eppendorf

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The Tip of the Iceberg: How Pipette Tips Influence Results

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Abstract

Pipettes are tools widely used in the lab and usually purchased with care. However, as pipette tips are only consumables, they are not usually selected with quality in mind. The standard ISO 8655 requires an extra calibration if pipettes and tips from different manufacturers are used. This study including tips from 15 different manufacturers proves that a pipetting system working perfectly with a certain tip exceeds the permissible error tolerances when a tip from a different manufacturer is used. Furthermore, we found that the calibration method influences the performance of the pipetting system: It is significant whether the calibration is done with or without tip change for each measured volume. Autoclaving impacts the tip dimension as well as the calibration result especially with small volumes. Eppendorf standard tips have been shown to perform within permissible error tolerances regardless of the calibration method or autoclaving. Here, the user is free to choose the method most suitable for his application.

Introduction

Within the scientific community, a rising number of experiments published cannot be reproduced by other groups. Incorrect pipette handling (e.g. holding the pipette at an angle during liquid aspiration) may be one reason for this.
A second source of error often not taken seriously is plastics. Consumables may lead to problems with analysis results, e.g. due to leachables, as well as incorrect pipetting volumes. This may result in non-reproducible data if experiments are repeated by other groups using other consumables.
Some problems with pipette tips are obvious like:

> Tips have to be pushed powerfully onto the pipette cone in order to achieve efficient tip fit
> Banana-shaped tips make it difficult to fill a plate with multichannel pipettes
> Pipetting of volumes below 1 µL on a solid surface is impossible because the liquid drop sticks to the outside of the tip.

In the same way that only the tip of an iceberg can be seen above water level, a number of other problems with pipette tips rather stay unknown. One example is decreased pipette accuracy when using tips not recommended by the pipette supplier.

The latter often stays unnoticed since problems with analysis results are usually linked to reagents, method and pipette but not to the pipette's consumable.
Moreover, calibrations are understood as "checking the pipette" instead of "checking the system". Thus, instead of checking the tips utilized in the lab only the tips recommended by the manufacturer are used for calibration. The ISO 8655-2 [1] defines the pipette and tip to be a system. It stipulates extra calibration for the use of other manufacturer's tips. But why does this standard put so much focus on a product that is to be discarded after use?
This publication shows the influence of tips on the pipetting result. It explains the origin of such influences and what to look for when purchasing tips not recommended by the pipette manufacturer. Basically, the main influencing factors are design/shape, production quality and material. As shown, these factors do not only influence the single pipetting result, they also have methodical consequences like varying results when calibrating with one/several tips or changing results by autoclaving.
The aim of this publication is to picture the iceberg underneath the water level: to generate understanding why the pipette and its tip form a system with both components having equal influence on pipetting results.

Materials and Methods

General material

In order to keep the random error as small as possible, electronic pipettes were used (the Eppendorf Xplorer® plus 50 - 1,000 µL and 0.5 - 10 µL). Tips from Eppendorf (epT.I.P.S.®) and 14 other manufacturers, system providers as well as generic tip manufacturers, were tested in the following experiments. Only 10 µL and 1,000 µL standard tips in racks were tested – except for manufacturer H which did not offer 10 µL standard tips in racks. Manufacturers K and N offered only 1,250 µL tips for 1,000 µL pipettes. In order to reconfirm findings, a pipette from tip manufacturer A was used to repeat calibration with 1,000 µL tips.

Calibration by gravimetric method

The performance of the system >pipette and tip< was determined by calibration using the gravimetric method as described in ISO 8655 [1] and the Eppendorf SOP [2]. Thereby two methods were applied:

a) Calibration according to ISO 8655-6 [1] using a new tip for each measurement
b) Calibration using one tip for each measured volume

The calibrations were performed with analytical balance Model XP26PC (METTLER TOLEDO®) in a draught-free room. Relative humidity was above 50 % r.H. and temperature was constant between 15 °C and 30 °C during the test. To ensure temperature equilibration, pipettes, tips and molecular grade water were stored inside the test room at least 2 h before calibration. Air temperature and atmospheric pressure were measured before and after testing in order to determine the Z correction factor.

The calibrations were performed at 100 % nominal volume and 10 % nominal volume. For each condition, two series of ten pipettings were performed. Eppendorf Xplorer pipettes were used in pipetting mode. Inaccuracy and imprecision obtained were compared to supplier specifications [3] and ISO 8655-2 [1]. Tests were performed by a skilled operator.

Dimensional measurements

The dimensions of the tips were measured with an optical 3D measuring machine (Vertex™ 311 UC, Micro-Vu) equipped with a high resolution CCD camera and a TP20 touch probe (Renishaw®). By combining video and touch probe, 2D and 3D measurements were performed with an accuracy of a few micrometers. This equipment is based on the contact-free measuring method which allows a fast dimension control of all different types of components (in term of shape and materials).

Microscopy of tip orifice

The tip orifices were examined using a microscope (Leica®) with 25-fold magnification and a digital camera DFC 280 (Leica).

Photometric determination of residual liquid volume

A tartrazine solution of 20 mg/mL was used to measure the residual liquid amount inside the tips after pipetting. 5 tips from each manufacturer were measured. Nominal volumes were used to pipette and dispense colored solution. Tips were prewetted 4 times with tartrazine solution. After the last dispensing the tip was rinsed with deionized water. The absorbance of the rinsing solution was then measured at 430 nm with an Eppendorf BioSpectrometer® kinetic using Eppendorf µCuvette® (d = 1 mm) and Eppendorf Uvette® (d = 10 mm) according to the volume tested. From the absorbance value obtained, the residual liquid volume was deduced by following formula:

$$RF_{\mu L} = \frac{(V_0 \cdot c_p)}{(c_0 - c_p)}$$

Where
- $RF_{\mu L}$: residual liquid [µL]
- V_0: volume tested
- c_p: tartrazine concentration of the residual liquid
- c_0: tartrazine concentration of the tested solution

Autoclaving

The tips were autoclaved at 121 °C for 20 min at an overpressure of 1 bar using Model 3840-EL-D (Tuttnauer®). After autoclaving, the tips were cooled to room temperature for at least 2 h before use.

Mouse-Embryo Assay (MEA)

The MEA test was performed by an external accredited lab (Toxikon, Bedford, USA) following a method described by ISO 10993 [4]. A medium, later brought into contact with the embryos, was in contact with the tips for three different durations: 10 pipettings, 4 h (±15 min) and 24 h (±2 h). Since the test is time-consuming only Eppendorf tip sizes usually used in cell culture labs, 200 µL and 1,000 µL (both sterilized: Biopur® purity grade) were tested.

Statistics

The calculation of systematic and random error was performed according to ISO 8655 [1] and Eppendorf SOP [2]. Some graphs display error bars in order to show the variation between tips. These error bars are derived by calculating the standard deviation. Correlation of the tip dimension and calibration results was performed by linear regression.

Results and Discussion

Influence of tips on the performance of the pipetting system

The >pipette and tip< system was perfectly within the error limits when Eppendorf tips were used. It was outside the specifications when used with tips from other manufacturers. As shown in fig. 1, the systematic error was exceeded in 4 cases with a volume of 1,000 µL (manufacturers C, E, K, N) and in 5 cases with a test volume of 1 µL (manufacturers A, E, F, H, M). With three of those 1,000 µL tips, the test volume not only exceeded the manufacturer specifications but also the wider maximum permissible systematic error as stated by the ISO 8655:2002 standard [1]. In contrast, the random error was increased noticeably but stayed within permissible tolerances.

