Comparison of the Precision of Measurements in Three Types of Micropipettes according to NCCLS EP5-A2 and ISO 8655-6

Hamid Alavi Majd[1,*], Jamal Hoseini[1], Hossein Tamaddon[2], Alireza Akbarzadeh Baghban[1]

[1] Department of Biostatistics, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Lab Sciences, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[*]Corresponding Author: e-mail address: alavimajd@gmail.com (H. Alavi- Majd)

ABSTRACT

Micropipettes or piston pipettes are used to make most volume measurements in fields such as health, chemistry, biology, pharmacy and genetics. Laboratories must ensure that results obtained using these instruments are reliable; therefore, it is necessary to calibrate micropipettes. Before the start of the calibration process**,** we must check the precision of measurements. The objective of this work is to compare several methods for calculating the precision of three kinds of micropipettes according to the reference value in ISO 8655-6. The medical tests will not have accurate results, if the volume of the liquid doesn't transfer precisely by micropipettes. Thus, the physician might potentially face problems in the disease diagnosis and its control. In the NCCLS EP5-A2, there is a method to specify and assess the precision of micropipettes by using CV (Coefficient of Variation). Also there are other methods to estimate and test the CV theory, in the formal statistics texts which could be applied to assess the micropipettes precision. In this research we evaluate the precision of lab micropipettes. Three brands of micropipettes, A, B and C are assigned to measure the distilled water mass by using accurate scale which is accurate up to 10^{-6} to measure 50-gram weights. The experimental environment is a metrology lab which is approved by Iran Standard and Industrial Researches Organization. A technician sampled at the beginning of the experiment and then after 2 hours, the same technician repeated the sampling. Overall, each micropipette is used to measure 40 times with 10-repeat times for single measurement in 28 work days. Common statistical methods are used to estimate and test the CV. Point estimation of CV for micropipettes A, B and C were 0.50%, 0.64% and 1.56%, respectively. Furthermore, the upper limit of 95% confidence bounds for these three micropipettes using the exact method were 0.53%, 0.69% and 1.65%, respectively. Micropipette A met the ISO 8655-6 standard level, but micropipettes B and C did not. On average**,** measurement errors in micropipettes B and C were respectively 30% and 3.11 times more than micropipette A. By using the approach of CLS EP5-A2 and confidence interval for CV, precision of the three micropipettes were compared. Only one of them met the ISO 8655-6 standard level, but the others failed.

Keywords: Coefficient of Variation; Micropipette; Measurement Error; ISO 8655-6; NCCLS EP5-A2

INTRODUCTION

Evaluation and monitoring the precision of medical laboratory equipment are very important. Ignoring the monitoring and control of measurement errors in laboratory equipment may reduce the accuracy of experiment results. In addition, before calibrating the equipment, we must check the precision of measurements. NCCLS EP5-A2 has proposed an exact method for evaluating the precision of laboratory equipment measurements [1]. Measurement errors are divided in two parts*:* systematic and random. Precision depends only on the distribution of random errors and is not related to the true value or the specified values. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation or as a CV (Coefficient of Variation) of the test results. According to ISO 5725-1, precision is the closeness of agreement between independent test results obtained under stipulated conditions and includes two components: repeatability and reproducibility [2]. In a variety of standard references such as ISO 5725-1 and ISO 2174-8, CV is used to compute the repeatability and reproducibility in order to control the measurement error in laboratory equipment. Less precision is reflected by large CV [2, &3]. CV is defined as the ratio of population standard deviation to its mean and estimated by sample CV. Iglewicz and Myers reviewed methods to estimate and compute the confidence interval for CV and suggested another method [4]. Also there are several methods which have proposed to estimate the value of CV [5-7]. Craig and Mark as well as Verrill and Johnson proposed some computational algorithms for CV confidence bounds [8, 9]. Tian used CV to evaluate precision and repeatability in medical research [10].

