The Tip of the Iceberg: How Pipette Tips Influence Results. Part 2: Perfect Geometry Makes a Difference

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Abstract

We performed a study including standard tips from different manufacturers in order to investigate the tip-related influences on the pipetting result. The study showed a dramatic impact of the tip on the pipetting accuracy. Small volume pipetting accuracy is significantly influenced by the quality of the tip orifice. Any impairment of geometry and shape can lead to retention of liquid.

Introduction

Tip-related influencing factors on pipetting results can be compared to an iceberg. Some influencing factors are easy to observe, like the necessity for high tip attachment forces in order to achieve a secure and tight fit of non-recommended tips on the pipette cone. Other factors stay rather unknown, like the geometry of the tip orifice.

Part 1 of this series of Application Notes described pipette and tip as a system which may not perform within permissible error tolerances with all tips [1].

This is in concert with ISO 8655 [2] which regards pipette and tip as a system and the use of other tips than recommended as a system disruption. In part 1, the air-cushion size was detected to be the main influencing factor on bigger volumes like 1,000 µL. In contrast, with 10 µL tips other factors come into play. Here, the quality of the tip's orifice was found to be of high importance. For further investigation, the orifices of tips from different manufacturers were scrutinized and compared to calibration results [1,3].

Material and methods

The orifice microscopy of 10 µL tips from Eppendorf and three other suppliers (manufacturers E, F, and H) was performed using a microscope (Leica®) with 25-fold magnification and a DFC 280 digital camera (Leica).

Results and discussion

The zone where liquid leaves the tip during dispensing is very important for the accuracy of results. At this part of the tip, the drop cut-off occurs. Any imperfection of geometry or shape, e.g. by production errors, can lead to liquid retention. This especially plays a role with small volumes. A poor drop cut-off may not only impair the pipetting result but can make it impossible to dispense small volumes below 1 µL onto a solid surface.

Fig. 1 (see next page) shows examples of such production flaws. Tips from these manufacturers lead to the system performing outside permissible systematic error tolerances [1,3]. This underlines the direct influence of tip orifice geometry on pipetting of small volumes.

A good tip has a perfectly round geometry and uniform wall thickness. The orifice does not show flashes or loose material residues. Walls with varying thickness and not perfectly round geometry lead to deflection of liquid drops to the outside of the thinner wall. Flashes cause liquid retention. Such production flaws are generated within tip production by tools with low quality or long maintenance cycles and/or a non-optimized injection molding process.

Since the production obviously has a high impact on the geometry and shape of the tip, influencing the pipetting result, it makes sense to take a closer look at production. The next part of this series will focus on the production of pipette tips and, in this context, the use of additives.

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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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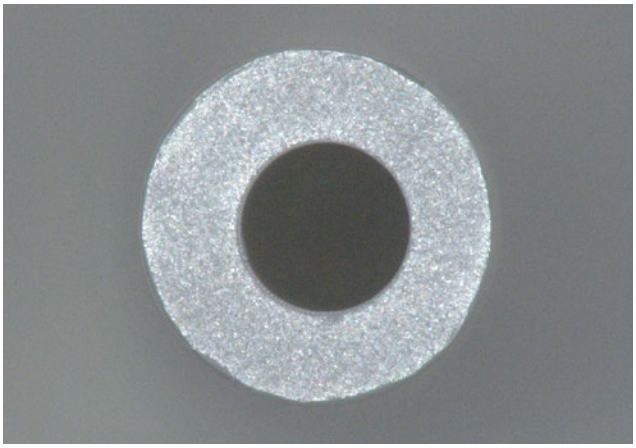
Missed part 1?

A rising number of published experiments cannot be reproduced by other groups. In general, only little attention is paid to the purchase of plastic laboratory consumables, like pipette tips and microtest tubes.

The fact that a pipette tip fits onto a pipette cone does not say anything about the pipetting accuracy of the pipetting system comprising the components "Pipette and Tip". We have studied this issue thoroughly in part 1 of our series "*The Tip of the Iceberg: How Pipette Tips Influence Results*".

