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Calibration Methods for Micro-Volumetric Apparatus

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Calibration Methods for Micro-Volumetric Apparatus K 0971-1989

1. Scope

This Japanese Industrial Standard specifies the calibration methods for micro-volumetric apparatus of capacity 5 to 5000 μl , hereinafter referred to as the "volumeter".

2. Definitions of Terms

For the purpose of this Standard, the following definitions apply. Other terms and definitions are given in JIS K 0211, JIS K 7109, JIS Z 8101 and JIS Z 8103.

- (1) volumeter This designates the volumeter in which the liquid of set definite capacity is able to be sucked and emitted by the operation of movable mechanism and there are variable capacity type, preset capacity type and fixed capacity type.
- (2) preset capacity type volumeter Volumeter with the method to fix the capacity by a clamping mechanism and equipped with plural clamps so as to be able to select the capacity.

3. Measuring Method for Calibration of Volumeter

3.1 Synopsis As for the liquid to be used, the water (distilled water or ion exchange water) shall be, in general, used⁽¹⁾ and the emitted mass of sucked water shall be measured to determine the volume by dividing with density.

Note ⁽¹⁾ In the case where the liquid other than water is used as liquid to be used, the density measured according to JIS Z 8804 shall be used.

Applicable Standards:

JIS K 0211-Technical Terms for Analytical Chemistry (General Part)
JIS K 7109-General Tolerancing Rules for Plastics Dimensions
JIS R 3503-Glass Apparatus for Chemical Analysis
JIS Z 8101-Glossary of Terms Used in Quality Control
JIS Z 8103-Glossary of Terms Used in Instrumentation
JIS Z 8804-Methods of Measuring Specific Gravity of Liquid

Reference Standard:

JIS K 0970-Piston Operated Micro-Volumetric Apparatus

3.2 Apparatus and Water

- (1) Chemical Balance As for chemical balance⁽²⁾, that with minimum scale value of $\frac{1}{1000}$ of the mass of water same as the setted value of volumeter to be calibrated or 10 μg or under and calibrated periodically (at every 3 months) with the grade 1 standard weight based on the Law of Metrology shall be used.

Note ⁽²⁾ An electronic force balance may be used.
- (2) Weighing Bottle As for weighing bottle, the cylindrical weighing bottle specified in JIS R 3503 or that with construction equivalent to this shall be used.
- (3) Thermometer The thermometer shall be that with the minimum scale value of 0.2°C or under.
- (4) Water The water shall be the distilled water or ion exchange water as given in the following.
 - (a) Distilled Water Use that of conductivity 2 $\mu\text{S}/\text{cm}$ (25°C)⁽³⁾ or under and with holding time of colour generated by potassium permanganate⁽⁴⁾ 60 min or over.
 - (b) Ion Exchange Water Refine with using the mixture of strongly acidic cation exchange resin (hydrogen ion type) and strongly basic anion exchange resin (Type I, hydroxide ion type) to make the conductivity to 1 $\mu\text{S}/\text{cm}$ (25°C)⁽³⁾ or under and the holding time of color generated by potassium permanganate⁽⁴⁾ to 60 min or over and filter with filter membrane with hole diameter 0.45 μm to use.

Notes ⁽³⁾ Flow the distilled water or ion exchange water directly through the liquid flowing type cell to measure the conductivity.

⁽⁴⁾ This designates the time since 0.2 ml of potassium permanganate solution ($\frac{N}{100}$) and 2 ml of sulfuric acid (1 + 1) added to 500 ml of water have been mixed by stirring sufficiently and are allowed to stand still until the color disappeared.

3.3 Test Conditions The test conditions shall be as follows:

- (1) Test Room Temperature This shall be the room temperature adjustable within $\pm 2^\circ\text{C}$.
- (2) Environment This shall be the place free from vibration and direct effect of wind of air conditioning.

3.4 Operation The operation shall be as follows:

- (1) Place the chemical balance, volumeter, water, weighing bottle, etc. in the test room since previous day to make constant temperature preliminarily.

- (2) Select the weighing bottle according to the capacity of water to be measured⁽⁵⁾.

Note ⁽⁵⁾ As for the size of weighing bottle, use that of volume within 50 times of setted capacity (when the setted volume is small, minimal obtainable one).

- (3) Read out the water temperature by thermometer.
- (4) Add water in the bottom of weighing bottle so that the depth may be of the order of 2 mm, put cover and measure the mass (tare) (W_1)⁽⁶⁾.