Micropipette is one of the most important tools in laboratories. Pipetting in the microliter range is now a current and necessary task for almost every field of chemistry. New dispensing systems allow experiments to be simpler and more automated, but at the same time new fields like genetics put heavier demands on the reliability of the results. It is; therefore, important to focus on calibration and uncertainty related with this kind of equipment. Before calibration we must check the precision of the equipment, because it is not possible to calibrate the micropipette precisely, if it does not have enough repeatability to transfer a determined volume of a liquid. Therefore, it is necessary to evaluate the repeatability of a micropipette. In conformity with Carl et al., distilled water was pipetted for several times; then by using very precise balances the weight of distilled water was measured [11]. The CV is applied to indicate precision and repeatability.

The main object of this paper is to evaluate and compare precision in three kinds of available micropipettes in medical labs according to NCCLS EP5-A2 by various statistical methods. Based on ISO 8655-6 reference value for CV was considered 0.006 [12].

MATERALS AND METHODS

In this paper, we used data obtained from an experiment to evaluate micropipette precision, based on NCCLS EP5-A2 by the gravimetric method. This method is a reference one which is recommended in ISO 8655-6 and applied to study the accuracy and precision of the micropipettes in the small volumes [11]. In the present research, three kinds of micropipettes which are common in medical diagnosis labs are used and are shown by A, B and C. A lab unit technician sampled the distilled water in a standard lab condition at the beginning of the work time and repeated the sampling two hours later. Overall, there were 40 measurements in 28 consecutive days, and in every measurement 10 times sampling was conducted for each three kinds of micropipettes. Based on ISO 8655-6, we used the precise balance named Prezia Model SMA-FR 262 with an accuracy of 10^{-6} to measure 50 grams, to weigh the mass of extracted distilled water by these micropipettes, based on ISO 8655-6. The research environment is one of the metrological labs approved by Iran Standard and Industrial Research Organization Inc. In order to transfer distilled water by micropipettes with a disposable tip, which are mostly made of polypropylene, and are attached to the micropipette, we sank the tip to the distilled water and when the water had reached the upper limit of the piston, the tip was taken out. According to ISO 8655-6, micropipettes were wetted 5 times to reach the equilibrium in humidity. Then, the tip was changed and was wetted again. The beaker net weight was measured, and then the distilled water in the pipette was ejected into the beaker. Again, the beaker weight was measured and the gained mass by comparison of these measurements was considered as transfer volume by the micropipette. This procedure was repeated 10 times and the beaker was cleaned after each volume increase. Formula (1) was used to determine the transferred volume by micropipettes was determined through the formula (1), suggested by ASTM.E.542-94 [13], and its relationship between the volume, mass, and density of the distilled water:

$$V_{20} = (\mathrm{I_L} - \mathrm{I_E}) \times \frac{1}{\rho_W - \rho_A} \times \left(1 - \frac{\rho_A}{\rho_B}\right) \times [1 - \gamma(t - 20)] \quad (1)$$

where:

V_{20}: volume (μl) in 20 degrees centigrade, $\mathrm{I_L}$: result of weighing after pipetting (mg), $\mathrm{I_E}$: result of weighing before pipetting (mg), ρ_W: water density $(mg/\mu l)$, ρ_A: air density $(mg/\mu l)$, ρ_B: density of mass pieces $(mg/\mu l)$, γ: mass cubic heat diastole coefficient of micropipette $(1/^oC)$, t: water temperature (℃). In equation (1), as the water density has fluctuations due to temperature and aerometer pressure, the fluctuation was taken into account when mass was being transformed into volume.

Statistical Methods

CV was considered as a criterion for precision. CV in population is defined by $R = \frac{\sigma}{\mu}$ (ratio of population standard deviation σ, to population mean μ). Because $E[\overline{X}] = \mu$ and $E[S^2] = \sigma^2$, therefore, $\frac{S}{\overline{X}}$ is a direct estimator for R. This estimator is used for measuring relative variation and is called Sample Coefficient of Variation (SCV). In this paper, for testing the hypothesis $R \leq R_0$, we used six different methods: Mc Key [14], David [15], Iglewicz and Myers [4], Craig and Mark [8], Lehman and Romano [16], Wei et al. [7].

