Accredited calibration and future demands for pipettors

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Already before the 1980s the first standards for hand-held pipettors were described. In the 1980s in addition to the European standards (German and British Standards), the "NCCLS Guidelines for determining performance of volumetric equipment" was published in the U.S coincidentally with the preparation of related International Standards Organization (ISO) and American Society for Testing and Materials (ASTM). Most of these standards described a gravimetric method and conditions for testing the pipettors.

Today, probably the most commonly used standard for pipettors and their calibration is the German DIN 12650 (1997). The DIN was appointed to be the responsible organization handling the secretariat when the new standard series for volumetric instruments was developed by a working group within the joint technical sub-committee ISO TC 48/SC1 and CEN/TC 332/WG1. The working group has developed ISO and EN standards for piston operated volumetric apparatus, which will be approved in parallel under ISO lead. As soon as the ISO 8655 - standard series becomes effective, the pipettor manufacturers most probably will begin to follow the new standard in their products and their quality assurance testing.

From the manufacturer. s point of view, the new future standard determines more carefully both the features of the pipettor and especially its performance. It differentiates the product type testing and the manufacturer´s quality assurance (factory) testing. It also states the different methods for testing the performance of the pipettor. Moreover, there is a clear difference between the performance level that the pipettor must have when it leaves the factory (controlled testing conditions) and the performance that is possible to reach by the user in various non-standard conditions (environmental conditions are not optimum or controlled). In addition to varying conditions, pipetting techniques may not be adhered to and the pipetted fluid is seldom purified water.

Fig. 1. Performance checking of a pipettor using the gravimetric method monitored by a computer.

Fig. 2. Each individual pipettor receives a QC-certificate, where most often the performance testing is done according to DIN 12650.

Performance testing

It is important to understand the difference between calibration and performance testing because most often the performance testing is thought to be calibration, which it is not. Performance testing is the actual testing procedure, verification of the pipettor by using the gravimetric, photometric or other test method. In other words, it is the check that the calibration of the pipettor is correct (Fig. 1). Usually, when arriving from the production line the pipettor fulfils the type specifications given by the manufacturer. The performance test confirms this. In case the results are incorrect the pipettor will need calibration. If calibration is needed, the pipettor will be performance tested again. After approved results, a quality control certificate with test results is printed for each individual pipettor (Fig. 2).

Factory testing and type testing for conformity

From the manufacturer´ s point of view the ISO/DIS 8655 standard differentiates the type testing that has to be done for each type of a pipettor and the actual factory testing (or manufacturer´s/supplier´s quality assurance test) which is done for each pipettor leaving the factory. The conformity testing of a pipettor means a systematic examination of whether the requirements of ISO 8655, especially in metrological sense, are fulfilled. Type testing is a test for conformity, a more thorough test to find out the specifications for each pipettor type (one or more specimens of a product representative, 100%, 50% and the lower limit of the useful volume range or 10% of the nominal volume, 10 measuring points, every channel in multi-channel pipettors etc.). On the other hand, the factory testing that can be specified by each manufacturer usually involves only one volume with, e.g. 5 measurements per pipettor simply because of costs. However, the manufacturer´s/supplier´s quality control test or factory test has to prove that even if the actual factory test is done only with one volume the pipettor fulfils the specifications of the type testing. For this the manufacturer can give out the supplier. s declaration (e.g. quality control certificate or calibration certificate).The type test specifications are normally given out in product instruction manuals and in separate marketing literature.

Testing by the user

The ISO 8655 standard clearly states the maximum permissible systematic error (the difference between the dispensed volume and the nominal volume or the selected volume) and random error (the scatter of the dispensed volumes around the mean of the dispensed volumes) for the pipettors. Notable is that there is an allowed difference between the maximum errors between the manufacturer and the user. Also the given Certificate of Conformity is always related to the entire volumetric apparatus including its metrological relevant accessories i.e. pipettor tips used. Therefore, it does matter, what kind of tips are used when the maximum error levels are defined. The users should establish their own acceptable maximum errors, which can be twice the error level mentioned in the standard at maximum. Also for technical service centers the specifications mentioned in the standard are valid. However, some manufacturers have specified the acceptable maximum error level not more than 50% higher than their own original type specifications. In many cases the error and confidence level is stricter than is allowed in the standard.