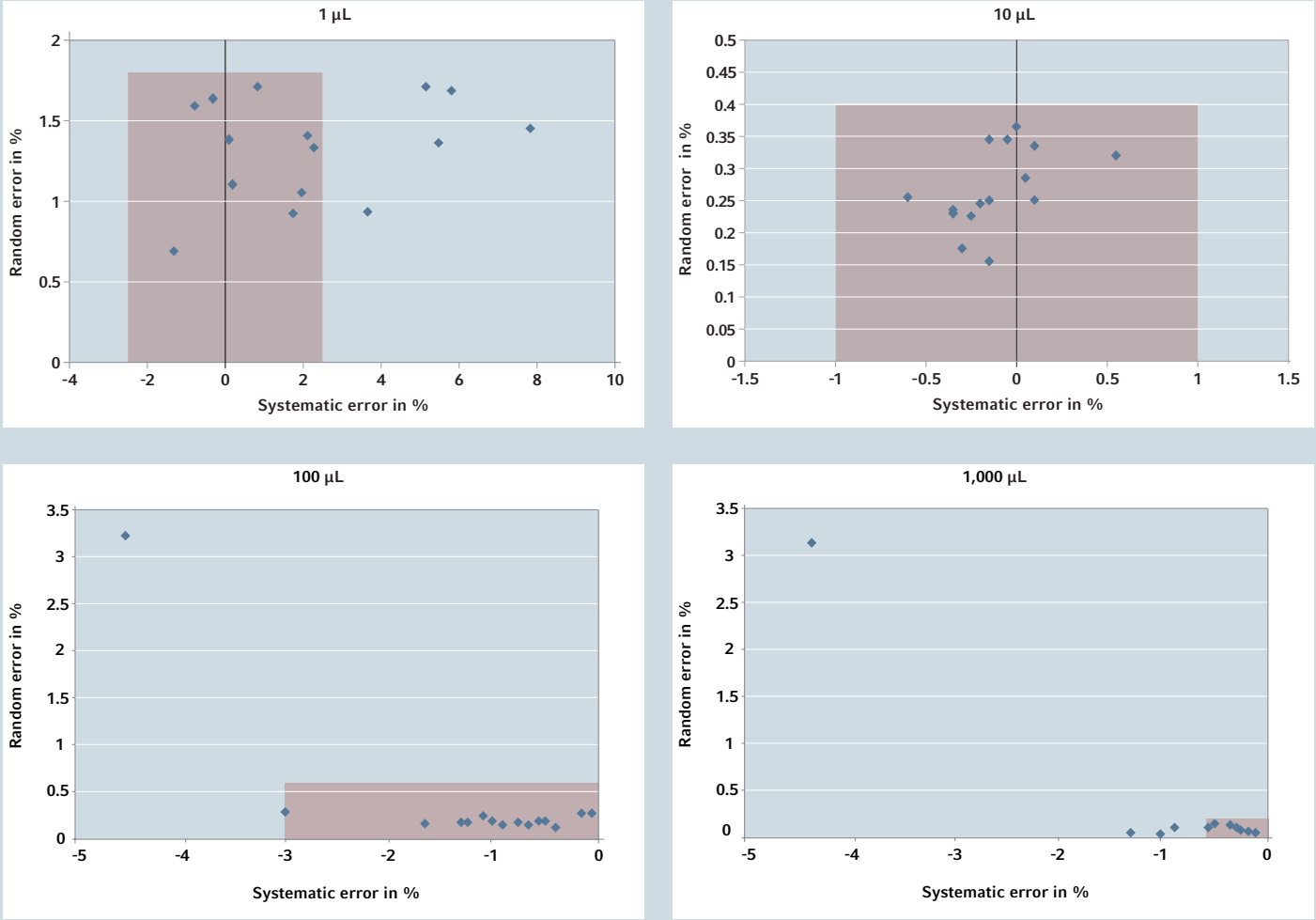


Figure 1: Calibration results using 10 µL tips and 1,000 µL tips from different manufacturers. The colored area shows the span of the maximum permissible errors stated for the Eppendorf Xplorer pipettes. All data points within the colored area are within the specifications.

If all calibration results are combined, a total number of 8 from 15 manufacturers exceeded the specifications. However, it cannot be assumed that all tips from a manufacturer are affected if with one tip the calibration result exceeded the permissible error limits. For example, the 10 µL tip from manufacturer K performs within error tolerances whilst the 1,000 µL tip from manufacturer K exceeds the pipette manufacturer's and ISO 8655 specifications.

Calibration results were found to be independent of the pipette manufacturer and were reconfirmed by calibrating with a pipette from another manufacturer (data not shown). This corresponds to the requirements of standard ISO 8655 [1]. All pipette users should be aware of the fact that when using tips not delivered by the pipette supplier, the manufacturer`s declaration or certificate of conformity does not apply.
The ISO 8655 [1] clearly states the pipette and tip to be a system. In case alternative tips are to be used the ISO 8655 part 2 [1] requires a conformity test first with tips from the pipette supplier. If the pipette passed the test, a second calibration with tips not supplied by the pipette manufacturer has to be performed.
Coming back to the calibration results and taking a closer look at the volumes being most impaired: We found a clear difference between 10 µL and 1,000 µL results.

Skim reading

With 8 from 15 tip suppliers, the systematic error was exceeded. Three cases did not even comply with the maximum permissible errors according to ISO 8655.

With 1,000 µL tips, the nominal volume (1,000 µL) was found to be most affected whereas with 10 µL tips, the 10 % of nominal volume (1 µL) was found to exceed the technical specifications. From this finding, it can be deduced that the violation of systematic error limits has different reasons with 10 µL and 1,000 µL tips. Those main influencing factors are described in the following.

Tip design and its influence on pipetting results

The dimensional measurements of length and inner diameter (see fig 2) showed that some 1,000 µL competitor tips were longer than Eppendorf tips with the same inner diameter. These longer tips failed the calibration.

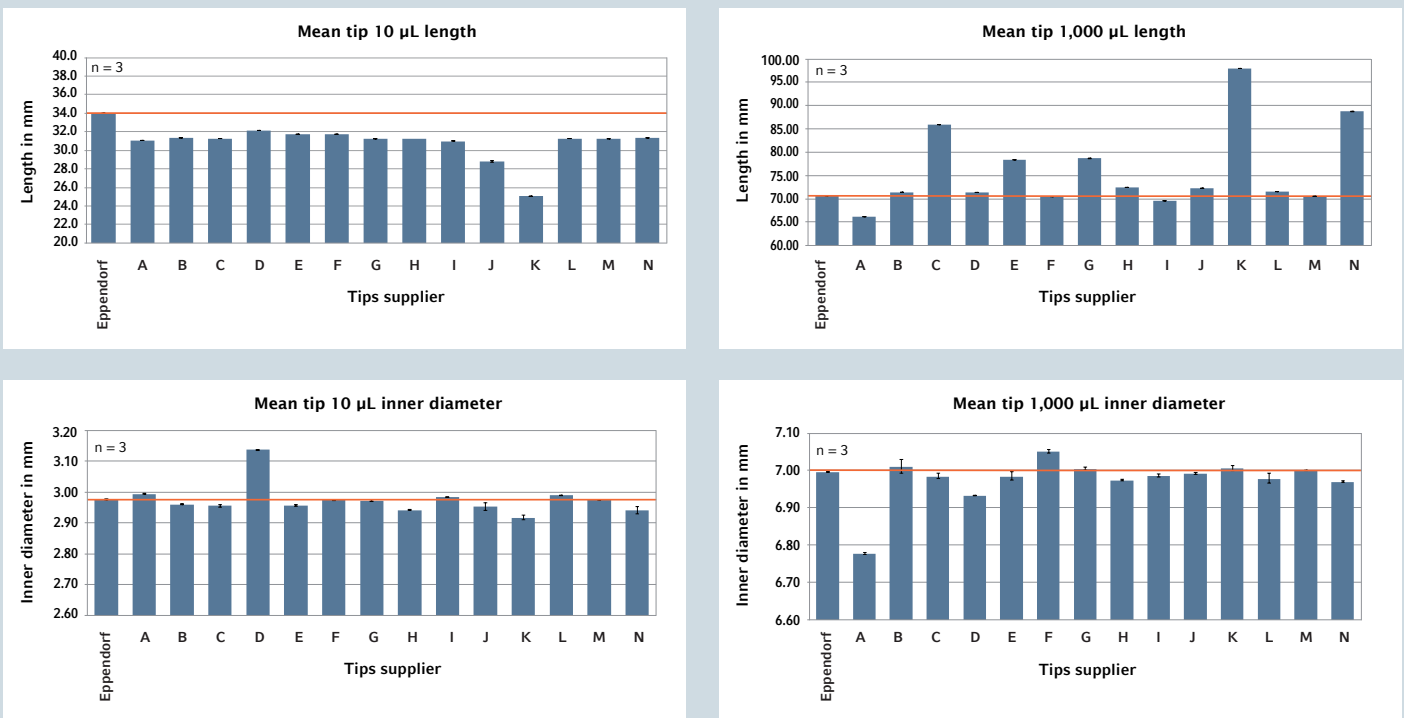


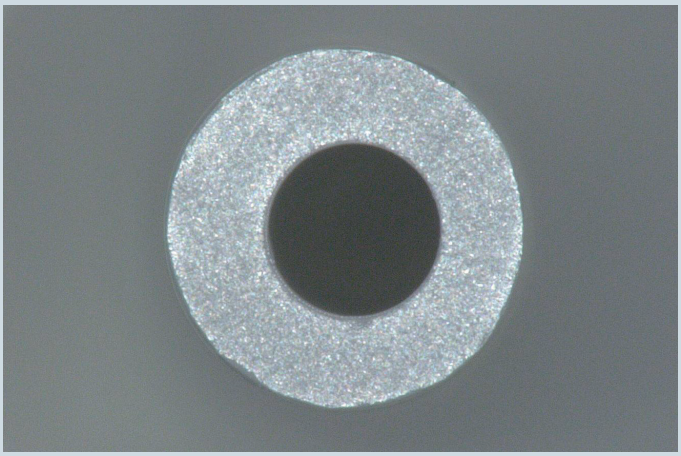
Fig. 2: Results of dimensional measurements of length and inner diameter of different manufacturer tips (10 µL and 1,000 µL models).