Mc Key's Method

Based on Mc Key's method about an approximation of sampling CV [14], we have $(1+\frac{1}{R^2})\frac{(n-1)\frac{S^2}{\overline{X}^2}}{(1+(\frac{n-1}{n})\frac{S^2}{\overline{X}^2})} \sim \chi^2_{n-1}$, and a $100(1-\alpha)$ confidence interval for $R = \frac{\sigma}{\mu}$ is:

$$\left[\frac{\frac{S}{\overline{X}}}{\sqrt{\left(\frac{\chi^2_{n-1,1-\frac{\alpha}{2}}}{n}-1\right)\left(\frac{S}{\overline{X}}\right)^2+\frac{\chi^2_{n-1,1-\frac{\alpha}{2}}}{n-1}}}, \frac{\frac{S}{\overline{X}}}{\sqrt{\left(\frac{\chi^2_{n-1,\frac{\alpha}{2}}}{n}-1\right)\left(\frac{S}{\overline{X}}\right)^2+\frac{\chi^2_{n-1,\frac{\alpha}{2}}}{n-1}}}\right] \quad (2)$$

David's Method

David's method is similar to Mc Key's,

except $(1+\frac{1}{R^2})\frac{\frac{S^2}{\overline{X}^2}}{(1+\frac{S^2}{\overline{X}^2})} \sim \chi^2_{n-1}$ [15].

Iglewicz's and Myers' Method

Based on Iglewicz and Myers, $E\left[\frac{S}{\overline{X}}\right] \simeq R$ and $Var\left(\frac{S}{\overline{X}}\right) \simeq \frac{1}{2n}R^2(1+2R^2)$[4]. By normal assumption for distribution of $\frac{S}{\overline{X}}$, we have:

$$P\left(Z_{\frac{\alpha}{2}} \leq \frac{\frac{S}{\overline{X}}-R}{\frac{R}{\sqrt{2n}}\sqrt{1+2R^2}} \leq Z_{1-\frac{\alpha}{2}}\right) \simeq 1-\alpha \quad (3)$$

Adjusted Noncenteral t Method

Craig and Mark used $S_n = \left(\frac{n-1}{n}\right)S$ instead of S and presented a method to build a confidence interval based on noncenteral t distribution [8].

Noncenteral t distribution

Lehman and Romano assumed that $X_1, \ldots, X_n$ is a random sample from normal distribution, so $\sqrt{n}\frac{\overline{X}}{S} \sim t_{n-1}(\sqrt{n}\frac{\mu}{\sigma})$ and therefore:

$$P\left(t_{n-1,\frac{\alpha}{2}}\left(\sqrt{n}\frac{\mu}{\sigma}\right) \leq \sqrt{n}\frac{\bar{X}}{S} \leq t_{n-1,1-\frac{\alpha}{2}}\left(\sqrt{n}\frac{\mu}{\sigma}\right)\right) = 1-\alpha. \quad (4)$$

Then a confidence interval is obtained by using noncenteral t distribution [16].

Exact Method

Under the assumption of normality, Wei et al. by using the following theorem found a confidence interval for μ/σ and also for $R = \frac{\sigma}{\mu}$. Then they inversed the results:

Theorem: If $0 < \alpha < 1$, $n \geq 2$, $-\infty < \mu < +\infty$ and $\sigma > 0$, then

$$P\{\mu/\sigma \leq h_{n,1-\alpha}(\overline{X}/S)\} = 1-\alpha \quad (5)$$

$$P\{h_{n,\alpha}(\overline{X}/S) \leq \mu/\sigma\} = 1-\alpha \quad (6)$$

Where $h_{n,\alpha}(\overline{X}/S)$ is a monotone increasing function of $\overline{X}/S$ [7].

