The Tip of the Iceberg: How Pipette Tips Influence Results. Part 1: Tip Fit Is Not All Users Should Look for

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Abstract

The fact that a tip fits onto a pipette cone does not say anything about the performance of the pipetting system comprising the components "Pipette and Tip". We performed a study including standard tips from 15 different manufacturers in order to investigate the tip-related influence on the pipetting result. The study results showed a dramatic influence of the tip on the pipetting accuracy.

The standard ISO 8655:2002 [1] recommends using pipette and tips from the same supplier. Our study results emphasize the validity of this recommendation and the need of calibration/pipette adjustment if other tips than recommended by pipette suppliers are to be used.

Introduction

Within the scientific community, a rising number of published experiments cannot be reproduced by other groups. In general, plastics are not taken seriously leading to problems with analysis results caused by e.g. leachables or incorrect pipetting volumes. This may lead to results not being reproducible if performed by other groups using other consumables.

Some problems with pipette tips are obvious, e.g. the need to push tips with force onto the pipette cone in order to achieve efficient tip fit. Other problems often remain unrecognized like decreased pipette accuracy when using other tips than recommended by the pipette supplier.

The ISO 8655-2:2002 standard [1] defines pipette and tip as a system, which requires extra calibration for the use of other manufacturers' tips. But why does this standard put so much focus on a product that is to be discarded after usage?

This series of articles answers this question. It shows the influence of tips on the pipetting result explaining the main tip-related impact factors.

Material and methods

General material

Eppendorf Xplorer® plus 50–1,000 µL and 0.5–10 µL were used. Racked 10 µL and 1,000 µL standard tips of Eppendorf and 14 other manufacturers have been tested. Exceptions: Manufacturer H did not offer racked 10 µL standard tips, manufacturers K and N offered only 1,250 µL tips for 1,000 µL pipettes.

Calibration by gravimetric method

The performance of the system "Pipette and Tip" was determined by calibration according to [1]. Environmental conditions were set according to requirements [1].

Calibration was performed using analytical balance Model XP26PC (Mettler-Toledo®) at 100 % nominal volume and 10 % nominal volume. Two series of 10 pipettings were performed. Systematic error and random error were determined for each series of 10 measurements and compared to specifications [1] and [2].

For further information please refer to [3].

Results and discussion

While being perfectly within error limits with Eppendorf tips, we found the system "Pipette and Tip" to be out of specifications when using other manufacturers' tips.

As shown in Fig. 1 and 2, the allowed systematic error was exceeded with 4 manufacturers' tips at a volume of 1,000 µL and with 5 manufacturers' tips at 1 µL test volume. These tips with 1,000 µL test volume exceeded not only the manufacturer specifications but also the wider limits for systematic error as stated by the ISO 8655:2002 standard [1]. The random error was noticeably increased but stayed within allowed tolerances.

When comparing the calibration results with the outcome of dimensional measurements, it becomes clear that with 1,000 µL the biggest impact factor is the air-cushion size: Those tips that produced error limits beyond the pipette

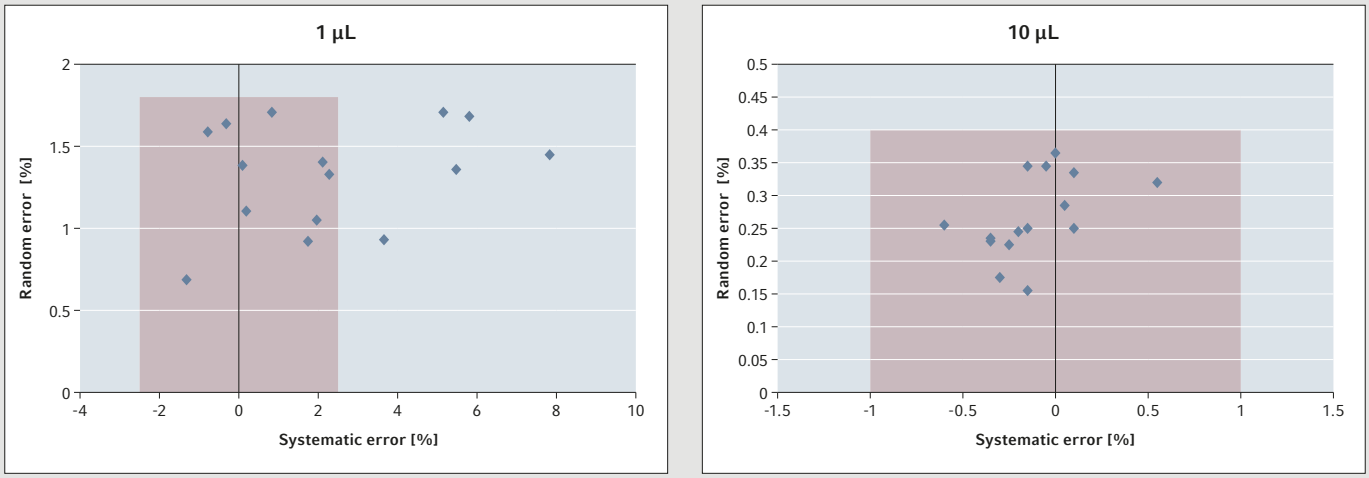

Fig. 1: Calibration results using 10 µL tips of different manufacturers. The red shaded area shows the span of the maximum permissible errors stated for the system "Pipette and Tip" by pipette supplier. All data points within the red shaded area are within the specifications.