In order to explain these findings, it has to be taken into account that pipettes in general are adjusted to a certain air cushion size and filling height of liquid within the tip. Longer, bigger or slimmer tips lead to an increased total size of air cushion and a different filling level [5]. If the dead air volume increases, the pipetting volume decreases. Additionally, an increased filling height (e.g. by slim and long geometry of tip) results in an increased hydrostatic pressure which has to be compensated and also leads to a decreased volume and higher systematic error [6]. Our data show that with 1,000 µL tips the shape-related influencing factors play an immense role. This effect is especially distinct at nominal volume because with 1,000 µL the biggest possible "weight" has to be moved by the air cushion. A linear correlation of tip length and calibration result for 1,000 µL resulted in $R^2 = 0.90$ excluding manufacturer E. When looking at the calibration results of manufacturer E which were found to be far out of the specifications with 100 µL and 1,000 µL volumes, we see that the tips dimensions do not solely explain the calibration results. As will be described by the next chapters, other factors like wetting and quality-related issues, for example, perfection of tip orifice, come into play.

In contrast to the results of dimensional measurements of 1,000 µL tips, all examined 10 µL tips had a smaller length than Eppendorf tips. With the exception of manufacturer D, all inner diameters were similar to Eppendorf tips. The 0.5-10 µL Eppendorf Xplorer pipette is adjusted by the manufacturer to the comparatively longer Eppendorf tips. Thus, the influencing factor "tip shape" does not affect the systematic error negatively. Thus the cases failing the calibration at 1 µL cannot be explained by the tip size/length. Other influencing factors impairing the pipetting result come into play (e.g. quality of tip orifice) and will be examined in the following.

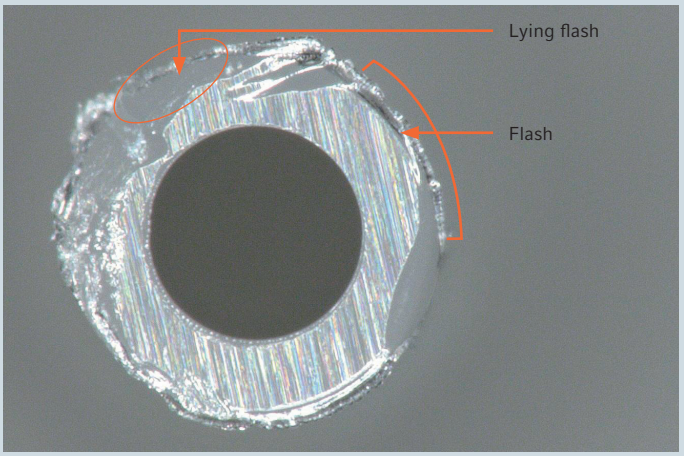
Quality of tip orifice

The zone where the 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 failures, leads to water retention. This especially plays a role with small volumes. A poor drop cut-off may not only impair the pipetting result but can also make it impossible to dispense small volumes: sometimes a drop leaves the tip, sometimes it doesn`t.
In order to give some examples of poor quality, fig. 3 (page 7) shows the tip orifice of generic manufacturer tips describing the error pattern. Tips of the displayed manufacturers exceeded the systematic error limit for calibration at 1 µL. The random error limit was kept by all manufacturers but suppliers F and H were very close to limit. As a comparison, an Eppendorf standard tip 10 µL is also displayed.



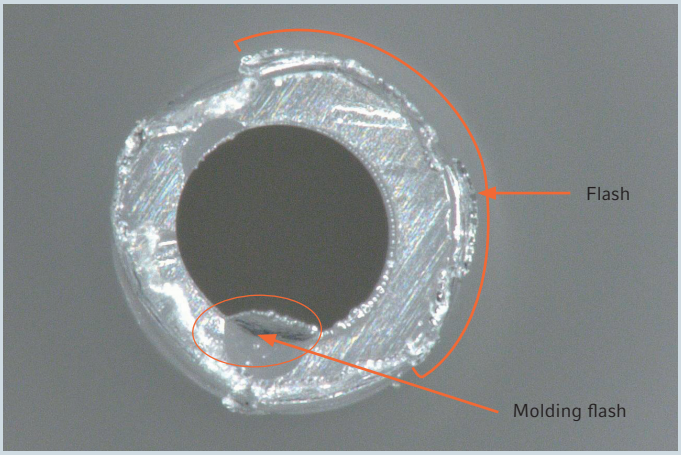
Eppendorf epT.I.P.S.® 10 µL

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



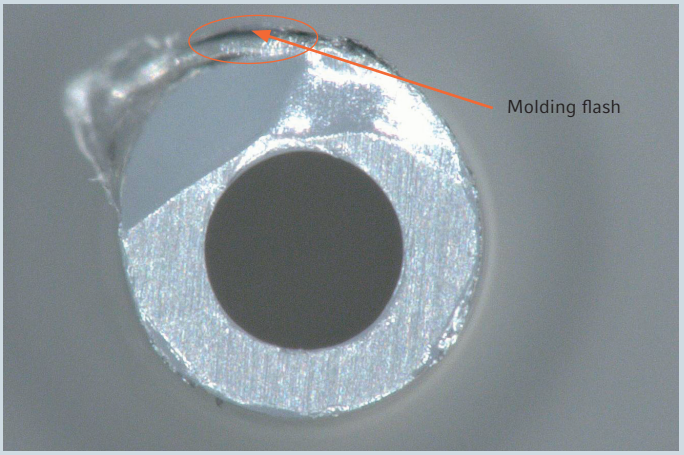
Manufacturer E

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 of exterior wall: Risk of liquid residue retention.



Manufacturer F

Problem 1: Flashes at interior and exterior wall caused by low 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 inaccurate liquid volume. Such an error pattern makes it impossible to dispense 0.8 µL sample onto a solid surface.



Manufacturer H

Problem 1: Molding flashes caused by a non-tightening tool or problems with the injection molding process where too much liquid PP has been injected.
Result: Water retention.
Problem 2: Noticeable core shift. Not all walls have the same thickness. This error pattern can be caused by, for instance, a poorly manufactured tool.
Result: Risk of deflection of water beside instead of into the target vessel.

Fig.: 3: Microscopic pictures of 10 µL tip orifices of different manufacturers. Manufacturers E, F and H failed the calibration at 1 µL by impaired systematic error. The examples have been chosen to explain production errors.

A good tip has a front phase with a defined wall thickness and surface structure in order to ensure a good drop cut-off. Production tolerances need to be very tight. A poor orifice is not perfectly round or has walls of differing thickness (fig. 3 competitor H). Liquid drops become deflected to the outside of the thinner wall. Furthermore, it shows "molding flashes" (fig. 3 supplier H) or thin "flashes" (fig. 3 supplier E and F) where liquid may be retained.

In addition, "lying flashes" (fig. 3 supplier E) can be observed. They influence the diameter of the orifice and again lead to liquid retention. From the production view, these flaws occur mainly if poor tools are used especially in combination with a non-optimized injection molding process. Generally, it is recommended to use tips which are made of a non-wetting plastic material with a flawless smooth orifice [5].

