The Tip of the Iceberg: How Pipette Tips Influence Results. Part 3: Not Every Tip Tolerates Every Treatment

MURIEL ART, VINCENT DUFEY, ION GLIGOR, EPPENDORF APPLICATION TECHNOLOGIES S.A., NAMUR, BELGIUM
ULRIKE GAST, LAURA KOCH, RONJA KUBASCH, EPPENDORF AG, HAMBURG, GERMANY

Abstract

The pipetting accuracy of pipette tips by certain manufacturers may suffer after autoclaving. This was not the case for Eppendorf tips. We describe the measures which Eppendorf takes in order to avoid the negative influences of autoclaving. Furthermore, we describe how the interference of biological assays by leachables from pipette tips is prevented. A MEA (Mouse Embryo Assay) test proves that sensitive biological systems are not influenced by Eppendorf pipette tips.

Introduction

We have shown in part 1 and 2 of this series (see BioNews No. 44 and 45) that pipette tips influence the performance of the system "pipette and tip" and thus the pipetting result. This was predominantly caused by the tip's shape and its production quality. Autoclaving is a widely applied method for decontamination of pipette tips but it imposes thermal stress. For this reason, calibration was used to determine whether autoclaving of pipette tips would influence the pipetting result.

Recent scientific literature reported evidence of disturbance of a broad range of biological assays caused by leachables, even for pipette tips [1, 2]. Examples include enzymatic and photometric assays as well as alterations in growth rates in cell culture [3]. Eppendorf does not make use of such additives and provides certificates to this effect. However, in order to obtain experimental proof, we have tested Eppendorf pipette tips for inhibition effects on the development of early stage embryos by performing a Mouse Embryo Assay.

Materials and methods

Autoclaving was performed by applying standard methods [4]. The influence of autoclaving on the performance of pipette tips by different manufacturers was determined by calibration [4] with the Eppendorf Xplorer® pipette. The MEA test was performed by an accredited and FDA-registered laboratory [4].

Results and discussion

Autoclaving is a widely applied method for the decontamination of labware such as pipette tips. Thus, one would expect autoclaving to have no impact on the tip's performance. On the contrary, Fig. 1 shows that autoclaving did exert a negative influence on the performance of the tips by manufacturers B, D, and I. Indeed, the system of pipette and tip passed calibration with tips by these manufacturers prior to autoclaving but exceeded the permissible error limits for the systematic error at 1 µL after autoclaving.

For the production of good tips, the impact of autoclaving is already taken into account during the construction phase. The type of polypropylene (PP) and the shape and surface structure decide how much a tip will shrink and in which direction this will occur. Recycled materials typically contain higher concentrations of leachables,

