.50002 g and its purity was 99.7% (according to its certificate).

$p = \frac{83.565 - 33.405}{50.1936269} = 0.999330056$ g/mL

$C_{glucose\ solution} = 0.50002\ x\ 99.7\%\ x\ \frac{100{,}000}{50.1936269} = 993.1937$ mg/dL

Working Solution Preparation

The weight of the volumetric flask (M3) and volumetric flask with glucose solution in it (M4) were recorded and the concentration of the glucose solution was calibrated according to Table 2.

Concentration and density of glucose solution and their measurement uncertainty

Density

The measurement uncertainty of the weighting process contains the position error, maximum permissible error, and repeatability. They all conform to rectangular distribution. According to the verification certificate of the electronic scales, these data were ± 0.5 mg, ± 0.5 mg, and 1 mg, respectively (the repeatability was expressed as internal in the calibration certificate). The density of the glucose solution was calculated according to formula (4), and the measurement uncertainty was recorded as u_ρ.

(1) Measurement uncertainty of the weight

$U_{scale\ A} = \frac{\sqrt{u^2_{position\ error} + u^2_{maximum\ permissible\ error} + u^2_{repeatability}}}{\sqrt{3}} = \frac{\sqrt{0.5^2 + 0.5^2 + \left(\frac{1.0}{2}\right)^2}}{\sqrt{3}} = 0.5$ mg

$U_{weight} = \sqrt{u^2_{scale\ A} + u^2_{scale\ A}} = \sqrt{0.5^2 + 0.5^2} = 0.7071$ mg

$\%U_{weight} = \frac{0.7071}{(83.565 - 33.405) \times 10^3}$ x 100% = 1.410 x 10^{-3}%

(2) Measurement uncertainty of volume

Constant temperature and humidity of the system was used in our reference laboratory and the temperature changes within 1°C. The dilatation coefficient of water was 2.1 x 10^{-4}/°C. It also conforms to rectangular distribution.

$U_{temperature\ effect}$ = 50.1936269 x 2.1 x 10^{-4} x 1 ÷ $\sqrt{3}$ = 0.006086 mL

u_v = 0.0052091 mL

$U_{volume} = \sqrt{u_v^2 + u^2_{temperature\ effect}} = \sqrt{0.005209^2 + 0.006086^2} = 0.008011$ mL

$\%U_{volume} = \frac{0.008011}{50.1936269}$ x 100% = 0.01596%

(3) The measurement uncertainty of density

$u_\rho = \sqrt{(\%u_{weight})^2 + (\%u_{volume})^2} = 0.016\%$

Concentration

The measurement uncertainty of the weighting process contains the position error, maximum permissible errors, and repeatability. They all conform to rectangular distribution. According to the verification certificate of the electronic scales, these data were ± 0.1 mg, ± 0.05 mg, and 0.1 mg, respectively.

$U_{scale} = \frac{\sqrt{u^2_{position\ error} + u^2_{maximum\ permissible\ error} + u^2_{repeatability}}}{\sqrt{3}} = \frac{\sqrt{0.1^2 + 0.05^2 + \left(\frac{0.1}{2}\right)^2}}{\sqrt{3}} = 0.07071$ mg

According to the certificate of SRM917c, the uncertainty of its purity was 0.3%, standard uncertainty was 0.15%. The uncertainty of the glucose solution was recorded as $U_{glucose\ solution}$.

Table 1. Preparation of working solutions.

Volume of glucose solution (mL)	Total volume (mL)	Concentration of working solution	
		mg/dL	mmol/L
0.50	10.00	50	2.78
1.00	10.00	100	5.55
2.00	10.00	200	11.10
3.00	10.00	300	16.65
4.00	10.00	400	22.20

Table 2. Calculation of the concentration of the working solution.

Theoretical concentration (mg/dL)	Volumetric flask number	Calibrated volume of volumetric flask (mL)	M3 (g)	M4 (g)	M4 - M3 (g)	Calibrated concentration (mg/dL)
50	CKS-229	9.952513	15.133	15.63	0.497	49.6305
100	CKS-230	9.939815	15.335	16.337	1.002	100.1877
200	CKS-231	9.934134	15.039	17.033	1.994	199.4895
300	CKS-232	9.927451	15.237	18.243	3.006	300.9374
400	CKS-233	9.959196	14.742	18.761	4.019	401.0687

Table 3. Absorbance value of glucose working solution.