Note ⁽⁶⁾ The operation of chemical balance shall be conducted according to the operation manual instruction of manufacturer.

- (5) Set the scale of volumeter to the capacity desired to emit and suck the water in volumeter by holding vertically. Next, take off the cover of weighing bottle weighed in (4), put the top end of tip lightly to its inner wall to emit the water and then put the cover to weighing bottle⁽⁷⁾⁽⁸⁾.

Notes ⁽⁷⁾ The time since the taking off of cover until the putting of cover shall be short as far as possible and approximately constant.

⁽⁸⁾ The details of operation of volumeter shall comply with the operation manual instruction of manufacturer.

- (6) Measure the mass of weighing bottle having been added with water (W_2).
- (7) Determine the volume of emitted water by the following formula:

$$V = \frac{W_2 - W_1}{d_w} \times \left[1 + \rho_a \left(\frac{1}{d_w} - \frac{1}{\delta} \right) \right]$$

where

V : volume of emitted water (ml)

d_w : density of water⁽⁹⁾⁽¹⁰⁾ (g/ml)

W_1 : mass of weighing bottle (tare) added with water in depth of order of 2 mm (g)

W_2 : W_1 and mass of water emitted from volumeter (g)

ρ_a : density of air (g/ml) (take as $\rho_a = 0.0012$ g/ml)

δ : density of weight of chemical balance (g/ml)

Notes ⁽⁹⁾ Determine the density of water from the table of water density of the year table of science (RIKA NENPYO).

⁽¹⁰⁾ In the case of used liquid other than water, measure the density d' according to Note⁽¹⁾ and replace d_w with d' .

4. Calibration Methods

The calibration method shall comply with the following (1) or (2):

(1) Calibration at Mean The calibration at mean shall be as follows:

- (a) Take the setted scales to be used for calibration in the neighbourhood of position of scale to be practical used. Determine the scale ranges of volumeter for calibration as $M_1, M_2, \dots, M_k$ (¹¹)(¹²).
- (b) Determine the emitted volumes at setted scales $y_1, y_2, \dots, y_k$ according to 3.
- (c) Determine the estimate of volume at setted scale (M') by the following formula to calibrate.

$$\hat{y} = M' - \bar{M} + \bar{y}$$

where $\hat{y}$: estimate of volume when liquid is emitted at setted scale (M') (μl)

M' : setted scale to be used for practical emission (μl)

$\bar{M}$: mean value of setted scales (M_i) (μl)

$$\bar{M} = \frac{M_1 + M_2 + \dots + M_k}{k}$$

$\bar{y}$: mean value of emitted volumes (y_i) at setted scales (M_i) (μl)

$$\bar{y} = \frac{y_1 + y_2 + \dots + y_k}{k}$$

Notes (¹¹) The setted scales may be the full range of scale to be used or one point.

(¹²) Conduct this calibration at mean for fixed volume type volumeter at its proper scale and for preset volume type volumeter at every scale to be used.

Informative Reference: Example of the Calibration at Mean

In the case where the measurements were repeated twice at setted value 20 μl and $M_1=20, y_1=19.97\mu\text{l}, M_2=20, y_2=19.90\mu\text{l}$ are obtained.

$$\bar{M} = \frac{20+20}{2} = 20$$

$$\bar{y} = \frac{19.97+19.90}{2} = 19.94$$

Determine the estimate of volume by the following formula:

$$\hat{y} = M' - 20 + 19.94 = (M') - 0.06 (\mu\text{l})$$

Determine the estimate of value when the liquid is emitted at the setted anew scale $M'=20$ by this formula as follows:

$$\hat{y} = 20 - 0.06 = 19.94 (\mu\text{l})$$

(2) Calibration by Linear Formula ($k > 2$) The calibration by linear formula shall be as follows:

- (a) Take the setted scales to be used for calibration in the neighbourhood of position of scale to be practical used. Determine the scale ranges of volumeter for calibration as $M_1, M_2, \dots, M_k$ (¹³).
- (b) Determine the emitted volumes at setted scales $y_1, y_2, \dots, y_k$ according to 3.1.
- (c) Determine the estimate of volume at setted scale (M') by the following formula to calibrate.

$$\hat{y} = \bar{y} + \hat{\beta}(M' - \bar{M})$$

where $\hat{y}$: estimate of volume when liquid is emitted at setted scale (M') (μl)

M' : setted scale to be used for practical emission (μl)