Calibration

Calibration is the adjustment of the pipettor to give out the selected volume. In practice, the length of the piston movement is adjusted to be in accordance with the selected volume in the display. In mechanical pipettors there is a calibration adjustment screw, which can be turned to change the calibration. Before doing that, the performance should, of course, be checked. If the measured result is out of the specification, the first things to check are the environmental conditions, the cleanliness of the pipettor (tip cone and piston with sealing) and the correct handling. If the pipettor is of good quality and well-maintained, actual calibration is seldom needed because in most cases deviations are caused by environmental conditions and/or handling. In mechanical pipettors, however, the speed and length of the movement can vary a lot depending on the user and technique, which of course wares out the pipettor in time, so that at certain stage recalibration may be necessary (especially after repair service or autoclaving). However, all pipettors should be checked, i.e. performance tested regularly, e.g. every 3 months.

Who does the performance testing and calibration?

Most mechanical pipettors are built so that recalibration is easy to do in-house. What comes to performance testing, in principle every laboratory should be able to do it. To test correctly, is another question. First, a semi-microbalance with readable graduation of 0.01 mg is needed. To control the humidity, the balance should be equipped with a humidity trap to prevent liquid from evaporating. The humidity should be kept between 60-90% during weighing. Water should be distilled

(ISO 3696, grade 3) and the temperature 20-25°C (constant to ±0.5°C). Moreover, the right technique should be applied for pipetting. For documentation purposes one should have software for pipettor testing and evaluation, which calculates the random and systematic error values automatically, and hopefully stores the values in memory for rechecking.

The demands for pipettor performance and calibration vary. Some laboratories are ISO 9001or GLP certified, even accredited by a notified body for performance, which means that their requirements for pipettor performance tractability of the calibration are also more strict than normally. Then, the best solution might be to employ an external pipettor calibration service for independent certification or invest heavily in equipment and labor costs.

Accredited calibration service

One could ask: Isn. t ISO 9001 certification enough for a laboratory? ISO9001 is the documentation of an organization. s quality management system and as such tells very little about its competence to provide accurate and reliable test data. However, ISO 9001 is a must if quality management is measured, but to determine also the technical competence to carry out testing and calibration, laboratory accreditation is needed.

Accredited calibration means that a customer can have confidence in technical competence of the body to carry out the calibration according to international standards. Accreditation is the significant evidence of competence to carry out both the testing and more important certification.

There are two sources of uncertainties in the calibration of pipettors, namely the uncertainty of the pipettor and the uncertainty of the gravimetric test method and its related apparatus. An accredited calibration service can differentiate between these uncertainties and give out the combined uncertainty budget of the measurement. Thereby, the customer knows exactly what is the performance of the pipettor and what is due to, for example, the uncertainty of the balance used. As an example, there are a big differences with standard analytical balances (readability of 0.1 mg or 0.01 mg) used. The proper and regular calibration of the balance used is essential and the biggest source of error when the uncertainty parameters are compared. The accredited calibration service uses equipment that minimizes the errors, and the uncertainty parameters are defined and documented. An example of the uncertainty budget of the Biohit calibration service is given in Table I. As seen it divides the uncertainty related to measuring process itself (the balance, water and air), the pipettor itself minimizing all the errors due to the measurement system. As a result, an accredited manufacturer is competent to release a calibration certificate where the uncertainties for the measurement involved are listed (Fig. 3). At the moment Biohit Plc. is one of the few accredited pipettor calibration laboratories in the world, simply because accreditation is a time-consuming process and costs money.

Fig. 3. The calibration certificate of an accredited calibration laboratory can bear the logo of an accreditation body, like FINAS (Finnish Accreditation Service), which is internationally recognized accreditation body and member of the EA (European Co-operation for Accreditation) and signatory of the MLA (European Multilateral agreement).

