Good Pipetting Practice

RAININ
Pipetting 360°

Pipetting Calibration and Technique

How they Affect Experimental Outcome

The use of pipettes to transfer liquids is a daily activity in most life science research labs. From academic labs involved in leading edge discoveries to testing labs that follow routine standard operating procedures, the data generated can be greatly influenced by the performance of the pipette and technique of the user.

Pipette performance is a function of many factors, including keeping the pipette well maintained in order to achieve the desired performance and periodically checking to ensure that it meets the desired specifications. The other major factor, technique, requires users to develop their pipetting skill, such that maximum performance is routinely achieved and data is reliably produced.

When the two key criteria of routine maintenance and user technique are met, inaccuracies arising from these variables are significantly reduced and reliable results obtained, no matter what the application.

A brief review of recent scientific literature indicates a constant stream of reminders that pipettes and pipetting technique can play a major role in the success or failure of an experiment. Likewise, the outcome of ignoring guidance on technique can result in significant loss of time and money, which are crucial for any lab.

Part of the GPP -
Good Pipetting Practice Series

METTLER TOLEDO

Pipette Calibration and Experiment Outcome

Many genomics experiments include a PCR or qPCR component that requires the careful addition of reaction components or preparation of a standard curve. Many publications indicate that not only careful pipetting, but also maintaining a calibrated pipette is essential if the data to be generated is accurate. Pennington and Edwards[1], using qPCR for gene expression studies in cultured cells and small tissue samples, recommend avoiding pipetting less than 2 µL since precise pipetting is vital to the success of the qPCR experiment. Toh et al[2], also using qPCR, recommend that readers specifically set aside a set of dedicated pipettes and have them calibrated on a regular basis. Grgicak et al[3] demonstrate that variability between standard curve dilutions has a significant impact on calibration curve stability and that using a single calibrated pipette showed minimal error in comparison to using either two pipettes or an uncalibrated pipette.

The underlying theme in this selection of papers is the importance of maintaining calibrated pipettes so that, at a minimum, the mechanical variability of the pipette is minimized as a result of routine professional calibration. This process can be enhanced by regular verification that the pipette in use meets the published specifications, a check that can be performed by using a high performance balance.

Pipette Technique and Experiment Outcome

Separately, many of these papers also provide guidance and reminders about pipetting technique that, if not followed, can also lead to substantial errors. For example, in Morga et al[4], the ability to obtain highly reproducible measurements with qPCR experiments depends on a number of factors, including the ability to perform "skilled pipetting."

In Vallania et al[5], Allele quantification was shown to be affected by pipetting errors during the process of DNA pooling. This was confirmed in further genome-wide association studies where inaccurate pipetting was shown to be a primary source of error.

Venegas et al[6], studying mitochondrial DNA with qPCR, indicated that inconsistencies with intra-run results were due to errors in pipetting of reagents, DNA template or primers, and that pipetting accuracy is very important.

Frendewey et al[7], studying cell screening and mouse genotyping by qPCR, indicate that differences in value between duplicate samples reflect differences in pipetting accuracy and reproducibility.

Life Technologies, a leading supplier of qPCR products, provides significant support to their platforms, including guidance on optimizing and troubleshooting. The guidance given in their qPCR protocols indicates that because low volume pipetting (<5 µL) negatively affects precision, they do not recommend it unless using pipettes designed for such volumes. The consequences of inaccurate pipetting of the test sample include high standard deviations and a number of errors that can occur when preparing the standard curve. Most of these lead to the production of an inaccurate standard curve, resulting in an artificially lower or higher amplification efficiency score, depending on whether the error is due to excess or deficit pipetting. This in turn can violate MIQE guidelines (Minimum Information for Publication of Quantitative Real-Time PCR Experiments).[8]

Simple calculations of pipetting error show the potential effects caused by these gross inaccuracies in a qPCR experiment. For example, if a 10 µL pipette is being used down to 5 µL, the mechanical accuracy is ± 0.075 µL. If the copy number in the 5 µL is 30,000, then the inherent copy number variability for the pipette alone (excluding user technique) can range from 30,450 down to 29,550 copies of DNA. And this assumes a well-calibrated and maintained pipette.

Depending on user skill, technique can add a range of ± 2 to 7%. The consequence of this additive error is a copy number range from 31,972 down to 28,072. The errors will accumulate during a dilution series and this accumulation can make significant differences in a standard curve and ultimately an assay.

Unlike genomics, which has a finite number of assay and detection techniques, proteomics has many detection systems with highly varying needs for volume, format and purity of protein. The analysis of the final sample in the detection system of choice results from a number of preparation steps involving pipetting, each step being capable of adding to the variance and inaccuracy of the data that is generated.

In an Alzheimer's disease study by Teunissen et al[10] there is a review of an inter-laboratory study that focuses on a specific biomarker assay. The clear outcome of the study is that even though each lab received the same sample and performed the same assay with the same materials, there was high variability in the results produced by the different labs. One of the areas of concern involved pipetting techniques, indicating that differences in technique contributed to the inter-lab variance.

An extension of the concern for technique includes making sure that the correct tips are securely fitted to the pipette to obtain a sufficient seal. For example, in the chapter "Immunoassays in Veterinary Plant-made Vaccines," Guzman et al[11] suggest that for their ELISA analysis not only is good pipetting technique essential, but the reader is reminded to "Always inspect the pipette and tips for correct seal, and ensure that consistent pipetting technique is used."

Recommendations: "Self Check" and External Calibration

Not only is operator technique important, but the physical capability of the pipette should be checked to verify that it meets the specification needs for the intended applications. In the work by Alamooti et al[12] using ELISA and flow cytometry studies, the authors state that the accuracy and reproducibility of all pipettes and technicians were checked every month by the gravimetric method. It is worth noting that only highly-trained and experienced service technicians are capable of performing truly accurate independent checks of individual pipettes.

Numerous organizations suggest variable frequencies for pipette calibration and checking. For example, ORA-LAB. 5.5 from the FDA suggests that all volumetric delivery devices, such as mechanical pipettes, be calibrated at a minimum of every six months.[13] In the review by Bertermann[14], the recommendation is for pipettes to be "calibrated according to documented procedures along with periodic checks to ensure proper ongoing performance."

Individual labs and researchers should evaluate their need for routine checks based on the sensitivity of their experiments to pipetting errors and to the risks they would assume if their data were compromised. The Risk Check Toolat www.mt.com/gpp is a useful tool for ascertaining pipetting risks.

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