Calibrated concentration of working solutions (mg/dL)	Average value of absorbance	Absorbance value 1	Absorbance value 2	Absorbance value 3	SD
49.6305	0.045866667	0.0449	0.0459	0.0468	0.000950438
100.1877	0.078666667	0.0789	0.0778	0.0793	0.000776745
199.4895	0.1596	0.1581	0.1599	0.1608	0.001374773
300.9374	0.243466667	0.2469	0.2402	0.2433	0.003353108
401.0687	0.326966667	0.3268	0.3289	0.3252	0.001855622

$$U_{\text{glucose solution}} = \sqrt{\left(\frac{0.07071}{0.5002 \times 10^3} \text{x } 100\%\right)^2 + 0.15\%^2 + \left(\frac{0.008011}{50.1936269} \text{x } 100\%\right)^2} = 0.15000015\%$$

Standard Curve Establishing

Absorbance values of glucose working solutions

The concentration of glucose working solutions were measured according to the measurement procedure recommended by the CDC. All values of absorbance were recorded to build the standard curve. The values of absorbance were tested three times. All the results were shown in Table 3.

Standard Curve

The standard curve was built according to the calibrated concentration and absorbance value of the working solutions. The standard curve was shown in Figure 1. The regression equation was y = 0.000807x + 0.001213 and the coefficient of association was 0.999179.

Evaluation of the measurement uncertainty of glucose by GUF method and the method introduced by quantifying uncertainty in analytical measurement (QUAM)

GUF method

Residual method was adopted in the evaluation of the measurement uncertainty of intercept and slope. All

Table 4. Residual method calculation.

Number	Theoretical concentration x_i	Calibrated concentration x_i	Absorbance y_i	Residual error e_i	e_i^2	$(x_i - \overline{x})^2$	x_i^2
x_{11}	50	49.63049877	0.0449	0.003337	0.000011	25,802.72569	2,463.186408
x_{12}	50	49.63049877	0.0459	0.004337	0.000019	25,802.72569	2,463.186408
x_{13}	50	49.63049877	0.0468	0.005237	0.000027	25,802.72569	2,463.186408
x_{21}	100	100.1877036	0.0789	-0.003013	0.000009	12,116.51965	10,037.57595
x_{22}	100	100.1877036	0.0778	-0.004113	0.000017	12,116.51965	10,037.57595
x_{23}	100	100.1877036	0.0793	-0.002613	0.000007	12,116.51965	10,037.57595
x_{31}	200	199.4895392	0.1581	-0.004513	0.000020	116.0624434	39,796.07625
x_{32}	200	199.4895392	0.1599	-0.002713	0.000007	116.0624434	39,796.07625
x_{33}	200	199.4895392	0.1608	-0.001813	0.000003	116.0624434	39,796.07625
x_{41}	300	300.9374362	0.2469	0.003587	0.000013	8,221.895573	90,563.34048
x_{42}	300	300.9374362	0.2402	-0.003113	0.000010	8,221.895573	90,563.34048
x_{43}	300	300.9374362	0.2433	-0.000013	0.000000	8,221.895573	90,563.34048
x_{51}	400	401.0686588	0.3268	0.002787	0.000008	36,406.88822	160,856.069
x_{52}	400	401.0686588	0.3289	0.004887	0.000024	36,406.88822	160,856.069
x_{53}	400	401.0686588	0.3252	0.001187	0.000001	36,406.88822	160,856.069

Table 5. Absorbance value of the measured sample.

Test number	Absorbance value
1	0.1772
2	0.1757
3	0.1736
4	0.1744
5	0.1773
6	0.1753
7	0.178
8	0.1699
9	0.1792
10	0.1798
Average value	0.17604
s	0.002942108
CV (%)	1.671272488

data were shown in Table 4. The residual error (e_i) and x (the average value of the calibrated concentration of all the working solutions) were calculated according to formula 6 and 7.

$$e_i = y_i - (a + bx_i) \quad (6)$$

$$\overline{x} = (X_{11} + X_{12} + X_{13} + \ldots \ldots + X_{53})/15 \quad (7)$$

$\overline{x} = 210.2627673$

$\sum e_i^2 = 0.000177$

$\sum (x_i - \overline{x})^2 = 247{,}992.2747$

$\sum x_i^2 = 911{,}148.7444$

Table 6. components of measurement uncertainty.

