



HEALTH EFFECTS LABORATORY Standard Operating Procedure

VERIFICATION AND CALIBRATION OF ACCURACY OF PIPETTING VOLUMES

Written by: ERP	Valid from: DD.MM.YYYY	Code: HEL11Q006
QA (name and sign): ESI	Last review: DD.MM.YYYY	Version: 1.0
Approved (name and sign): MDU	Page 1 of 3	

SCOPE

The scope of this SOP is to describe how to verify and calibrate the pipettes to ensure that dispensing is carried out with the intended accuracy. This means determining the difference between the dispensed volume and the selected volume.

RESPONSIBILITY

Verification of pipettes should be performed by the Study Director/Study Personnel before the experimental start of a GLP Study. The volume should also be checked after the experimental end date. If a failure is detected during the verification, corrective actions should be taken. An assessment of the impact on data obtained since the last “good” verification should be done.

CONDUCT

- 1) The pipette is checked with the maximum volume (nominal volume), the minimum volume or 10% of the maximum volume, whichever is higher. For example, Pipette 0.5-10 μl is tested at 10 μl and 1 μl .
- 2) Put carefully a tip to the pipette and pre-wet the tip 5 times with distilled water
- 3) Carefully enter the liquid to the tip, holding the pipette vertically.
A series of ten pipettings of distilled water is performed with both volumes.
- 4) The pipette volume is weighed on an analytical balance on a tared foil with closed sliding lock-gate on the balance and noted on HEL11Q006 FORM.
- 5) Calculate conversion from mass to volume (see formula below).
- 6) If the average calculated results are within the selected limits, the adjustment of the pipette is correct.
- 7) If the average calculated results are not within the selected limits, the pipette needs to be adjusted.
- 8) Adjust the pipette volume by turning the pipette key tool clockwise to increase or counterclockwise to decrease the volume. After adjustment, check the calibration once more according to the instructions given above.
- 9) Sign and date the log book for pipettes (remember to note pipette number) and send the written form to archive.

If a pipette has been dropped to the floor, damaged, autoclaved or if deviations in pipetted volume are suspected, the pipette should be controlled and if any deviation it should be calibrated.



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Page 2 of 3

Conversion of mass to volume

V = (w+e) · Z

V = volume (µl)

w = weight (mg)

e = evaporation loss (mg) – if you use flask with lid e=0

Z = conversion factor (mg/µl)

for distilled water in normal condition(T=23°C, P095kPa)

Z=1.0032µl/mg (see also table 1 below)

Table 1: Values of the conversion factor Z (µl/mg), as a function of temperature and pressure, for distilled water:

Table with 8 columns: Temp °C, Air pressure kPa* (80, 85, 90, 95, 100, 101, 105) and 8 rows of conversion factor values ranging from 1.0026 to 1.0040.

*1kPa = 10 hPa

Accuracy (systematic error)

Accuracy is the difference between the dispensed volume and the selected volume of a pipette.

A = V - Vo

A = Accuracy

V = mean volume

Vo = numeric value

Accuracy can be expressed as relative value: A = 100% x A/Vo

Precision (random error)

Precision refers to the repeatability of the pipettings. It is expressed as standard deviation (s) or coefficient of variation (cv). In addition to the features of the pipette, laboratory practice and user experience are the main factors that affect precision

S = sqrt((sum(Vi - V)^2) / (n - 1))

S = Standards deviation

V = mean volume

n = number of measurement



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Page 3 of 3

Standard deviation can be expressed as a relative value (CV) $CV = 100\% \times S/V$

Table 2: Selected limits

Range	Volume μl	Accuracy (Maximum permissible systematic error)		Precision (Maximum permissible random error)	
		μl	%	S.d. μl	CV (%)
0,3-3 μl	3	$\pm 0,125$	$\pm 4,2$	0,075	2,5
	0,3	$\pm 0,125$	± 42	0,075	25
0,5-5 μl	5	$\pm 0,125$	$\pm 2,5$	0,075	1,5
	0,5	$\pm 0,125$	± 25	0,075	15
1-10 μl	10	$\pm 0,120$	$\pm 1,2$	0,080	0,8
	1	$\pm 0,120$	± 12	0,080	8,0
3-30 μl	30	$\pm 0,50$	$\pm 1,7$	0,20	0,7
	3	$\pm 0,50$	± 17	0,20	6,7
5-50 μl	50	$\pm 0,50$	$\pm 1,0$	0,20	0,4
	5	$\pm 0,50$	± 10	0,20	4,0
10-100 μl	100	$\pm 0,80$	$\pm 0,8$	0,30	0,3
	10	$\pm 0,80$	$\pm 8,0$	0,30	3,0
30-300 μl	300	$\pm 4,0$	$\pm 1,3$	1,5	0,5
	30	$\pm 4,0$	± 13	1,5	5,0
100-1000 μl	1000	$\pm 8,0$	$\pm 0,8$	3,0	0,3
	100	$\pm 8,0$	$\pm 8,0$	3,0	3,0
0,5-5ml	5000	$\pm 40,0$	$\pm 0,8$	15,0	0,3
	500	$\pm 40,0$	$\pm 8,0$	15,0	3,0
1-10ml	10000	$\pm 60,0$	$\pm 0,6$	30,0	0,3
	1000	$\pm 60,0$	$\pm 6,0$	30,0	3,0

For more information see instruction manual no. 44.

REFERENCES

Calibration manual for Finn Pipettes: <http://www.pipettecalibration.net/pipette-calibration-files/Guide-To-Pipetting-2.pdf>