According to ISO 8655-6, reference value R_0 is equal to 0.006 [12]. By using the above mentioned statistical methods, we calculated 95% confidence upper bound for each micropipette. Then, based on the method proposed by Verrill and Johanson [9], we tested hypothesis of equality of CV in three micropipettes and we built 95% confidence bounds for ratio of each pairs of them. For testing the hypothesis $R \leq R_0$ in each micropipette by exact method, we used a FORTRAN program from Wei et al. [7]. Other calculations were done by using Craig and Mark [8].

Hypothesis test for equality of CV in three micropipettes and confidence bounds for the ratio of each pair of them were obtained by a FORTRAN program from Verrill and Johanson [9].

RESULTS

The main goal of this paper is to evaluate the precision of three kinds of micropipettes, usually used in medical laboratories and compare it with a reference value based on ISO 8655-6. Before statistical analysis, we should check our data for outlier values detection. We used Dixon test to detect outlier values based on Ozanne [17]. This test indicated that there were no outlier values in our data. Assumption of normality was checked by Kolmogorov-Smirnov nonparametric test. As we can see in Table 1, the fluctuation in CV for micropipettes A and B is approximately similar, but they are different for micropipette C. Also CV in micropipette C is approximately 2 and 3 times greater than micropipettes A and B, respectively.

Table 1. Descriptive statistics for CV in 40 times measurements

Micropipette	Maximum	Minimum	Mean	Range	SD
A	0.00792	0.00187	0.00394	0.00605	0.0014
B	0.01149	0.00227	0.00537	0.00921	0.0019
C	0.02029	0.00229	0.00677	0.01800	0.0035

Coefficients of variation for micropipette A in 40 runs are more stable and are less than the values for micropipette B. In Figure 1, it is shown that the values of CV for micropipettes A and B are very similar and most of them are less than the reference value according to ISO 8655-6, but the values of CV for micropipette C is clearly different from the other two.