Uncertainty components	Value	Measurement uncertainty	Probability distribution	Type of uncertainty
A	0.17604	0.002942108	normal	A
a	0.001213	0.001826	normal	A
b	0.000807	0.0000074	normal	A
$C_{glucose\ solution}$	993.1937	1.48979	normal	A

Table 7. Data used in the measurement of uncertainty.

C_j	A_j	B_0	B_1	$B_1 * C_j$	$B_0 + B_1 * C_j$	$A_j - (B_0 + B_1 * C_j)$	$[A_j - (B_0 + B_1 * C_j)]^2$
49.6305	0.0449	0.001213	0.000807	0.040052	0.041264814	0.003635187	0.000013
49.6305	0.0459	0.001213	0.000807	0.040052	0.041264814	0.004635187	0.000021
49.6305	0.0468	0.001213	0.000807	0.040052	0.041264814	0.005535187	0.000031
100.1877	0.0789	0.001213	0.000807	0.080851	0.082064474	-0.003164474	0.000010
100.1877	0.0778	0.001213	0.000807	0.080851	0.082064474	-0.004264474	0.000018
100.1877	0.0793	0.001213	0.000807	0.080851	0.082064474	-0.002764474	0.000008
199.4895	0.1581	0.001213	0.000807	0.160988	0.162201027	-0.004101026	0.000017
199.4895	0.1599	0.001213	0.000807	0.160988	0.162201027	-0.002301027	0.000005
199.4895	0.1608	0.001213	0.000807	0.160988	0.162201027	-0.001401026	0.000002
300.9374	0.2469	0.001213	0.000807	0.242856	0.244069482	0.002830518	0.000008
300.9374	0.2402	0.001213	0.000807	0.242856	0.244069482	-0.003869482	0.000015
300.9374	0.2433	0.001213	0.000807	0.242856	0.244069482	-0.000769482	0.000001
401.0687	0.3268	0.001213	0.000807	0.323662	0.324875441	0.001924559	0.000004
401.0687	0.3289	0.001213	0.000807	0.323662	0.324875441	0.004024559	0.000016
401.0687	0.3252	0.001213	0.000807	0.323662	0.324875441	0.000324559	0.000000

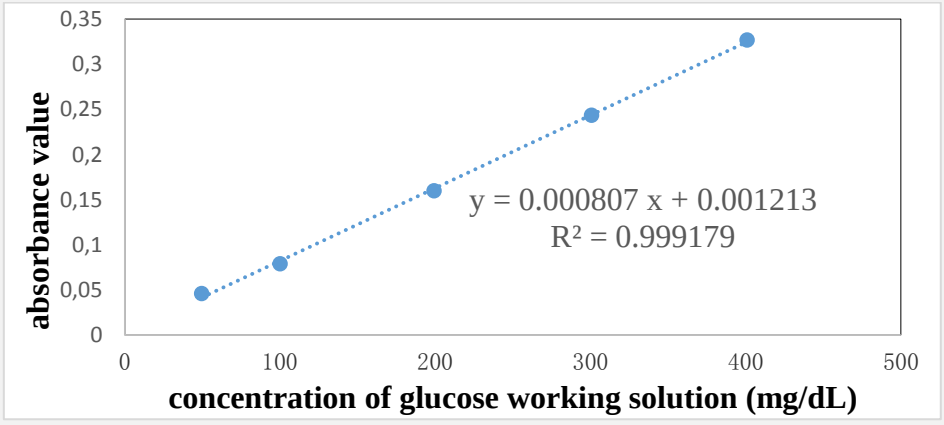

Figure 1. Standard curve of glucose measurement.

