eppendorf

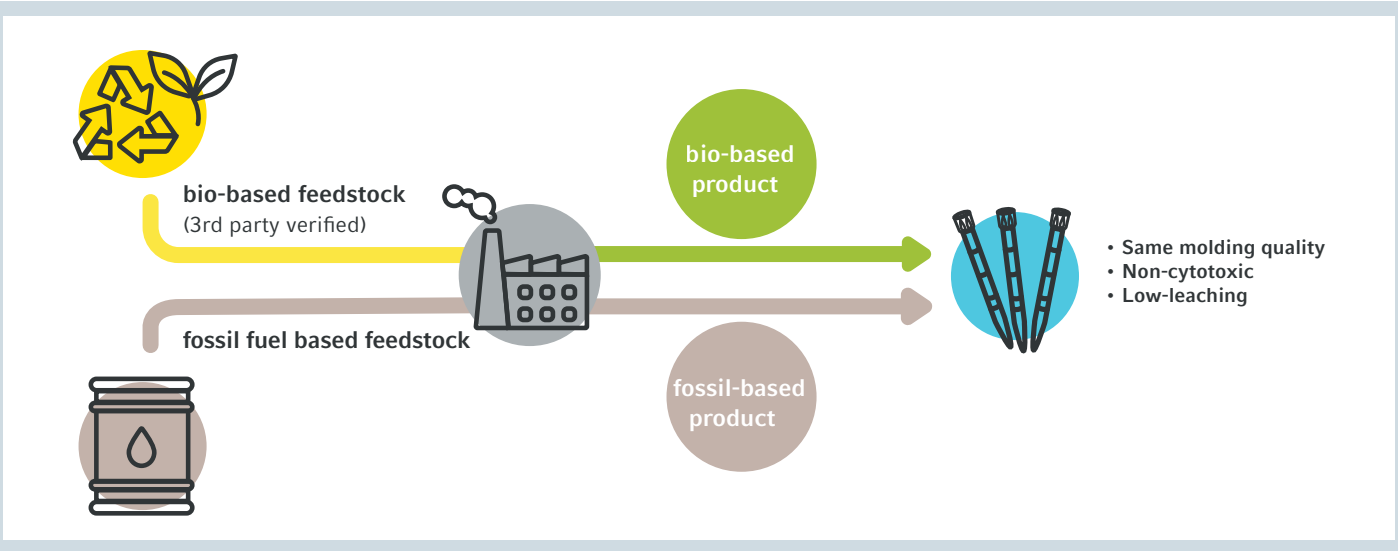
APPLICATION NOTE No. 477

2nd Generation Material - 1st Class Performance Vol. 2: Comparison of Quality and Safety Parameters, Cytotoxicity and Leaching Levels of epT.I.P.S.® BioBased and epT.I.P.S.® Standard

Author
Jana Schmidt[1], Estelle Deboever[2], Jean-François Hoet[3] and Muriel Art[3]
[1]Eppendorf SE, Hamburg, Germany
[2]Eppendorf Application Technologies, Namur, Belgium · [3]Eppendorf Core Test Lab, Namur, Belgium

Abstract

In this study, the new Eppendorf epT.I.P.S.® BioBased Biopur® pipette tips made from bio-based feedstocks were compared to standard epT.I.P.S. Biopur, made from fossil-based feedstocks.
There were no significant differences noticeable between the pipette tips performance in any of the executed experiments. This indicates that the more sustainable, bio-based material from renewable feedstocks offers equal properties as fossil-based material. Interestingly, compared to pre-sterilized pipette tips tested from other manufacturers, epT.I.P.S., BioBased and standard epT.I.P.S. showed the lowest leaching levels. In total, three different parameters were evaluated to test for an equal performance of the two tip versions: tip orifice quality, cytotoxicity according to ISO 10993-5 and ISO 10993-12 norms, and leachable lixiviation.



Introduction

Pipette and tip build a system that allows for precise, accurate and reliable results of any laboratory procedure including liquid handling steps. Whilst the pipette can be used for years, the plastic tip must be exchanged with each liquid handling step, making them a relevant source of waste in a laboratory, typically produced from fossil-based polypropylene. With the global need to be more sustainable, such lab consumables are thus an important factor that must be considered when trying to reduce waste and the usage of fossil-born products [1]. Eppendorf Tubes® were the first lab consumables manufactured from bio-based feedstocks [2], opening the door for more sustainable labware. They are now followed by **E**ppendorf's **T**otally **I**ntegrated **P**ipetting **S**ystem, epT.I.P.S. BioBased. These pipette tips are manufactured from ≥ 90 % renewable feedstocks in order to significantly reduce the amount of fossil resources required for their production. The plastic used for their manufacturing is ISCC certified [3], where the plastic can be traced back to biological waste material which is attributed to these tips via the ISCC mass balance approach (figure 1).