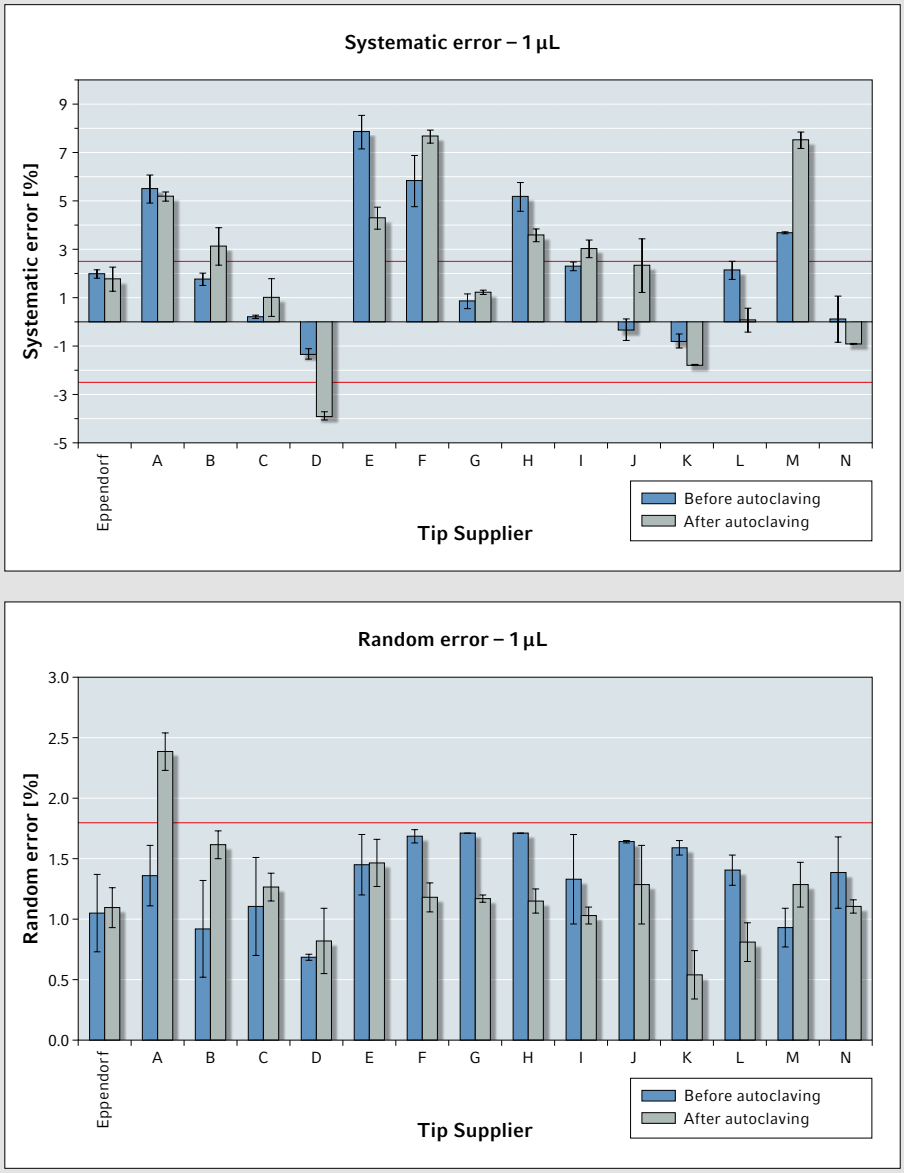

Fig. 1: Calibration results at 1 µL with non-autoclaved and autoclaved 10 µL pipette tips by different manufacturers. Each red line marks the error limits for the Eppendorf Xplorer pipette.

The Tip of the Iceberg: How Pipette Tips Influence Results. Part 3: Not Every Tip Tolerates Every Treatment

Results for MEA test of Eppendorf tips			
	Extraction time		
Pipette tip	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.® 50–1000 µL Biopur® Batch-Nr. D157726P	90 %	87 %	100 %

Table 1: Results of the MEA test with epT.I.P.S. in Biopur quality. The test is considered passed if the test item has no effect on the growth and development of at least 80 % of tested embryos.

and they may also alter the tip characteristics, for example shrinking behavior during autoclaving. For these reasons, Eppendorf does not use recycled material, nor does Eppendorf reuse material from discarded products.

Furthermore, Eppendorf is very careful with the addition of additives to the polypropylene. Plastics in general need additives in order to ensure certain desired characteristics, e.g. prevention of fast decomposition. These additives cannot be avoided. On the other hand, additives such as slip agents, biocides, and plasticizers are only included to make the production process faster and cheaper. Such additives can be avoided – if the producer has the required expertise and ability to allow a more expensive production process.

Eppendorf pipette tips are made of pure, virgin polypropylene. The material is free from any plasticizers, UV stabilizers, latex, slip and antistatic agents, silicones, bisphenol A, and biocides. Mouse embryos are very sensitive to molecules such as additives from plastics and are therefore good indicators of toxicity such as that posed by leachables. We found no influence on the growth of two-cell embryos towards the blastocyst stage by the Eppendorf pipette tips (Table 1).

Conclusion

The quality of production directly influences tip performance at the user's lab bench. Production does not start with melting the plastics but rather with the tip's design phase. It is absolutely necessary to consider other uses of the product besides pipetting water, such as autoclaving. The international standard ISO 8655 [5] requires re-calibration of the system pipette plus tips if tips of other manufacturers are used. Based on our results, we recommend that laboratories using autoclaved alternative tips calibrate the system after autoclaving. In contrast, with Eppendorf tips, no negative influence on the calibration result was observed after autoclaving. Therefore additional calibration is not required.

Expertise and the determination not to minimize production costs at the expense of quality are essential in the production of pipette tips which will not leach additives. Eppendorf provides certification for the absence of additives which interfere with biological assays – and rightfully so: It has been shown that the epT.I.P.S. examined here did not influence a sensitive biological system.

In the fourth part of this series the influence of tip change on pipetting results will be explained (BioNews No. 47).

Literature

[1] Olivieri A, Degenhardt OS, McDonald GR, Narang D, Paulsen IM, Kozuska JL, Holt A: On the disruption of biochemical and biological assays by chemicals leaching from disposable laboratory plasticware. *Can J Physiol Pharmacol* 2012; 90(6):697-703

[2] Watson J, Greenough EB, Leet JE, Ford MJ, Drexler DM, Becastro JV, Herbst JJ, Chatterjee M, Banks M: Extraction, identification, and functional characterization of a bioactive substance from automated compound-handling plastic tips. *J Biomol Screen* 2009; 14(5):566-72

[3] Grzeskowiak R, Gerke N: Eppendorf White Paper 26: Minimizing the influence of plastic consumables on the laboratory worflows. www.eppendorf.com

[4] Art M, Dufey V, Gast U, Gligor I, Koch L, Kubasch R: Application Note 354: The Tip of the Iceberg: How Pipette Tips Influence Results. www.eppendorf.com/appnote354

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