Measurement uncertainty of residual error:

$$s_{y/x} = \sqrt{\sum_{i=1}^{n} e_i^2 / (n-2)} = \sqrt{0.000177 / (15-2)} = 0.00369$$

Measurement uncertainty of slope:

$$s_b = \frac{s_{y/x}}{\sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2}} = \frac{0.00369}{\sqrt{247992.2747}} = 0.0000074$$

and $S_b\% = S_b/b \times 100\% = 0.916976\%$

Measurement uncertainty of intercept:

$$s_a = s_{y/x} \sqrt{\frac{\sum_{i=1}^{n} x_i^2}{n\sum_{i=1}^{n} (x_i - \bar{x})^2}} = 0.00369 \times \sqrt{\frac{911148.7444}{15 \times 247992.2747}} = 0.001826$$

and $r_{a,b} = -\bar{x}\frac{S_b}{S_a} = -210.2627673 \times \frac{0.0000074}{0.001826} = -0.8521$

The sample to be measured was also treated and the absorbance value of the measured sample was recorded as A. The value of glucose concentration in the sample could be calculated through the standard curve. The absorbance value of sample was tested 10 times and the results were shown in Table 5. All the components of the uncertainty were combined to obtain the measurement uncertainty according to Table 6. Because the value of intercept was small, the measurement uncertainty was not considered in the evaluation process.

$\%u_c = \sqrt{1.671^2 + 0.916976^2 + 0.15000015^2} = 1.912\%$

U_c = 1.912% × 12.02 mmol/L = 0.2298 mmol/L

U_{rel} = 0.2298 mmol/L × 1.96 = 0.450408 mmol/L

Method introduced by quantifying uncertainty in analytical measurement (QUAM)

According to our standard curve, the value of intercept (B0) was 0.001213 and the value of slope (B1) was 0.000807. The measurement uncertainty was evaluated according to Table 7, where Cj was the values of calibrated concentration of glucose working solutions, Aj was the absorbance values of glucose working solutions.

$$S = \sqrt{\frac{\sum_{j=1}^{n} [A_j - (B_0 + B_1 \times C_j)]^2}{n-2}} = 0.0036038313$$

$$S_{xx} = \sum_{j=1}^{n} (C_j - \bar{c})^2 = 82664.101996152$$

$$u(c) = \frac{S}{B}\sqrt{\frac{1}{P} + \frac{1}{n} + \frac{(c-\bar{c})^2}{S_{XX}}} = 1.8298805777$$

The factors considered in the evaluation of measurement were the concentration of the glucose solution, the absorbance of the samples, and the fitting of standard curve (intercept and slope).

$\%u_c = \sqrt{(\frac{1.8298805777}{216.638} \times 100\%)^2 + 1.671^2 + 0.15000015^2}$ = 1.878%

U_c = 1.878% × 12.02 mmol/L = 0.2257 mmol/L

U_{rel} = 0.2257 mmol/L × 1.96 = 0.442372 mmol/L

DISCUSSION

At present the most authoritative guide in the evaluation of the measurement uncertainty is the "Guide to the expression of uncertainty in measurement" (GUM). In this paper, measurement uncertainty was evaluated according to the principle expressed in GUM. At the same time, this paper mainly studied the measurement uncertainty of building the standard curve, so the principle in the European association for chemical documents issued by the quantification of the Uncertainty of Measurement (Quantifying Uncertainty in Analytical Measurement, QUAM) was also adopted.

Standard curve method is widely used in various industries, the evaluation of the measurement uncertainty of linear fitting has been widely researched [13]. It has been widely applied in the field of clinical laboratory. Dissolving the reagents and samples, dilution of samples, and building standard curves are common operations in our daily work. What bothers us is how to obtain accurate results [14-16]. In this paper we tried to evaluate the measurement uncertainty according to the measurement model. The absorbance value, linear fitting, dissolution, and dilution process were mainly considered in the process of measurement uncertainty evaluation.

In this study, we tried to build the standard curve according to the calibrated concentration of a glucose working solution. In the process, the weight method was used in the dissolving and dilution of the glucose reference material. This way the testing results could be more accurate. Our results showed that the dilution process of the sample results in measurement uncertainty to a certain extent. During our work this source of uncertainty should be considered and the calibration process is necessary.

For the study of uncertainty in the process of linear fitting, we need to consider many factors: whether the fitting result is in accordance with the requirements, whether the slope and intercept are related, and so on [17,18]. However, in the field of laboratory medicine, related contents of research were rarely reported. According to the literature, the precondition of linear fitting is that the uncertainty of reference material is small enough [19-21]. In our experiment the certified refer-

ence material of glucose was used to build the standard curve. Its uncertainty was 0.3%, small enough to meet the requirements. Two methods were used to evaluate the measurement uncertainty in the liner fitting. The results showed that they have good consistency. In our daily work, they should be selected according to our needs.

Declaration of Interest:

The authors declare that they have no competing interests.

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