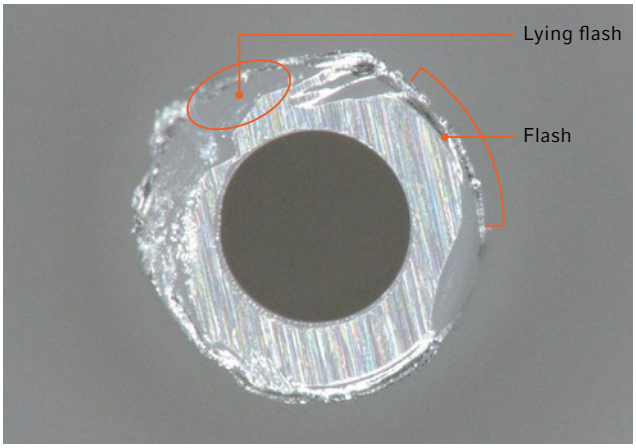
If you missed part 1, then check out BioNews No. 44 in our archive at **www.eppendorf.com/bionews**.

The Tip of the Iceberg: How Pipette Tips Influence Results. Part 2: Perfect Geometry Makes a Difference



Eppendorf epT.I.P.S.® 10 µL

The orifice has a good geometry and the function is not negatively influenced by production errors.



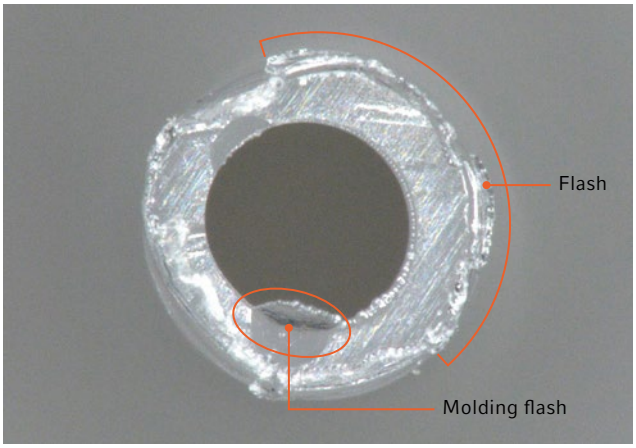
Manufacturer E

Problem 1: Lying flashes caused by non-harmonized ejection molding process. Cavity has not been fully filled with liquid PP.

Result: Risk of deflection of water drop because of varying diameter of frontal area

Problem 2: Flashes at exterior wall

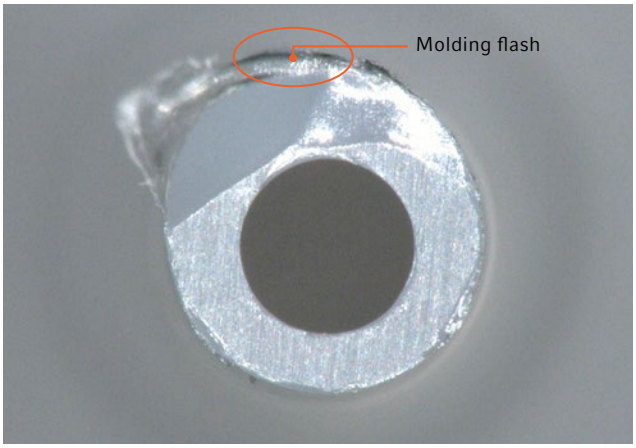
Result: Risk of keeping liquid residues



Manufacturer F

Problem 1: Flashes at interior and exterior wall caused by long maintenance cycles of tool.

Result: Risk of liquid residues and risk of PP particles falling into sample. Additionally, particles inside the tip displace water leading to wrong liquid volume. Such an error pattern makes it impossible to dispense e.g. 0.8 µL sample onto a solid surface.



Manufacturer H

Problem 1: Molding flashes caused by a non-tightening tool or problems with injection molding process where too much liquid PP has been injected.

Result: Liquid retention

Problem 2: Noticeable core shift. Not all walls have the same thickness. This error pattern can be caused by e.g. a poorly manufactured tool.

Result: Risk of deflection of liquid beside instead of into the target vessel.

Fig. 1: Microscopic pictures of 10 µL tip orifices of different manufacturers. Manufacturer E, F, and H failed the calibration by impaired systematic error. The examples have been chosen to explain production errors. PP=polypropylene.

Literature

[1] The Tip of the Iceberg: How Pipette Tips Influence Results. Part 1: Tip Fit Is Not All Users Should Look for. BioNews 44, January 2016. www.eppendorf.com/bionews

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

[3] Application Note 354: The Tip of the Iceberg: How Pipette Tips Influence Results. www.eppendorf.com/applications

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