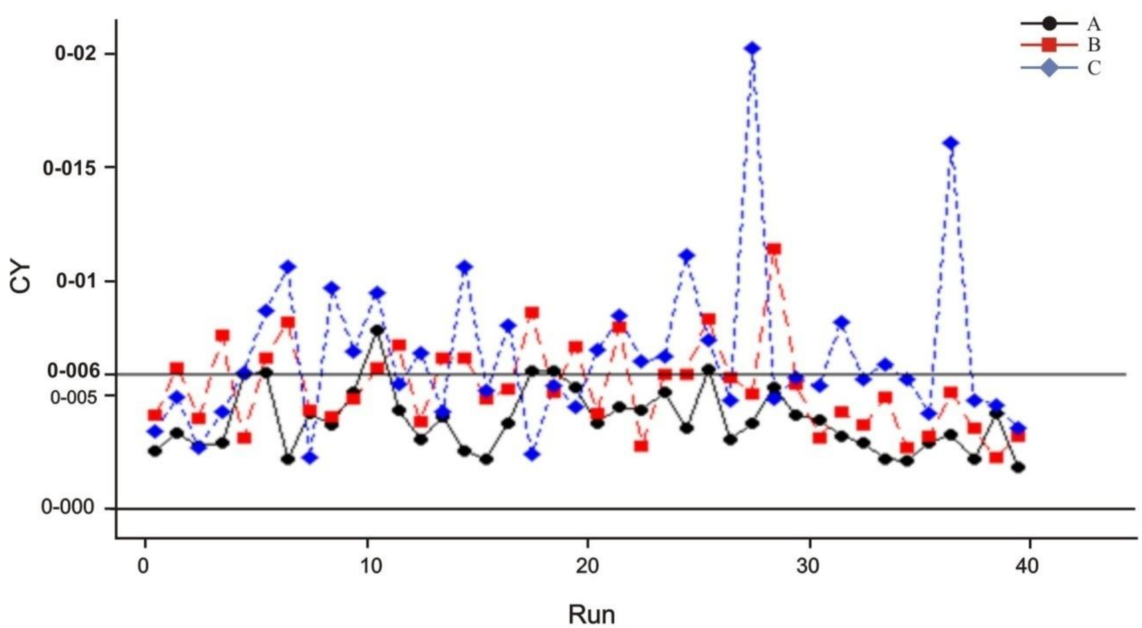

Figure 1. Values of CV for three micropipettes in 40 runs

Point estimations for CV in micropipettes A, B and C were 0.005, 0.0064 and 0.0156, respectively. Upper limit of 95% confidence interval for three kinds of micropipettes by using six statistical methods are shown in Table 2. As we can notice in Table 2, the reference value of 0.006 is not in the confidence interval of micropipette A, but it is in confidence interval of micropipettes B and C. Therefore, we can conclude that micropipette A conforms to ISO 8655-6, but micropipettes B and C do not conform to this standard.

Table 2. Upper limit of 95% confidence interval for micropipettes A, B and C

Estimation Method	Micropipette A	Micropipette B	Micropipette C
Mc Key	0.0053779	0.007035	0.0194952
Daivid	0.0053865	0.007048	0.0196133
Iglewicz and Myers	0.0053721	0.007005	0.0178149
Craig and Mark	0.0053725	0.007013	0.0180779
Lehman and Romano	0.0053793	0.007022	0.0181005
Wei et al.	0.0053240	0.006893	0.0165800

Hypothesis test for equality of CV in the three kinds of micropipettes showed that there is significant difference among these micropipettes ($p<0.05$).

In Table 3, 95% confidence bounds are presented for the ratio of each pair of micropipettes. The value of 1 is not present in any of the confidence bounds, thus we can conclude that the most precise micropipette is A ($p<0.05$). Point estimation for the CV ratio in micropipette B to A is equal to 1.295; therefore, it can be concluded that CV in micropipette B is approximately 30 percent greater than micropipette A. Point estimation for the r CV ratio in micropipette C to A is equal to 3.114, so it means that precision of micropipette A is approximately 3 times greater than the precision of micropipette C. Also micropipette B is significantly more precise than micropipette C ($p<0.05$). Point estimation for the CV ratio in micropipette C to B is equal to 2.405, so one can conclude that the precision of micropipette B is approximately 2 times greater than the precision of micropipette C.

Table 3. Maximum likelihood estimations (MLE) and 95% confidence bounds for the ratios of CV in three kinds of micropipettes

Ratio of CVs	MLE	95%CI
B / A	1.295	(1.138 , 1.473)
C / A	3.114	(2.737 , 3.452)
C / B	2.405	(2.114 , 2.736)

DISCUSSION

In the present paper, we computed the precision of three kinds of micropipettes usually used in medical labs, by gravimetric method and compared them with a reference value according to ISO 8655-6 [12]. CV was considered as an index for quantifying the precision. Six statistical methods were used for estimation and hypothesis test about the CV. Based on upper limit of 95% confidence interval for CV in each micropipette; we concluded that the micropipette A is more precise than the others. Many other researchers have conducted methods to assess the precision and accuracy of lab equipment, for example using PIPETTE software, Ozanne evaluated the precision and accuracy of lab micropipettes [17]. Bastista et al. used four gravimetric methods for calibrating micropipettes according to several ISO standards [18]. Also, Bastista et al. examined and compared the calibration of 1000 micro-liter micropipettes in six regional metrological institutes [19].

Estimation and hypothesis test methods proposed by Vangel [5] and Wei et al. [7] were used in the present research. Also some algorithms based on Craig and Mark [8] as well as Verrill and Johanson [9] were applied for some calculations. Results showed that the precision of micropipette A was significantly more than the others ($p<0.05$). CV as an indicator for random measurements in micropipette B was 30 percent greater than micropipette A. In general, micropipette A was more precise than the other two.