$\bar{M}$: mean value of setted scales (M_i) (μl)

$\bar{y}$: mean value of emitted volumes y_i at setted scales (M_i) (μl)

$\hat{\beta}$: estimate of sensitivity of proportionality (μl)

Calculate $\hat{\beta}$ by the following formula:

$$\hat{\beta} = \frac{(y_1 - \bar{y})(M_1 - \bar{M}) + (y_2 - \bar{y})(M_2 - \bar{M}) + \dots + (y_k - \bar{y})(M_k - \bar{M})}{(M_1 - \bar{M})^2 + (M_2 - \bar{M})^2 + \dots + (M_k - \bar{M})^2}$$

Note (¹³) In the case where the data are determined repeatedly at the same setted scale, treat the repetition as independent datum.

Informative Reference: Example of the Calibration by Linear Formula
In the case where in the range of scale 5 to 20 μl the setted value $M_1 = 5, y_1 = 4.9 \mu\text{l}$, $M_2 = 10, y_2 = 9.7 \mu\text{l}$, $M_3 = 20, y_3 = 19.5 \mu\text{l}$ were obtained.

$$\bar{M} = \frac{5+10+20}{3} = 11.67$$

$$\bar{y} = \frac{4.9+9.7+19.5}{3} = 11.37$$

$$\hat{\beta} = \frac{(4.9-11.37)(5-11.67) + (9.7-11.37)(10-11.67) + (19.5-11.37)(20-11.67)}{(5-11.67)^2 + (10-11.67)^2 + (20-11.67)^2} = 0.974$$

Determine the estimate of volume ($\hat{y}$) by the following formula:

$$\hat{y} = 11.37 + 0.974(M' - 11.67) (\mu\text{l})$$

From this formula, the esteem of volume when the liquid is emitted at the setted anew value $M' = 20$ ($\hat{y}$) is as follows:

$$\hat{y} = 11.37 + 0.974(20 - 11.67) = 19.48 (\mu\text{l})$$

Informative Reference 1 Relation between Calibration and Errors of Volumeter

1. At the Beginning

This Informative Reference indicates the determination of errors required at the judgement of necessity of calibration, evaluation of effectivity of calibration, etc.

2. Determination of Errors of Emitted Volumes

2.1 Determination of Errors of Calibrating Operation As the error of calibrating operation when the user calibrates, determine the estimate of error variance ($\hat{\sigma}^2$) according to 4.

2.2 Determination of Error in Practical Use In order to determine preliminarily the error to be thought of the generation by the using condition and environment when the user uses the volumeter, take up the quantities influencing the emitted volume as error factors and determine the estimate of error variance ($\hat{\sigma}'^2$) as the error at use according to 5.

2.3 Determination of Error of Standard This designates the error of the value of volume to be the reference of calibration when the calibration is conducted and take as this error of standard the error composed of error of chemical balance and error of density to be the base for the determination of value of volume and determine the estimate of this error variance ($\hat{\sigma}_0^2$) according to 6. However, in the case where the error of standard is not determined, the rightful error of emitted volume is not taken as to have been determined.

3. Indication of Error of Emmitted Volume

3.1 Indication of Error in the Case when the Calibration Is Conducted The error of estimate of emitted volume ($\hat{y}$) contains the error of calibrating operation, error in practical use and error of standard. The calculation of error of emitted volume shall, in general, comply with the 2 sigma method utilizing the additivity of these error variances as shown in the following.

The 2 sigma designates the approximate existence range of emitted volume. Namely, taking up the error variance of calibration error ($\hat{\sigma}^2$), error variance in practical use ($\hat{\sigma}'^2$) and error variance of standard ($\hat{\sigma}_0^2$) determined according to 4. to 6., the error of emitted volume integrating these ($\hat{\sigma}_T$) and estimate of volume ($\hat{y}$) shall be indicated as follows:

$$\hat{y} \pm 2\hat{\sigma}_T$$

where
$$\hat{\sigma}_T = \sqrt{\hat{\sigma}^2 + \hat{\sigma}'^2 + \hat{\sigma}_0^2}$$

Example: In the case where the error variances determined according to 4. to 6. are $\hat{\sigma}^2 = 3.03$, $\hat{\sigma}'^2 = 4.18$, $\hat{\sigma}_0^2 = 0.98$ in each and the estimate by calibration by linear formula is ($\hat{y}$) = 504, the indication is as follows:

$$504 \pm 5.7 \mu\text{l}$$

$$[504 \pm 2 \sqrt{3.03 + 4.18 + 0.98}]$$

3.2 Indication of Error in the Case of Use without Calibration The error variance of use (σ'^2) determined according to 5. shall be used and the estimate of volume ($\hat{v}$) shall be indicated as follows.

$$M \pm 2\sqrt{\sigma'^2} (\mu\text{l})$$

4. Determination of Error of Calibrating Operation of Volumeter

4.1 Method of Measurement to Determine Error

4.1.1 Selection of Setted Scales and Levels Take the setted scales used in the calibration $M_1, M_2, \dots, M_k$ as levels.