Water retention on inner surface

Water retention, so-called "wetting" is not only influenced by the tip's orifice but also by its material and inner surface. If the inner surface is uneven or the tip is made of an unfavorable material, liquid will be retained on the surface inside the tip. However, a completely smooth inner surface is not the only solution for minimizing water retaining effects. The combination of material composition and surface structure decides on the water retention. Tips are generally made from a plastic called polypropylene (PP). However, PP is not PP. Each tip manufacturer has its own secret formula for the PP it uses to produce pipette tips. From cooking we know that the secret behind a good cake is good ingredients. This also applies to the PP for tips. The mixture of ingredients determines the water repellent characteristics. PP in general is hydrophobic. This can be observed by pipetting drops of water onto different surfaces (e.g. glass and plastic). The rounder the drop, the higher the surface energy and the less the wetting effect. Within this study, we found differences in volume of retained liquid between different tip manufacturers. As shown in fig. 4 for 1,000 µL tips, manufacturers E, J, K, M showed three-fold higher water retention than Eppendorf tips. A rather big surprise was the result for the tip E2 which is the "low retention" version of the tip E. Similar results were determined for the 10 µL tips (data not shown). The results may look dramatic but if the retained volume is proportioned to the nominal volume used it becomes clear that the impact of the wetting factor is not high enough to solely explain the calibration results from fig. 1.

However, since pipetting errors sum up, like holding angle of pipette during uptake or different temperatures of pipette and liquid, high water retention can cause a pipetting system to be out of specifications.

Besides the lowest wetting, the Eppendorf tips also showed the smallest standard deviation. A small standard deviation points to a small variation between tips and high reproducibility which can be important for sensitive analyses.

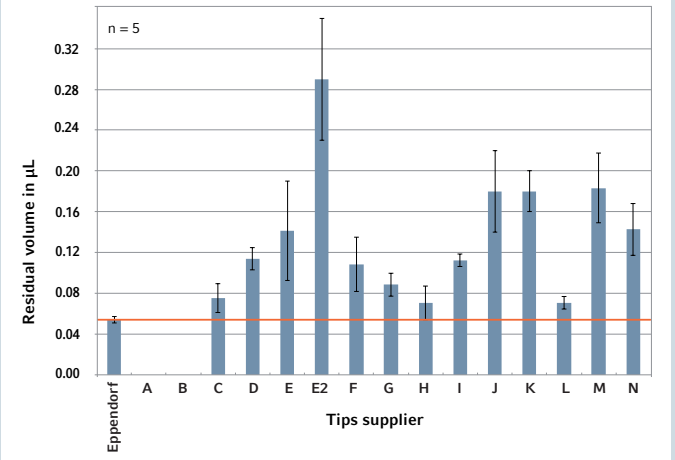


Fig. 4: Volume of water retained in 1,000 µL tips of different manufacturers after pipetting 1,000 µL. Competitors A and B did not fit tightly onto pipette cone.

Tip fit – Influence of sealing rim on creation of an air-tight system

It is a basic requirement of a tip to fit tightly onto a pipette cone in order to generate an air-tight system. The term "tip fit" applies in two different contexts: firstly, does the tip physically fit onto the pipette cone. Secondly, does this tip fit provide a tight sealing.

The physical fit of a tip onto a cone is mainly influenced by the design and shape of its contact zone with the pipette cone. The tip diameter is not the only factor. The connection between tip and pipette cone needs to be tight enough to prevent air passing through. If the tip fit is not tight, the system does not aspirate enough liquid and may leak. In the worst case, the pipette is obviously dripping but incomplete tightness is often not recognized during daily lab work. It was shown that the pipetting error increases by up to -0.6 % systematic error and 0.8 % random error with the generic tips used [7]. The ISO 8655:2002 [1] part 2 (Appendix B) benchmarks the influence of non-tight pipette tips with 0.5 - 50 % of nominal volume.

In order to ensure a tight fit on the pipette cone, most tips are equipped with a sealing rim. The position as well as the quality of this sealing rim is important for the tip fit. If the sealing rim is positioned too low, the cone may not reach it. If the sealing rim is too thick, it takes high forces to fit the tip onto the pipette cone. This has a negative effect on ergonomics and pipetting comfort. The consistent quality of the sealing rim itself is important for the tightness of the system. Its thickness must not vary significantly from tip to tip.

For example with 20 µL (filtered) tips of manufacturer G, we found differences in thickness of the sealing rim. This meant that some tips from the box fit onto the pipette cone, others did not. With those who fit onto the cone some enabled a tight system and others did not (data not shown). Fig. 5 shows three of these tips.

A testing prod was used to illustrate this: it was inserted into the tip without applying pressure. The position where it stopped was marked using a scalpel.

Fig. 5: Insertion depth of a testing prod in 20 µL tips of manufacturer G. All tips were taken from same box.

Variations in the quality of the sealing rim mainly originate from three different production failures: 1) the tools used to produce a tip are not equally shaped or are maintained with too infrequently, 2) the injection molding process is not balanced enough to ensure that the sealing rim is perfectly shaped (e.g. not enough plastics injected), 3) application of excessive production tolerances in quality control in order to save money in production.
A manufacturer with a good quality control sets very tight production tolerances and monitors continuously if tips comply with production tolerances. Products exceeding the production tolerances are discarded. Installing wider production tolerances means less products are discarded but more sold – with a lower overall quality.
Manufacturers of pipettes and tips, so-called system providers, offer their customers a widely unnoticed additional service: They produce a system instead of single parts. This means that the production tolerances of the pipette cone are aligned to the production tolerances of the tips and vice versa. If the production tolerance of the pipette cone reaches the maximum and the production tolerance of the tip sealing area reaches the minimum both still fit tightly together. Coordination of production tolerances is a feature which a non-system provider cannot achieve. However, this is not the end of the story. System providers manufacture

Skim reading

System providers manufacture a system instead of single parts of it. They have a natural interest in tight production tolerances complying regardless of batch. By this they ensure the system being within stated error limits. Generic tip provider's tips fitting on several manufacturer pipettes do not ensure this.

according to ISO 8655-2002. Thus, they have a natural interest to ensure (and certify) that the manufactured pipette/tip is within the published error tolerances on the date of purchase. This means that system providers have to take care that the production tolerances are tight enough in order to be able to certify the system being within published error limits – regardless of the batch. Manufacturers only producing tips do not have to comply with this requirement thus have the freedom to apply wider production tolerances. Knowing this, the question arises how Eppendorf gains and maintains a high tip quality.

Reproducible tip quality – How Eppendorf generates and maintains a high-quality standard of tips

As already described in the previous chapters, a poor tip quality has a negative effect on the pipetting result. We have shown that the quality of features like tip shape, wetting, orifice and sealing rim play a role. Besides these factors, the tip-to-tip quality, meaning the uniformity of tips within one box, between boxes and between batches is important. Tips of poor tip-to-tip-quality not only show an increased standard deviation (e.g. for wetting, compare fig. 4, manufacturer E) or increased random error in calibration using a new tip for each measurement (compare fig. 6 competitors F, G, H J, K with 1 µL calibration). Pipetting results may be impaired by methodical factors like calibration with/without tip change (to be discussed in a later chapter).
In production, the most important factors for tip quality are: the injection molding process, tool quality, material, small production tolerances and compliance with these.

First of all, the **design** does not only give the tip a certain look. Design should be based on the product's function. Accordingly, at Eppendorf, pipette tips are primarily designed to work perfectly. For example, not only the tight fit onto the pipette cone is important. The angles of the tip shape in combination with tip length affects how secure and smoothly the liquid rises within the tip or if the surface tears and the liquid jumps into the pipette cone. The angle, wall thickness and diameter of the tip orifice play an important role for the drop cut-off. Thus, even before production, within the design process, already a huge know-how is needed to create a tip with good functionality.
Pipette tips are produced by an **injection molding process**. Within this process the plastic material is melted and forced with high pressure (injected) into the cavity of a tool having the shape of the pipette tip. After a cooling time, the pipette

tip is taken out of the tool and subsequently processed (placed in boxes or bags, quality control etc.). Injection molding is a highly complex process influenced by numerous factors. The most important factors are temperature, pressure, time, material composition and characteristics of the tool. The art is the optimal harmonization of all factors. At Eppendorf, a lot of care is taken when producing a new batch. When the production of a certain tip model is started, the machine is run until the system with all its influencing factors becomes tuned. Tips manufactured before this point of "perfect tuning" is reached are discarded because an imperfect harmonized injection molding process leads to imperfect products. One can imagine that this is a cost-intensive process to ensure high product quality.

The **tools** used for the injection molding process are the "sacred core" of the production process. Their perfection of shape and surface is of significant importance.
Eppendorf even has a department which is solely responsible for producing and maintaining tools. During tip production, the tools have to withstand a pressure of over 1,000 bar and a closing force of over 100 tons. This means a very high load for the tools. Consequently, the maintenance cycles of these tools play an important role in the product quality. It is possible to use the tools until they are not dimensionally stable. However, it is better is to establish a maintenance protocol at short intervals in order to have the maintenance done before the tool becomes dimensionally affected. The tool maintenance is a complex process: the production has to be stopped and the tool has to be disassembled from the machine. The tool itself then becomes disassembled before inspection, maintenance and, if needed, exchange of components can be performed.
From a production point of view, this is a time-, man-power and material-consuming process, in short: cost-intensive. Since the tools are one of the keys to products of high quality, Eppendorf sets very short maintenance intervals.