Conclusions

The trend today is to look for reassurance that the purchased products fulfil the laboratory requirements and meet the high end-user expectations. This often leads to testing or measurement of the characteristics against a standard or specification. To be able to test accurately and reliably, a laboratory needs to have qualified, experienced personnel, properly calibrated test equipment and controlled test environment. Proper sampling and sound test procedures with tractability are as important. In short, the testing laboratory should have the full competence to perform calibration according to international standards.

The new ISO 8655 becoming an international standard unifies the wide variety of different standards and recommendations for pipettors. It clearly states the pipettor specifications, both for manufacturers, technical services and users, the testing methods, testing conditions, and even the influence of the different possible mistakes in pipetting. This is especially for the benefit of the user because all manufacturers most probably start following the same standard to be able to fulfill the growing demands.

Most of the laboratories today are ISO 9001 or GLP certified and the trend is to get accredited, too. To ensure that the pipettors used in the laboratory fulfill the standards and are calibrated

correctly one should use an accredited calibration service. By accredited calibration the customer can see the real performance of the pipettor. The calibration certificate given and signed by the accredited calibration service separates the uncertainties of the balance and the test method and, thus, eliminates the sources of error. As a result, the customer can have full confidence using the pipettors in their tests.

Table I. An example of the uncertainty budget of the Biohit calibration service

BIOHIT UNCERTAINTY BUDGET										
Balance:	serial 11305654				Pipettor:	Biohit Proline 50 - 200 µl. serial AQ30836				
Parameter		Interval	Distribution	Standard	Sensitivity		Sensitivity		Sensitivity	
					50µl		100µl		200µl	
		+/-		uncertainty	coefficient	c*u(x)	coefficient	c*u(x)	coefficient	c*u(x)
balance	uncertainty	8µg	rectangular	4.62µg	1nl/µg	4.62nl	1nl/µg	4.62nl	1nl/µg	4.62nl
	linearity	4µg	rectangular	2.31µg	1nl/µg	2.31nl	1nl/µg	2.31nl	1nl/µg	2.31nl
1st value	reproducibility	1µg	rectangular	0.58µg	1nl/µg	0.58nl	1nl/µg	0.58nl	1nl/µg	0.58nl
2nd value	reproducibility	1µg	rectangular	0.58µg	1nl/µg	0.58nl	1nl/µg	0.58nl	1nl/µg	0.58nl
1st value	resolution	1µg	rectangular	0.58µg	1nl/µg	0.58nl	1nl/µg	0.58nl	1nl/µg	0.58nl
2nd value	resolution	1µg	rectangular	0.58µg	1nl/µg	0.58nl	1nl/µg	0.58nl	1nl/µg	0.58nl
	temperature drift	1.00E-06K-1	rectangular	5.77E-07µg	1nl/µg	5.77E-07nl	1nl/µg	5.77E-07nl	1nl/µg	5.77E-07nl
water	temperature	0.1K	rectangular	0.06K	10nl/K	0.58nl	20nl/K	1.15nl	40nl/K	2.31nl
air	temperature	0.1K	rectangular	0.06K	-0.23nl/K	-0.01nl	-0.46nl/K	-0.03nl	-0.91nl/K	-0.05nl
	pressure	10hPa	rectangular	5.77hPa	0.06nl/hPa	0.34nl	0.12nl/hPa	0.68nl	0.24nl/hPa	1.36nl
	relative humidity	10%	rectangular	5.77%	-0.01nl/%	-0.03nl	-0.01nl/%	-0.07nl	-0.02nl/%	-0.14nl
delivering	cubic									
device	expansion									
	coefficient	9.00E-05K-1	rectangular	5.20E-05K-1	-1.00E+05nl*K	-5.20nl	-2.00E+05nl*K	-10.39nl	-4.00E+05nl*K	-20.78nl
	temperature	2K	rectangular	1.15K	-4.5nl/K	-5.20nl	-9nl/K	-10.39nl	-18nl/K	-20.78nl
standard uncertainty associated with the volume V20						9nl		16nl		30nl
measured with the gravimetric measuring system										
precision on the calibration						63nl		126nl		157nl
standard uncertainty of the calibration						64nl		127nl		160nl
expanded uncertainty, coverage factor k=2						127nl		254nl		320nl

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