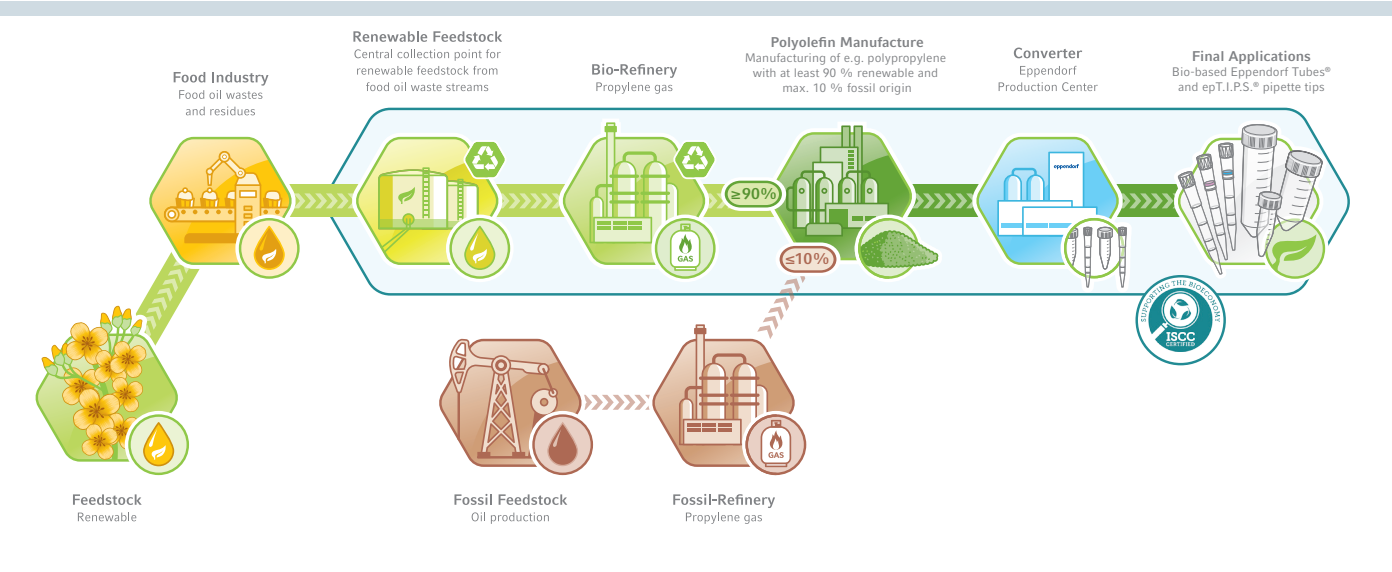


Figure 1: From food industry waste to Eppendorf Tubes® BioBased and epT.I.P.S.® BioBased: food oil wastes get collected and refined to form polypropylene, the material for the consumables. The plastic used for this manufacturing is ISCC certified, where the plastic can be traced back to biological waste material which is attributed to these tips via the ISCC mass balance approach [3].

This study tested three crucial parameters of standard epT.I.P.S. Biopur and epT.I.P.S. BioBased, Biopur to assure comparability of standard, fossil-based and bio-based epT.I.P.S.: The orifice quality, the potential material cytotoxicity and leaching levels. The latter was also compared to main competitors offering pre-sterilized pipette tip reloads.

Material and Methods

The following pipette tips were evaluated in all three assays:

> epT.I.P.S.® BioBased, Biopur®, Reloads, 2-200 µL (order no. 0030 075 439)
> epT.I.P.S.®, Biopur®, Racks, 2-200 µL (order no. 0030 075 234)
> pre-sterilized, 200 µL non-filtered pipette tips from other manufacturers

Additionally, the following tips were evaluated in orifice quality:

> ep Dualfilter T.I.P.S.® BioBased, PCR clean/sterile, Reloads, 50-1000 µL (order no. 0030 081 099)
> ep Dualfilter T.I.P.S.®, PCR clean/Sterile, Racks, 50-1000 µL (order no. 0030 078 578)

Microscopy of tip orifices

The tip orifices were examined using a digital microscope (Keyence, VHX-7000) with 50-fold magnification. Observations were done in triplicates. Only one representative tip orifice for each tip version is shown.