As described previously, pipette tips in general are made of **Polypropylene** (PP) which is a compound consisting of a certain mixture of ingredients.

Skim reading

A high-quality tip can only be manufactured by cost-intensive production:

> All freshly manufactured tips of a new batch are discarded until the point of perfect tuning of the injection molding process is reached.

> Cost-intensive tool maintenance stopping production is performed at short intervals.

> Use of virgin PP ensuring known product characteristics at the user's lab.

Every tip manufacturer has its own recipe in order to gain best functionality in terms of e.g. water repellent characteristics or rigidity. Regardless of its exact composition, the material needs to be highly pure. Eppendorf does not use recycled material nor reuses material from discarded products as their characteristics are unknown and the injection molding process can then easily be impaired leading to a poor-quality product. In addition, after production a recycled material may alter the tip characteristics e.g. by shrinking behavior during autoclaving or "wetting". As will be described later in this article, additives making the production process easier or faster are decreased to a minimum or completely omitted. The PP composition is aligned to the characteristics of the injection molding process. This means that a certain material composition needs certain parameters (pressure, temperature, etc.) in order to gain a perfect product. It takes approx. 1.5 years to establish an injection molding process with a new material. This elaborate qualification process is cost-intensive but results in a high-quality tip containing an absolute minimum of additives.

The fourth pillar of producing high-quality tips is the **quality control** of the manufactured products. At its production site, Eppendorf has a department for quality control with two laboratories. The first lab focuses on dimensional control while the other controls the applicative quality of the manufactured products. Both labs work closely together. At Eppendorf, quality control already begins by checking the incoming raw material before use. During the production process, the following checks are performed:

a) Directly after injection molding, still on the machine, the first quality checks are performed. Therefore, the operator of the injection molding machine or automated line not only monitors the production but also quality aspects.

   Thus, he is responsible for product quality and checking the products constantly ("system of self-inspection") for flashes, molding flashes, concentricity, etc. In case tips do not pass this first quality control level, a complex process of investigation is initialized which can end in tips being discarded.

b) After passing the first quality control level products are checked by the two quality labs during and after the production process of a batch:

   > at the beginning of a batch

   > several times during production of the batch

   > 24 h after production (after shrinking)

   > after sterilization (certain purity grades)

Skim reading

At Eppendorf, quality control already starts with the raw material, proceeds with product control at the machine during manufacturing and ends with extensive applicative and dimensional tests in the lab.

This quality control is focused on e.g. measuring concentricity, filter position (filter tips), measurement of orifice diameter, determination of sealing on pipette cone, wetting behavior and gravimetric measurement of accuracy and precision on Eppendorf pipettes (calibration).

Methodical influences (1/2): Pipette calibration with or without tip change

The standard ISO 8655:2002 part 6 states that during a calibration the tip has to be changed a) after the initial pre-wetting step and b) after each measurement. This means a high consumption of tips (33) for one pipette. The question arises if this is really necessary and if there is a difference between performing a complete calibration with one tip or using a new tip for each measurement. Since the standard addresses the system of pipette and tip, it recommends using a new tip for each measurement in order to represent variances in tip quality. Within this study, we have performed both: a calibration with tip change and a calibration with one tip in total.

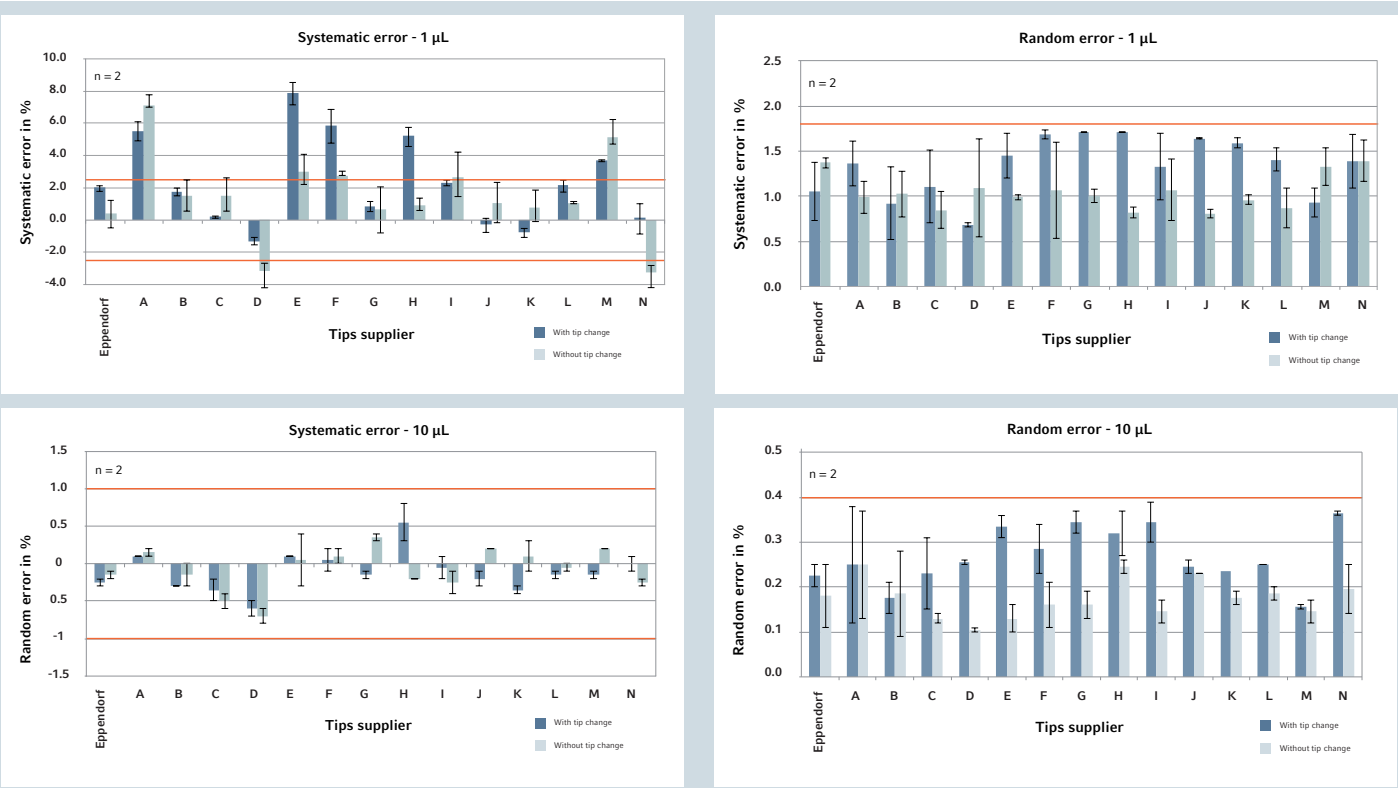


Fig. 6: Results of calibration with and without tip change using 10 µL tips of different manufacturers. The orange line displays the error limits of the Eppendorf Xplorer pipette.

Looking at the results for the 10 µL tip, we found an influence of calibration method by tip change with systematic and random error (fig. 6). At 1 µL, manufacturers E, F, H were clearly negatively influenced in systematic error by tip change and exceeded the permissible error tolerances for the pipetting system. In contrast, suppliers A and M showed a better performance when calibrating with tip change but exceeded the permissible error tolerances with both calibration methods. Such differing results for calibration had already been reported by Wenk et al. [8] investigating 24 pipettes of six manufacturers with generic and recommended tips.

Although this publication is very old, we reconfirmed the finding that Eppendorf tips are less influenced by the calibration method than tips from other manufacturers.

The random error of both calibrated volumes, 1 µL and 10 µL, shows a clear increase with tip change. Such an increase in random error has been published for 20 µL [9]. The random error uncovers all non-systematic influences. It depicts an influencing factor with varying impact. We found the random error to be within manufacturer specifications but greatly influenced by the tip change with 10 µL tips. Thus manufacturers E, F, G, H, J, K, L showed an increase in random error at 1 µL whereas suppliers D, E, F, G, H, I, L, N showed an increase at 10 µL. Since the system stayed the same during the complete calibration we deduce that this increase is evoked by poor tip-to-tip quality.