In view of the importance of precision in micropipette measurement and its influence on the results of experiments in medical labs, it is necessary to study about evaluation and also modeling the relation between measurement error of micropipette and results of experiments.

The conclusion of the present research has indicated that some of the available micropipettes, are not in conformance with the related standards, and careful monitoring is needed in this subject.

ACKNOWLEDGEMENT

This research was performed by the cooperation between the Department of Biostatistics in Shaid Beheshti University of Medical Sciences and Pishgaman Andazeshenasi Daghigh (PAD), an approved metrological company in Tehran. The authors are grateful to the director and personnel of the company for providing the research budget and their invaluable cooperation.

REFERENCES

1. NCCLS EP5-A2:2004. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, Second Edition. NCCLS, Pennsylvania, USA.
2. ISO 5725-1:1994: Accuracy (Trueness and Precision) of Measurement Methods and Results, Part 1: General Principles and Definitions. International Organization for Standardization, Geneva, Switzerland.
3. ISO 2174-8:2004: Guidance for the Use of Repeatability, Reproducibility, and Trueness Estimates in Measurement Uncertainty Estimation. International Organization for Standardization, Geneva, Switzerland.
4. Iglewicz B, Myers R.H. Comparisons of approximations to the percentage points of the sample CV. Technometrics 1970; 12: 166-169.
5. Vangel MG. Confidence interval for a normal coefficient of variation. The American Statistician 1996; 50: 21-26.
6. Wong ACM, Wu J. Small sample asymptotic inference for the coefficient of variation: normal and no normal models. Journal of Statistical Planning and Inference 2002; 104: 73– 82.
7. Wei L, Wan K.P, Wei K.H. Exact Confidence Bounds for the Coefficient of Variation of a Normally Distributed Population. International Journal of Statistics and Systems 2006; 1: 81-86.
8. Craig A.W, Mark E.P. modeling the coefficient of variation in factorial experiments. Communication in Statistics Theory and Methods 2002; 31(3): 463-476.
9. Verrill S, Johanson R.A. Confidence Bounds and Hypothesis Tests for Normal Distribution Coefficients of Variation. Communication in Statistics – Theory and Methods 2007; 36: 2187-2206.
10. Tian L. Inferences on the Common Coefficient of Variation. Statistics in Medicine 2005; 24: 2213-2220.
11. Carl A.B, Edward R.A, David E.B. TITZ Textbook of clinical chemistry and molecular diagnostics. Fourth ed. Elsevier: 2006.
12. ISO 8655-6:2002: Piston-operatic volumetric apparatus-part2: Piston pipettes, International Organization for Standardization, Geneva, Switzerland.
13. ASTM E 542-94:1994: Standard Practice for Calibration of Laboratory Volumetric Apparatus. American Society for Testing and Materials, West Conshohocken, USA.
14. McKay A.T. Distribution of the Coefficient of Variation and the Extended *t* Distribution.

Journal of the Royal Statistical Society 1932; 695–699.

15. David FN. Note on the application of Fisher's k-statistics. Biometrika 1949; 36: 383–393.

16. Lehmann E.L, Romano J.P. Testing Statistical Hypotheses. Third Edition, New York: Springer; 2005.

17. Ozanne G. Computer Program to evaluate the accuracy and the precision of micropipettes. Journal of Microbiological Methods 1995; 21: 33-43.

18. Bastista E, Sabrosa A, Ferriera M, Castanheira I and Van der Veen A.H.M. Calibration of micropipette: Test methods and uncertainty analysis. Measurement 2007; 40: 338-342.

19. Bastista E, Filipe E, Mickan B. Volume Calibration of 1000 µl Micropipettes, Inter-Laboratory Comparison. Accreditation and Quality Assurance 2008; 13: 261–266.