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Validation of the choice of methods to evaluate the uncertainties of the calibration of micropipettes by the Monte-Carlo simulation method

Mona MAKHLOUF (Aequo Ltd - mona.makhlouf@aequoltd.com)

Context :

There are three methods for assessing the uncertainties of piston-operated pipettes « micropipette » calibration :

- ✓ ISO / TR 20461:2000
- ✓ DKD R 8-1:2011
- ✓ LAB GTA 90:2017

Declaration laboratries uncertainties for calibration of micropipettes with air curshion using the same standards équipments have a variabilities between methods up to six times greater.

Searching the best method of evaluations uncertainties for calibration micropipette taking into account the risks of having adequate comparison results and the consideration of the various factors of influence having an impact on the results.

Objectives :

The aim of this study is to :

- ✓ Demonstrate the risk taken for the adoption of a method for determining measurement results
- ✓ Choose the best method for evaluation uncertainties of the calibration micropipette results
- ✓ Evaluate the advantages and limits of tools GUM and Monte Carlo simulation methods

Methods :

This study is based on the exploitation of the results of the uncertainty budget presented in the guidelines LAB GTA 90 and DKD R 8-1 for a nominal value 100 µL.

The exploitation and interpretation of the expended uncertainties of the three methods ISO / TR 20461:2000, LAB GTA 90:2017 and DKD R 8-1:2011 as described in their respective standards or guidelines. The calculation was made using the Tanaka formula for the determination of the density of water and the simplified formula published by the BIPM at CIPM-2007 for the determination of the density of air.

Method 1 – ISO 20 461 : 2000 :

Calculation was made by the mathematical model :

$$V_{20} = \frac{m}{\rho_G} \cdot \frac{\rho_G - \rho_a}{\rho_w - \rho_a} \cdot [1 - \gamma(t_M - t_{M20})] \qquad \text{(V20 : 1)}$$

With :

m : Mass of the test liquid (corresponding to the difference of the balance readings)

ρ_G: Density of the standard weights used to calibrate the balance (equal to 8000 kg/m^3)

ρ_w: Density of the water used as a test liquid

ρ_a: Air density

γ : Cubic coefficient of expansion of the material from which the pipette is made

t_M: Temperature of the piston-operated pipette during measurement

t_{M20}: Piston-operated pipette reference temperature of 20 °C

Method 2 – DKD R8-1:2011 :

Mathematical model used was :

$$V_{20}\,(2) = (V20 : 1) + \xi_{operator} + \xi_{\text{repetability}} \qquad (V_{20} : 2)$$

$\xi_{operator}$: volume error caused by handling (estimate of 0,07% of nominal value)

$\xi_{\text{repetability}}$: standard deviation of the mean value of a series of 10 individual measurements

The volume error caused by handling include Mechanical influences , Operator-based influences , Hand warmth and Transport.

Method 3 – LAB GTA 90 : 2017 :

$$V_{20}\,(3\text{-}1) = (V20 : 1) + \xi_{inter\_operator} + \xi_{\text{bias\_method}} \qquad (V20 : 3\text{-}1)$$

$\xi_{inter\_operator}$: inter operators error

$\xi_{\text{biais\_method}}$: biais method error

With correction :

$$V_{20}\,(3\text{-}2) = (V20 : 3\text{-}1) + \xi_{operator\_effect} + \xi_{\text{others\_factors}} \qquad (V20 : 3\text{-}2)$$

$\xi_{operator\_effect}$: error of operator effect

$\xi_{\text{others\_factors}}$: others influent corrected factors

Equipments :

The equipments used for calibration was :

- ✓ Balance comparator 20 g / resolution : 0,1 µg
- ✓ Thermometer for measuring temperature of water : resolution 0,01 °C
- ✓ Temperature of air : resolution 0,1 °C
- ✓ Pressure transmitter : resolution 0,1 hPa
- ✓ Hygrometer : resolution 1% RH

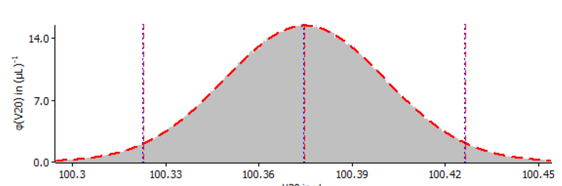
Software :

Gum workbench version 2.4.1 is the calculation and simulation software that was used for the determination of the results using both the GUM method and the Monte Carlo simulation method.