Although the increase in random error did not exceed the permissible error limits, it may easily become problematic due to the fact that pipetting errors sum up. A high random error caused by the "hardware" leaves it to very skilled personnel to pipette within permissible error tolerances. This means that all factors influencing the pipetting, e.g. environmental conditions or pipetting skills, are needed to be at optimum in order to achieve calibration results within the permissible error tolerances. For this study, we used an electronic pipette since this pipette has the lowest possible random error. Using a manual pipette instead may already change the result to a system outside the limits for random error. But what does this mean for the daily lab routine? The theoretical consequence of the conclusion would be the use of one generic tip for all pipettings at least within one analysis. This handling would improve the reproducibility of single pipettings and thus the comparability of analysis results e.g. for different samples. However, due to contamination of samples this handling is not viable. Users should employ tips recommended by the pipette manufacturer or at least define the impact of imprecision by performing calibration with/without tip change.

Skim reading

Eppendorf tips were less affected by the calibration method. We deduce that the increased random error of the other manufacturers is caused by varying tip-to-tip quality. A highly increased random error induced by the tips leaves it to very skilled personnel to pipette within the permissible error tolerances.

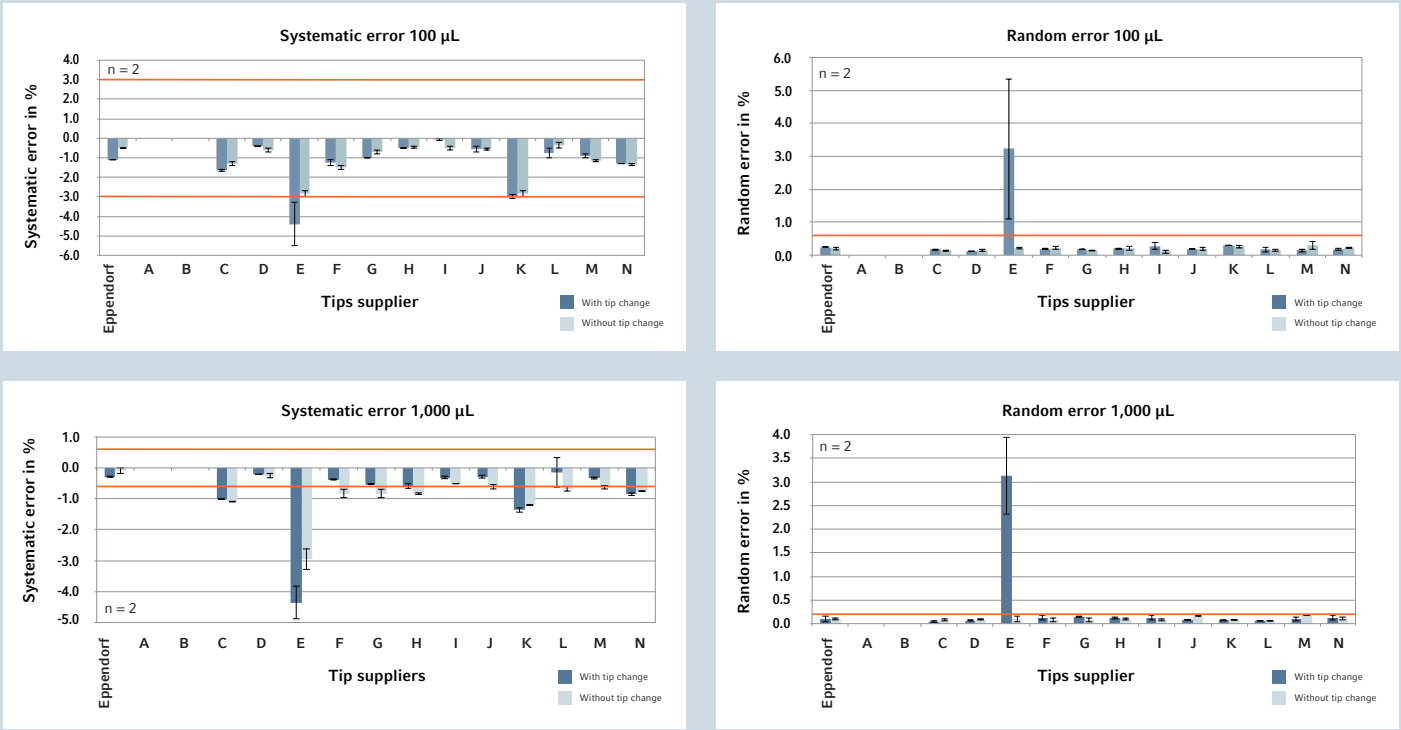


Fig. 7: Results of calibration with and without tip change using 1,000 µL tips from different manufacturers. With this tip size, tips from supplier A and B fell off the cone after a few pipettings. The orange line displays the error limits of the Eppendorf Xplorer pipette.

With the 1,000 µL tips, the most distinct result was determined when calibrating with manufacturer "E" (fig. 7). This pipette system exceeded by far the permissible tolerances for systematic and random error at 100 µL as well as at 1,000 µL if the tips were changed for each measurement. If one tip was used for the complete calibration, the calibration results of supplier E improved although they were not within error limits in all cases. This manufacturer not only exceeds the error tolerances (compare discussion of fig 1 and fig 2), its tip-to-tip quality is so poor that it clearly impairs the calibration result. This finding is furthermore underlined by a very high standard deviation.

With the exception of manufacturer E, the differences between a calibration with/without tip change were in general less distinct than with 10 µl tips. This also illustrates that production quality is especially important for small tips/volumes. Here, it is significant that all tips of one box are manufactured with lowest production tolerances in order to generate lowest differences between tips: a box with uniform tips.

In general, when looking at all four measured volumes (1 µL, 10 µL, 100 µL, 1,000 µL) a total of 13 tip suppliers exceeded the permissible errors. The only tips showing no influence of the calibration method (changing the tip or using one tip for complete calibration) on the calibration result nor exceeding the permissible error tolerances were from supplier B and Eppendorf. Here, the tip-to-tip quality is so high that no impact on the calibration method was found. With such tips, the user does not have the need to check the variance of the system – as stated by ISO 8655 [1] – but may focus on the pipette. With such tips, it is possible to calibrate with just one tip [2].

Methodical influences (2/2): Autoclaving – how a common decontamination method affects tips

Users who need sterile products have two possibilities to achieve sterility: either they purchase sterile products or they decontaminate pipette tips by autoclaving. The first choice is safeguarded by a professional quality assurance.

At Eppendorf, a batch of sterile tips does not leave the manufacturer before the test has been passed. The sterilization process is validated and the testing is performed by an external, accredited laboratory. Accredited labs fulfill highest requirements in terms of confidence in process as well as traceability giving maximum proof in the results. The tests are performed batch-specific. This means every newly produced sterile batch is tested. The result of the test is published in a certificate which can be downloaded by the user 24 hours a day just by entering the batch number on the homepage.

It is rather difficult for users to set up a comparable quality assurance. Instead of testing the effectiveness of the decontamination method, in most cases an established method becomes "trusted" and is not further scrutinized. Thus, the sterility is unknown. In case autoclaving conditions differ from standard methods (121 °C, 2 bar total, 20 min), e.g. by application of shorter times than requested by some tip suppliers, a test for autoclaving effectiveness becomes even more important. Pipette tips without filter are – if not declared otherwise – usually autoclavable. However, it has to be taken into account that PP, based on its composition becomes soft at approximately 110 - 120 °C (melting temperature of approximately 160 - 180 °C). Thus, one can imagine that autoclaving according to standard method does stress the material. In the worst examples, tips have been reported to have a closed orifice after autoclaving with standard methods. In order to find autoclaving-triggered dimensional changes the autoclaved and non-autoclaved tips were measured by 3-D laser measurement and used in a calibration.

Skim reading

Sterilization processes performed by users are usually not monitored. Thus the sterility is questionable. The benefit of purchasing sterile products from a manufacturer is an assured sterility. At Eppendorf, no sterile batch leaves the production site before the external accredited laboratory gives a green light.