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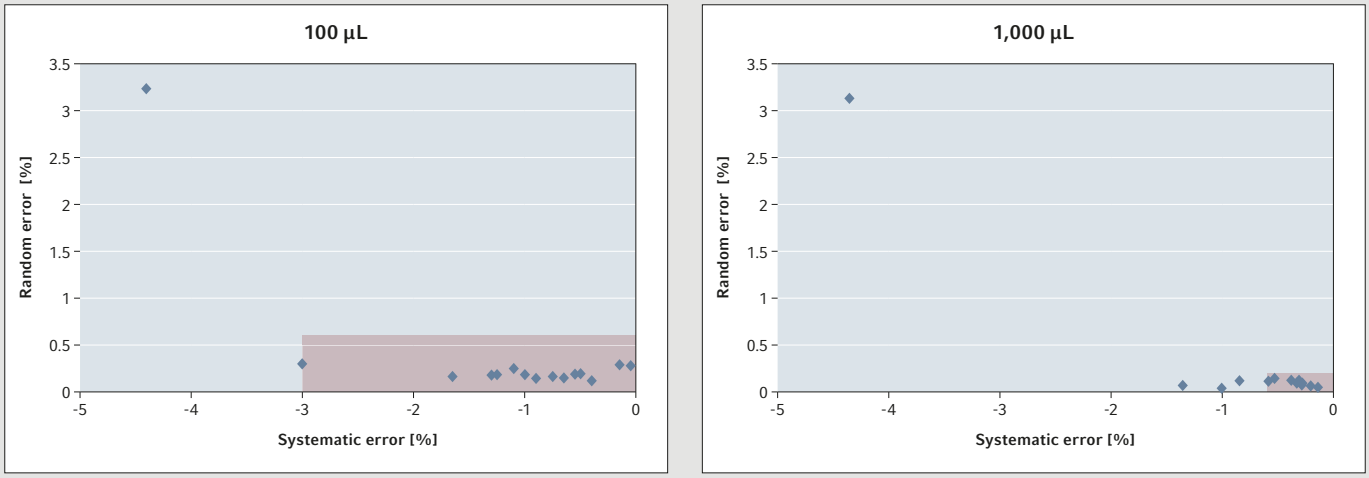

Fig. 2: Calibration results using 1,000 µL tips of different manufacturers. The red shaded area shows the span of the maximum permissible errors stated for the system "Pipette and Tip" by pipette supplier. All data points within the red shaded area are within the specifications.

specifications were longer although they had a similar inner diameter [3].

Larger tips offer a larger air-cushion. Since air-cushion pipettes are adjusted to a certain air-cushion size, an increase in air-cushion volume has a negative impact on the pipetting result, especially with nominal volume.

The calibration results have been found to be independent of the pipette manufacturer and were reconfirmed by calibrating with another manufacturer's pipette (data not shown).

In contrast to 1,000 µL tips, the impact of "air-cushion size" does not influence the 10 µL tips to an important degree. With this tip model, other influencing factors such as geometry and quality of tip orifice are more important. These influencing factors are discussed in more detail in [3] and our next publication of BioNews.

The results are conclusive that precaution is needed if tips with a design differing from the tip recommended by the pipette supplier are to be used. Elongated tips are an example. Within the operating manual, Eppendorf advises the user to adjust the pipette if such tips are to be utilized. In the case of manual pipettes, this can be done by user adjustment. More conveniently, with electronic Xplorer pipettes, this is performed by just choosing the tip from the options menu. Most non-system tip providers inform about tip fit on different pipettes. However, the fact that a tip physically fits onto a pipette cone does not say anything about the system's performance. If other manufacturers' tips are to be used, the only user's chance to learn about inaccurate pipetting results is by calibration.

This is supported by statements from the standard [1] which generally recommends using pipette and tip of one manufacturer. In case this is impossible, the user is required to calibrate first with the tips recommended by the pipette manufacturer ("conformity testing" to ensure the system is working correctly), and secondly to calibrate with other manufacturers' tips [1].

Conclusion

That a tip physically fits onto a pipette cone does not mean that the system performs within the manufacturer's specification. We have shown for 1,000 µL and 10 µL tips that the system's performance can be heavily influenced by the tip.

Manufacturer-wise pipettes are supplied adjusted to a certain air-cushion size. The tip design, however, directly influences the air-cushion size. Especially with bigger volumes such as 1,000 µL, this happens to a degree affecting the system's accuracy. With small volumes, other impacting factors come into play which will be discussed in more detail in the next edition of BioNews.

Literature

[1] DIN EN ISO 8655:2002, parts 1, 2, 6. Piston-operated volumetric apparatus. Beuth-Verlag, Berlin, Germany.

[2] Eppendorf Xplorer® plus operating manual. www.eppendorf.com/manuals

[3] Application Note No. 354: The tip of the iceberg: How pipette tips influence results. www.eppendorf.com/applications

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