Cytotoxicity

Preparation of Liquid Extracts

Controls and all tips tested were decontaminated with isopropanol 70 % and were handled aseptically during all following steps. Materials were cut in small pieces, placed into glass vessels and covered with completed medium (4 mM MEM glutamine, 100 UI/mL penicillin, 100 µg/mL streptomycin, 10 % FBS) in a 3 cm^2/mL surface-to-volume ratio. Extraction conditions for the tip material were 37 °C for 72 hours, compliant with the ISO norms 10993-5 [4] and ISO 10993-12 [5], and 50 °C for 24 hours as well as 30 min at 37 °C to simulate the usually shorter contact time between tip and liquid. The extracts were used for cell culture growth immediately after the incubation. Each experiment was performed in triplicates.

Cell Culture

L929 cells were cultured in the completed medium (ATCC, 30-2003) in a humidified atmosphere of 5 % CO_2 and digested by 25 % Trypsin/EDTA to get a single cell suspension. After inactivation of Trypsin-EDTA, cells were centrifuged and diluted in fresh culture medium to obtain a $1x10^5$ cells/mL suspension. Of this culture 200 µL/well were seeded and cultured as described for 24 h to form a monolayer. The culture medium was discarded and replaced with the liquid extracts (see Preparation of Liquid Extracts) and cultured for 48 h. The potential cytotoxicity was evaluated qualitatively (Cell Morphology) and quantitatively (MTT assay).

Cell Morphology Evaluation

Cell morphology was examined under the microscope (10x magnification, EVOS FL Auto 2) and morphological changes (e.g., detachment, cell lysis, vacuolization, etc.) were assessed. For each condition, the morphological status of cells was graded according to table 1. A grade of more than 2 is considered as a cytotoxic effect, according to the named ISO standards.

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmic granules, no cell lysis, no deduction of cell growth.
1	Slight	Not more than 20 % of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50 % of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50 % growth inhibition observable.
3	Moderate	Not more than 70 % of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50 % growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

Table 1: Qualitative morphological grading (ISO 10993-5, [4])

MTT assay

After the morphology assessment, the liquid extract culture medium was discarded and replaced by 50 µL/well freshly prepared MTT solution (1 mg/mL). Cells were incubated for 2 h (37 °C, 5 % CO_2). Absorbance of each culture was measured at 570 and 650 nm (ref). A blank measurement was performed using Completed medium.
Yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) is reduced to purple formazan by a cellular enzyme and NADH. Thus, a color change of the culture is a direct indicator for cell viability [6].

As described in the stated ISO norms, a viability reduced below 70 % (compared to the blank) was considered as a cytotoxic effect [4,5]. The error indicates the standard deviation of the mean of the three replicates.

The measured absorbance was correlated to the number of viable cells as follows:

$$\text{rel. viability} = \frac{100\ \% \times OD_{570e}}{OD_{570e}}$$

OD_{570e} is the mean value of the measured optical density of the test sample

OD_{570b} is the mean value of the measured optical density of the blank

Leaching

One pipette tip was put inside a glass tube and was fully covered by 8 mL of ethanol, 99.9 % p.a.. The glass tube was sealed with aluminum foil and placed into a tube rack inside a shaker at a 45 ° angle. The samples were shaken at 140 rpm at 60 °C for the indicated period. A 200 µL sample was transferred into an Eppendorf UVette® and measured at 260 and 280 nm. Ethanol incubated in the same manner without a tip served as a blank. Three tips were assayed per experiment, and each experiment was performed in triplicates, the error indicates the standard deviation.

Results and Discussion

Tip orifice quality

The shape of a pipette tip influences the accuracy of pipetting experiments [7]. The intact form and uniform wall-thickness of the tip's orifice are thus important parameters to check when evaluating a pipette tip's quality.

Optical analysis of the standard epT.I.P.S. and the epT.I.P.S., BioBased revealed the identical and exact shape and quality for both evaluated volume variants (Figure 2). Neither molding errors, lying flashes, nor cavities were visible. Likewise, the dimensions were identical to 10^{-2} mm and represent the given dimensions for epT.I.P.S. [8].