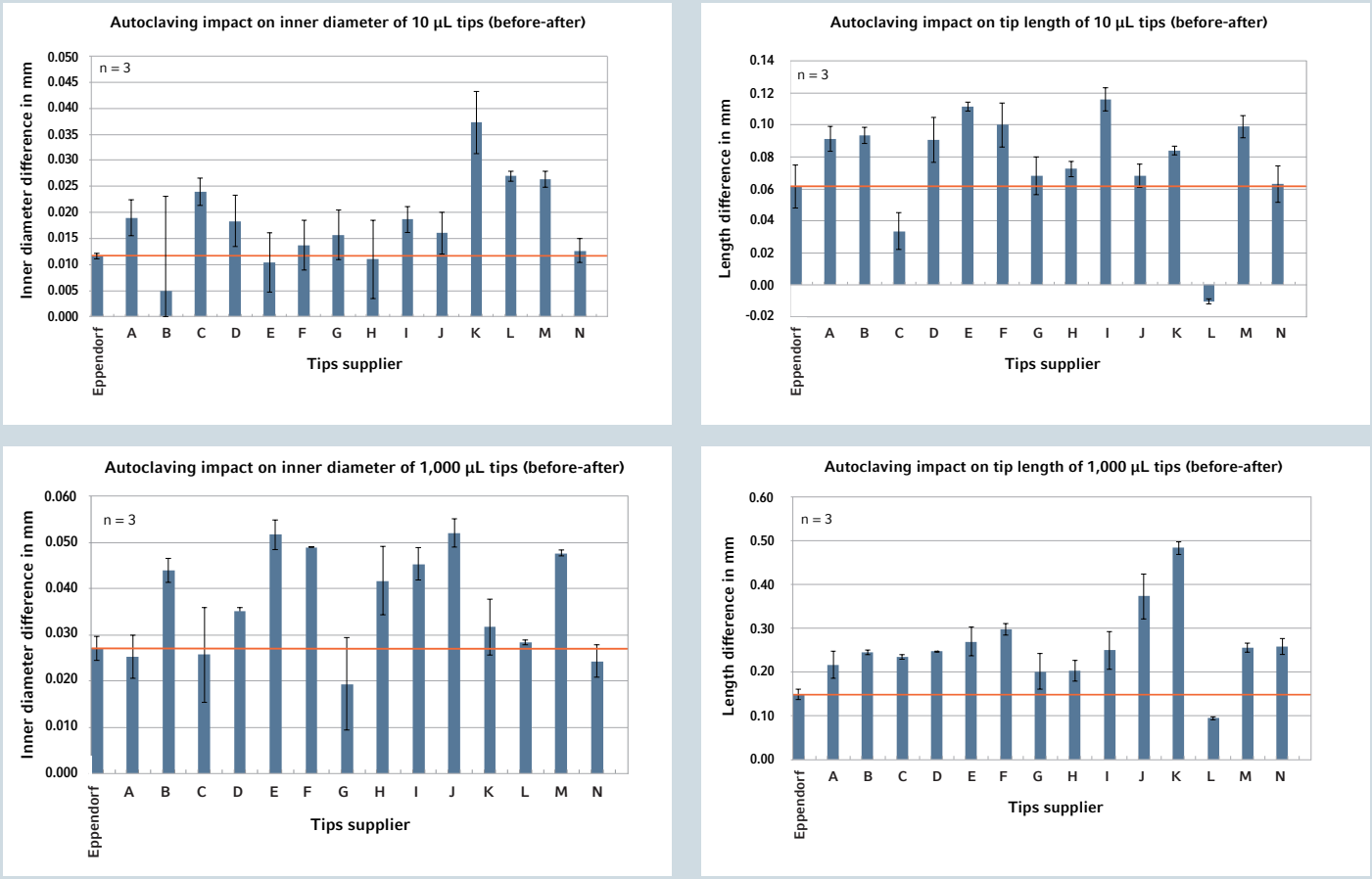


Fig. 8: Dimensional changes of different manufacturer pipette tips induced by autoclaving.

Fig. 8 shows that autoclaving influenced the dimensions of pipette tips while its implication differed between tip manufacturers and tip models. In most cases, the tips shrank in length and inner diameter. Only the 10 µL tip of manufacturer "L" expanded (tip length). In the case of a good tip, the impact of autoclaving is already taken into account within its construction phase. The material composition, tip design and surface structure decide if under heating and cool down a tip shrinks or expands - and in which direction this occurs: in diameter or in length. With the 1,000 µL tip of supplier D, the tip could not be used after autoclaving because the inner diameter shrank and in combination with the design of the sealing rim it became impossible to push the tip onto the pipette cone. Thus, the influence of autoclaving on the pipetting result by subsequent calibration could not be evaluated for this manufacturer. Besides the tip fit of supplier D, the biggest impacts on dimensional changes induced by autoclaving are the variability of reaching the original dimensions and the changed air cushion size. Since the tips shrank, a change in the air cushion size had a rather decreasing effect on systematic error. This was confirmed by calibration results. As displayed in tab. 1, an autoclaving-induced influence on the calibration result was determined only with 1 µL volume. It was discussed previously that small volumes like 1 µL are more affected by other factors than the air-cushion size. Here the geometry and shape of the tip orifice play a more important role. The autoclaving had a negative influence on one of these factors as three more manufacturers (B, F, I) exceeded the permissible error tolerances for systematic error at 1 µL (tab. 1). Besides these findings, the results of calibration after autoclaving reconfirmed the results of the first calibration with tips not autoclaved.

	Before autoclaving							
	1,000 µL tip model				10 µL tip model			
	1,000 µL		100 µL		10 µL		1 µL	
Manufacturer	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %
Eppendorf	V	V	V	V	V	V	V	V
A	/	/	/	/	V	V	X	V
B	/	/	/	/	V	V	V	V
C	X	V	V	V	V	V	V	V
D	V	V	V	V	V	V	V	V
E	X	X	X	X	V	V	X	V
F	V	V	V	V	V	V	X	V
G	V	V	V	V	V	V	V	V
H	V	V	V	V	V	V	X	V
I	V	V	V	V	V	V	V	V
J	V	V	V	V	V	V	V	V
K	X	V	V	V	V	V	V	V
L	V	V	V	V	V	V	V	V
M	V	V	V	V	V	V	X	V
N	X	V	V	V	V	V	V	V

	After autoclaving							
	1,000 µL tip model				10 µL tip model			
	1,000 µL		100 µL		10 µL		1 µL	
Manufacturer	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %
Eppendorf	V	V	V	V	V	V	V	V
A	/	/	/	/	V	V	X	X
B	/	/	/	/	V	V	X	V
C	X	V	V	V	V	V	V	V
D	X no fit	X no fit	X no fit	X no fit	V	V	X	V
E	X	X	X	X	V	V	X	V
F	V	V	V	V	V	V	X	V
G	V	V	V	V	V	V	V	V
H	V	V	V	V	V	V	X	V
I	V	V	V	V	V	V	X	V
J	V	V	V	V	V	V	V	V
K	X	V	V	V	V	V	V	V
L	V	V	V	V	V	V	V	V
M	V	V	V	V	V	V	X	V
N	X	V	V	V	V	V	V	V

Tab. 1: Overview of changes in calibration result after autoclaving tips of different manufacturers. Gray marking: tips did not fit tightly onto pipette cone.

On the whole, it was found that autoclaving may have negative effects on the calibration result with some manufacturers. For those laboratories using autoclaved tips it is recommended to check the performance of the pipetting system after autoclaving the tips. However, such a calibration is only needed if other manufacturer tips are used. With Eppendorf tips, no negative influence on the calibration result was observed after autoclaving of tips.

Leachables

In certain application areas, e.g. MALDI-TOF, traces are to be analyzed. Strong or aggressive chemicals like acetonitrile are used in order to extract the molecule to be determined. As shown in the past e.g. organic solvents have the ability to extract additives from polypropylene. Such additives may mimic peaks. Beyond this widely known finding, recent scientific literature reported evidence of disturbance of a broad range of biological assays caused by leachables. Examples are enzymatic, receptor binding and photometric assays as well as alterations in growth rates in cell culture [10].

In general, based on the extraction method, two types of molecules transferring from the plastics into the sample are distinguished: Leachables and extractables. Extractables are all substances which can be taken out of the plastic by applying maximum stress to the plastic, e.g. by combining hot temperature with a strong chemical. Leachables are substances that transmit from the plastic into the sample under normal laboratory use. The latter is of much more interest for laboratory personnel.