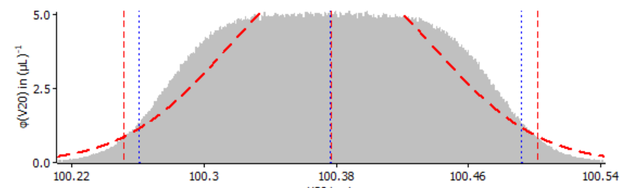
Technical specifications for simulation :

- ✓ Simulator : OMCE V:1.2.14.1
- ✓ Number of Monte Carlo Trials : 2000000
- ✓ Block size : 10000 runs

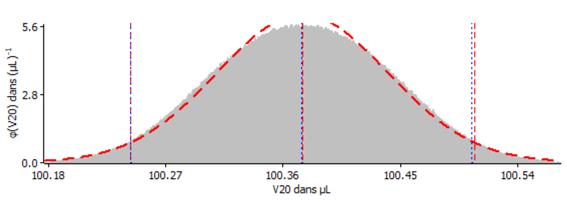
Results of GUM Workbench simulations :



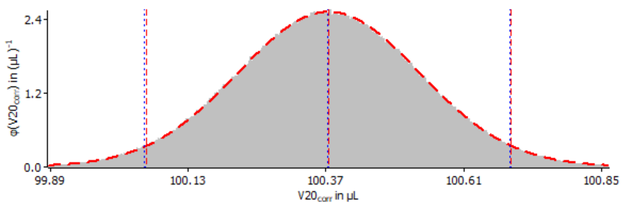
V20 – ISO /TR20461 (I)



V20 – DKD R 8.1 (II)



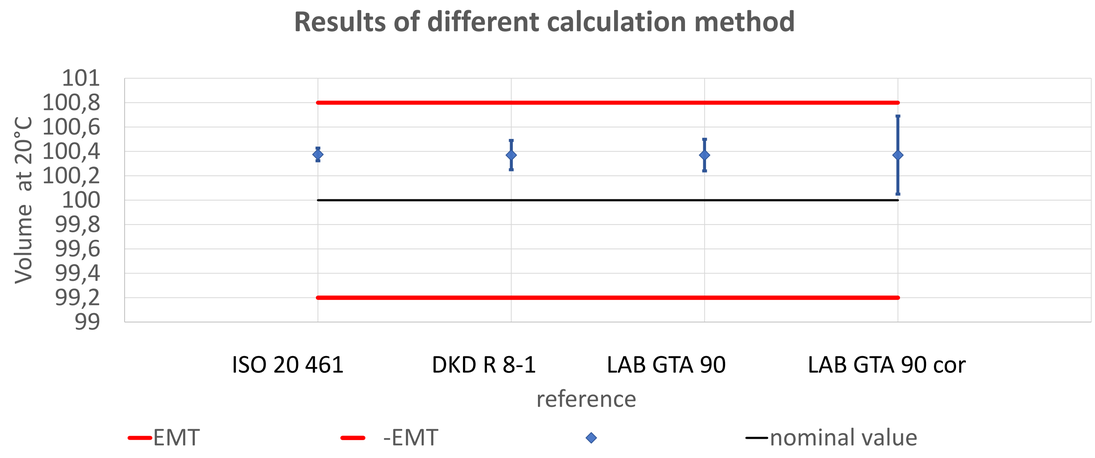
V20 – LAB GTA 90 without corrections (III)



V20 – LAB GTA 90 with corrections (VI)

Comparaisons results :

Reference methods	ISO/TR 20461	LAB GTA 90 without correction	LAB GTA 90 with linear corrections	DKD R 8.1
Mean Value :	100.375 µL	100.375 µL	100.37 µL	100.377 µL
Standard Uncertainty :	0.026 µL	0.067 µL	0.16 µL	0.064 µL
Coverage Interval (p=0.9545) :	[100.323, 100.427] µL	[100.244,100.506] µL	[100.06, 100.69] µL	[100.261,100.493] µL
Expanded Uncertainty Interval (p=0.9545) :	(+0.052, -0.052) µL	(+0.13,-0.13) µL	(+0.32, -0.32) µL	(+0.12,-0.12) µL



Conclusion :

The simulation method using a theoretical mathematical model is not sufficient to validate the measurement uncertainty evaluation method, in the case of the simulation results of the mathematical model given by ISO 20461.

The two guidelines Lab GTA 90 without the application of operator and inter-laboratory comparison corrections and the DKD R-8.1 are similar, the results of their simulations show that other influencing factors must be taken into account in order to have a mathematical model that better encompasses the measurement result, better analysis of influence factors and consideration of other factors will be added such as correction due to piston air chamber volume and operator effect.

The LAB GTA 90 used with correction value is a very broad estimate of uncertainty, the simulation results are very good but the expanded uncertainty determine does not allow to give a conformity ($\boldsymbol{MPE^* < \xi_j \pm U < MPE}$) taking into account the risks related to the results delivered.

comparison between the GUM method and the simulation method using experimental results is the best method.

**** MPE : Maximum permissible error***

References :

Software GUM Workbench 2.4.1 - Metrodata GmbH

ISO/TR 20461:2000 « Determination of uncertainty for volume measurements made using the gravimetric method ».

LAB GTA 90 revision 01 « étalonnage des instruments volumétriques à piston ».

EURAMET Calibration Guide n°18 Version 4.0 « Guidelines on the calibration of non-automatic weighing instruments ».

DKD-R 8-1 : 12/2011 « Calibration of piston-operated pipettes with air cushion ».

Expert Report DKD-E 8-2 : 05/2013 « Analysis of influencing parameters on calibration of piston-operated pipettes with air cushions ».