4.1.2 Selection of Error Factors and Levels Take up as the error factors (R) not the error factors companying with measuring operation but the factors causing differences in the situation of calibrating operation. Take up as levels preferably three levels or over. It is allowed to take by combining error factors.

Example: There are the calibration operators and repetition distant by day(s) and 4 levels of two calibration operators and repetition of twice had been taken.

4.1.3 Measurement Conduct the measurements assigned with setted scales, error factors and repetition of twice to conduct continuously the suction and emission to determine the emitted volumes (y) as shown in Informative Reference 1 Table 1.

Informative Reference 1 Table 1. Table of Measurement and Measured Data

Error factor	Repetition	Setted scales				
		M_1	M_2	M_3	...	M_k
R_1	1	y_{111}	y_{211}	y_{311}	...	y_{k11}
	2	y_{112}	y_{212}	y_{312}	...	y_{k12}
R_2	1	y_{121}	y_{221}	y_{321}	...	y_{k21}
	2	y_{122}	y_{222}	y_{322}	...	y_{k22}
.	.	.	.	.	.	...
.	.	.	.	.	.	...
.	.	.	.	.	.	...
.	.	.	.	.	.	...
.	.	.	.	.	.	...
.	.	.	.	.	.	...
R_r	1	y_{1r1}	y_{2r1}	y_{3r1}	...	y_{kr1}
	2	y_{1r2}	y_{2r2}	y_{3r2}	...	y_{kr2}

Example: For the volumeter of 100 to 1000 μl , the data of Informative Reference 1 Table 2 have been obtained.

Informative Reference 1 Table 2. Example of Measured Data of Volumeter of 100 to 1000 μl

Error factor	Re-petition	Setted scales		
		$M_1=200 \mu\text{l}$	$M_2=500 \mu\text{l}$	$M_3=1\ 000 \mu\text{l}$
R_1	1	196.4	497.6	995.3
	2	199.3	498.8	998.5
R_2	1	197.2	496.1	996.0
	2	197.8	498.0	993.9
R_3	1	198.7	493.9	998.5
	2	199.6	496.1	996.3

4.2 Calculation of Errors

4.2.1 Preparation of Table of Deviations Determine the value of deviation $y' = y - M$ by the Informative Reference 1 Table 2 and array as shown in Informative Reference 1 Table 3.

Informative Reference 1 Table 3. Values of Deviations $y' = y - M$

Error factor	Re-petition	Setted scales				
		M_1	M_2	M_3	...	M_k
R_1	1	y_{111}'	y_{211}'	y_{311}'	...	y_{k11}'
	2	y_{112}'	y_{212}'	y_{312}'	...	y_{k12}'
R_2	1	y_{121}'	y_{221}'	y_{321}'	...	y_{k21}'
	2	y_{122}'	y_{222}'	y_{322}'	...	y_{k22}'
.		.	.	.	...	.
.		.	.	.	...	.
.		.	.	.	...	.
R_r	1	y_{1r1}'	y_{2r1}'	y_{3r1}'	...	y_{kr1}'
	2	y_{1r2}'	y_{2r2}'	y_{3r2}'	...	y_{kr2}'
Sum		y_1'	y_2'	y_3'	...	y_k'

Example: Example of measured data of volumeter of 100 to 1000 μl

Informative Reference 1 Table 4. Deviations obtained from Informative Reference 1 Table 2

Error factor	Repetition	Setted scales		
		200 μl	500 μl	1 000 μl
R_1	1	- 3.6	- 2.4	- 4.7
	2	- 0.7	- 1.2	- 1.5
R_2	1	- 2.8	- 3.9	- 4.0
	2	- 2.2	- 2.0	- 6.1
R_3	1	- 1.3	- 6.1	- 1.5
	2	- 0.4	- 3.9	- 3.7
Sum		-11.0	-19.5	-21.5