Plastics in general need additives in order to ensure certain desired characteristics. Such substances cannot be avoided and are known to be most likely non-critical for assays. For example, a plastic that is exposed to UV-light and lacks UV-protective additives will become rigid and brittle after short exposure. Such a pipette tip would break when becoming attached to the pipette cone. On the other hand, there are additives which just ease the production process by making it faster and cheaper. Examples for such additives are slip agents (easier and faster removal from mold), biocides (preventing microorganism growth on plastic) and plasticizers (altering mechanical properties). These additives are known to affect various assays but can be avoided in production. Due to huge know-how in the production of plastics, Eppendorf does not need to make use of such production-related additives. All other additives are decreased to an absolute minimum. Eppendorf certifies that is does not use those additives evidently interfering with biological assays: slip agents, biocides and plasticizers. Of course, this is only possible because of large know-how, the very carefully optimized injection molding process and the high quality in production.

Skim reading

Additives easing production process (plasticizers, biocides, slip agents) are known to disturb biological assays. Thus Eppendorf avoids their use. Additives needed for product characteristics (e.g. not becoming brittle) are decreased to a minimum. Consequently a sensitive MEA test showed no influence on growth of embryo.

But do leachables play a role for pipette tips? Due to the very short period of contact, the time window for transmission of such substances is very short. Recent scientific literature discusses that there may be effects after increasing the number of pipetting steps indicating a cumulative effect [11, 12].

For this reason, a MEA test (Mouse-Embryo-Assay) was performed by an external accredited and FDA-registered lab. Cell embryos are very sensitive to influencing molecules, e.g. additives, from the plastics thus being a very good indicator for toxicity such as that posed by leachables. During this test, the product to be tested becomes extracted by the medium used for growing two-cell embryos towards blastocyst stage. It is then evaluated if the growth of the embryo is increased or decreased by the medium. In the case of the test with Eppendorf tips, the 50-1,000 µL Biopur and 2-200 µL Biopur tips were tested under varying contact/incubation times: 10 pipettings, 4 hours of incubation and 24 hours of incubation. No influence on the embryo was determined in any of the tests, the Eppendorf tips passed all 6 tests (tab. 2). A positive control prevented growth of two cell embryos thereby validating the functioning of the system.

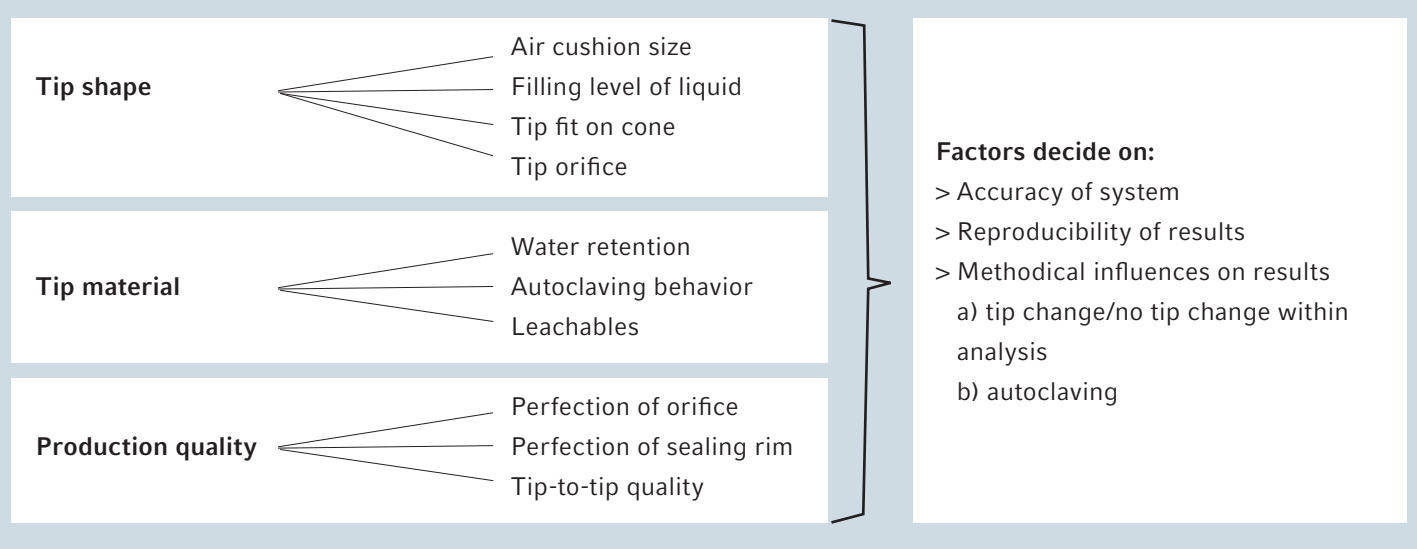
Results for MEA test of Eppendorf Tips

	Extraction time		
Consumable	**10 pipettings**	**4 h ± 15 min**	**24 h ± 2 h**
epT.I.P.S.® 2-200 µL Biopur® Batch-Nr.: D158054Q	90%	90%	87%
epT.I.P.S.® 50-1,000 µL Biopur® Batch-Nr.: D157726P	90%	87%	100%

Tab. 2: Results for MEA test of Eppendorf 50-1,000 µL Biopur and 2-200 µL Biopur tips. The test is passed if the test item has no effect on growth and development to at least 80 %.

Conclusion

Within the scientific community, there are a rising number of studies which cannot be reproduced by other groups. One possible reason may be that the influencing factors of pipette tips are not taken into account – just like recognizing only the tip of an iceberg. We showed within this study that tips from different suppliers can alter the pipetting result and its reproducibility. Thereby, different influencing factors become effective:



Some non-system providers offer tables showing on which pipettes certain tips fit. Our results show that a tip fitting onto a pipette cone does not say anything about the pipetting result. Furthermore, our results underline that it does not make much sense to use a "universal" tip if the pipette does not become calibrated (and, if needed, adjusted). It is incumbent upon the user to proof that the system performs within specifications.
Tips are an important component of the system and they are optimized for the pipette they are produced for.

Accordingly, the ISO 8655 [1] regards the pipette and tip to be a system. It requires an extra calibration when alternative tips are to be used. Our results are evidence that this requirement is meaningful and we strongly recommend naming the tip used within publications and to calibrate (if needed: adjust) the pipettes if other manufacturer tips have to be used.
As a final overview, tab. 3 displays the results of all experiments done within this study. It shows that Eppendorf tips keep the promise of highest quality performing best overall.

	With tip change (one tip per measurement) / Not autoclaved								Without tip change (one tip for complete calibration) / Not autoclaved								With tip change (one tip per measurement) / Autoclaved								Conclusion of all results
	1,000 µL tip model				10 µL tip model				1,000 µL tip model				10 µL tip model				1,000 µL tip model				10 µL tip model				
	1,000 µL		100 µL		10 µL		1 µL		1,000 µL		100 µL		10 µL		1 µL		1,000 µL		100 µL		10 µL		1 µL		
Manufacturer	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	Systematic error %	Random error %	
Eppendorf	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
A	/	/	/	/	V	V	X	V	/	/	/	/	V	V	V	X	/	/	/	/	V	V	X	X	X
B	/	/	/	/	V	V	V	V	/	/	/	/	V	V	V	V	/	/	/	/	V	V	X	V	X
C	X	V	V	V	V	V	V	V	X	V	V	V	V	V	V	V	X	V	V	V	V	V	V	V	X
D	V	V	V	V	V	V	V	V	V	V	V	V	V	V	X	V	X no fit	X no fit	X no fit	X no fit	V	V	X	V	X
E	X	X	X	X	V	V	X	V	X	V	V	V	V	V	X	V	X	X	X	X	V	V	X	V	X
F	V	V	V	V	V	V	X	V	X	V	V	V	V	V	X	V	V	V	V	V	V	V	X	V	X
G	V	V	V	V	V	V	V	V	X	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	X
H	V	V	V	V	V	V	X	V	X	V	V	V	V	V	V	V	V	V	V	V	V	V	X	V	X
I	V	V	V	V	V	V	V	V	V	V	V	V	V	V	X	V	V	V	V	V	V	V	X	V	X
J	V	V	V	V	V	V	V	V	X	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	X
K	X	V	V	V	V	V	V	V	X	V	V	V	V	V	V	V	X	V	V	V	V	V	V	V	X
L	V	V	V	V	V	V	V	V	X	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	X
M	V	V	V	V	V	V	X	V	X	V	V	V	V	V	X	V	V	V	V	V	V	V	X	V	X
N	X	V	V	V	V	V	V	V	X	V	V	V	V	V	X	V	X	V	V	V	V	V	V	V	X

Tab. 1-3: Summary of results of this study. The orange colored cross indicates that a tip of this manufacturer did not pass at least one of the calibrations. Gray markings: tips which did not fit tightly onto pipette cone.

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