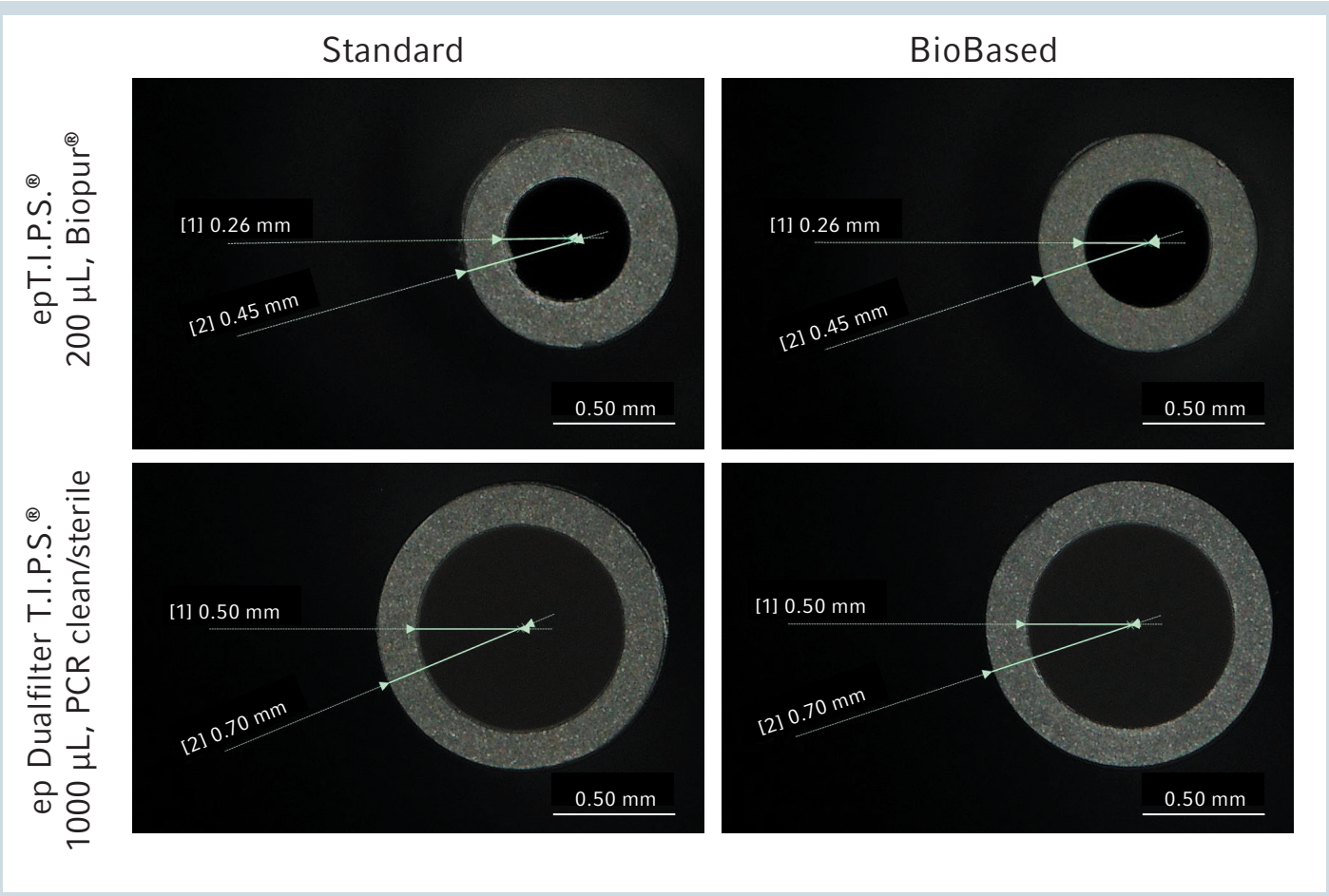


Figure 2: Orifice comparison of epT.I.P.S.® made from fossil-based (left) and bio-based (right) polypropylene. No cavities, molding errors or lying flashes are visible for either. Radii of the orifices inner [1] and outer [2] circles are identical.

Cytotoxicity

Cell Morphology

To test material cytotoxicity, ISO 10993-5 [4] and ISO 10993-12 [5] standards were followed, growing L929 cells in liquid extracts of the tested pipette tips. Cell viability was examined qualitatively with microscopy and quantitatively with the MTT assay.