4.2.2 Calculation of Error Variances of Calibrating Operation Determine the error variance of calibrating operation (σ^2) by the following procedure:

(1) In the Case of the Calibration at Mean

$$S_m = \frac{(y_1' + y_2' + \dots + y_k')^2}{2 \times k \times r}$$

$$S_T = y_{111}'^2 + y_{112}'^2 + \dots + y_{kr2}'^2$$

$$V_e = \frac{S_T - S_m}{2 \times k \times r - 1}$$

Determine the error variance of calibrating operation by the following formula:

$$\sigma^2 = V_e (\mu\text{l})^2$$

(2) In the Case of the Calibration by Linear Formula

$$\bar{M} = \frac{M_1 + M_2 + \dots + M_k}{k}$$

$$\bar{y} = \frac{y_1' + y_2' + \dots + y_k'}{2 \times k \times r}$$

$$S_m = \frac{(y_1' + y_2' + \dots + y_k')^2}{2 \times k \times r}$$

$$S_\beta = \frac{[(y_1' - \bar{y})(M_1 - \bar{M}) + (y_2' - \bar{y})(M_2 - \bar{M}) + \dots + (y_k' - \bar{y})(M_k - \bar{M})]^2}{2 \times r [(M_1 - \bar{M})^2 + (M_2 - \bar{M})^2 + \dots + (M_k - \bar{M})^2]}$$

$$V_e = \frac{S_T - S_m - S_\beta}{2 \times k \times r - 2}$$

where $\bar{y}$: mean emitted volume of data (μl)

S_m : magnitude of effect of general mean (μl)

S_T : magnitude of total effect contained in data (μl)

S_β : magnitude of effect of proportionality to setted scales (μl)

V_e : error variance (μl)²

Determine the error variance of calibrating operation (σ^2) by the following formula:

$$\sigma^2 = V_e(\mu l)^2$$

Example: Determine the error variances of calibrating operation in the calibration at mean and the calibration by linear formula from the deviations in Informative Reference 1 Table 4.

(1) In the Case of the Calibration at Mean

$$S_m = \frac{[(-11.0) + (-19.5) + (-21.5)]^2}{3 \times 3 \times 2} = 150.22$$

$$S_T = (-3.6)^2 + (-0.7)^2 + \dots + (-3.7)^2 = 200.30$$

$$V_e = \frac{200.30 - 150.22}{18 - 1} = 2.95$$

Determine the error variance of calibrating operation (σ^2) by the following formula:

$$\sigma^2 = V_e(\mu l)^2 = 2.95(\mu l)^2$$

(2) In the Case of the Calibration by Linear Formula

$$\bar{M} = \frac{200 + 500 + 1\,000}{3} = 566.67$$

$$\bar{y} = \frac{(-11.0) + (-19.5) + (-21.5)}{3} = -17.33$$

$$S_m = \frac{[(-11.0) + (-19.5) + (-21.5)]^2}{3 \times 3 \times 2} = 150.22$$

$$S_T = (-3.6)^2 + (-0.7)^2 + \dots + (-3.7)^2 = 200.30$$

$$\hat{S}_B = \frac{\begin{aligned} & [(-11.0) - (-17.33)](200 - 566.67) + [(-19.5) - (-17.33)] \\ & \times (500 - 566.67) + [(-21.5) - (-17.33)](1\,000 - 566.67) \end{aligned}}{6 \times [(200 - 566.67)^2 + (500 - 566.67)^2 + (1\,000 - 566.67)^2]} = 8.10$$

$$V_e = \frac{200.30 - 150.22 - 8.10}{18 - 2} = 2.62$$

Determine the error variance of calibrating operation (σ^2) by the following formula:

$$\sigma^2 = V_e(\mu l)^2 = 2.62(\mu l)^2$$

5. Determination of Errors in Practical Use of Volumeter

5.1 Method of Measurement to Determine Errors

5.1.1 Selection of Setted Scales and Levels In order to determine the average error for plural setted values of practically used volumeter⁽¹⁾, select the levels of setted scale M_1 , M_2 , M_3 in the range of upper and lower limits of setted scale. Take M_1 and M_3 at positions near to upper and limits in the range and M_2 at approximate middle.

Note (1) Conduct the measurement for fixed capacity type at its proper capacity and for preset capacity type at every setted scales.

Example: In the case of volumeter with scale range 5 to 25 μl and using range 5 to 25 μl

M_1 : 5 μl , M_2 : 15 μl , M_3 : 25 μl