For all conditions, the cell morphology was not affected when cultured in presence of the extracts (Table 2, Figure 3). Neither fossil-based nor bio-based material induced any morphological changes under the extraction conditions tested.

Extraction Conditions		37 °C 30 min		37 °C 72 h		50 °C 24 h	
tip material		Standard	BioBased	Standard	BioBased	Standard	BioBased
replicate	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0

Table 2: Cell morphology grading of the L929 cell culture according to ISO 10993-5 and ISO 10993-12 norms [4, 5]. Cell culture media to grow the cells was incubated with pipette tips as indicated prior to usage to generate a liquid extract. Neither standard nor epT.I.P.S., BioBased (green) show a cytotoxic effect on cell morphology.

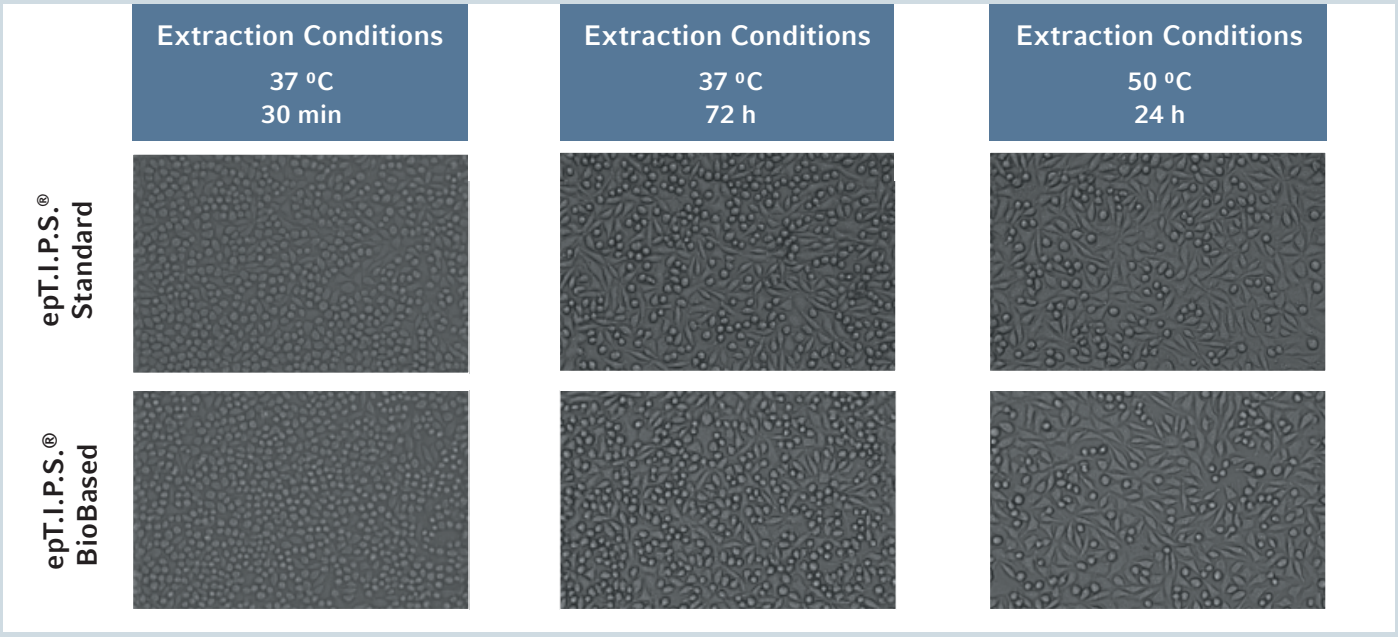


Figure 3: L929 cell culture grown in pipette tip extracts generated under the indicated conditions, respectively. Neither standard nor epT.I.P.S. BioBased influence cell morphology

Cell Viability

Following the stated ISO norms, the viability of cells grown in the described extracts was quantified using the MTT assay which allows measuring the metabolic activity of the culture as an indicator for proliferation and viability. A reduction in relative viability of below 70 % indicates a cytotoxic effect of the tested materials.

Cell-viability in the tested extracts was very high with a relative viability genuinely above 70 %, reaching 85 % or more, regardless of the extraction condition or the material evaluated (figure 4). This quantification unambiguously confirms the morphology observations.
This shows again, that the epT.I.P.S. material has no cytotoxic effect, as well as that fossil-based and bio-based material do not differ in this quality.

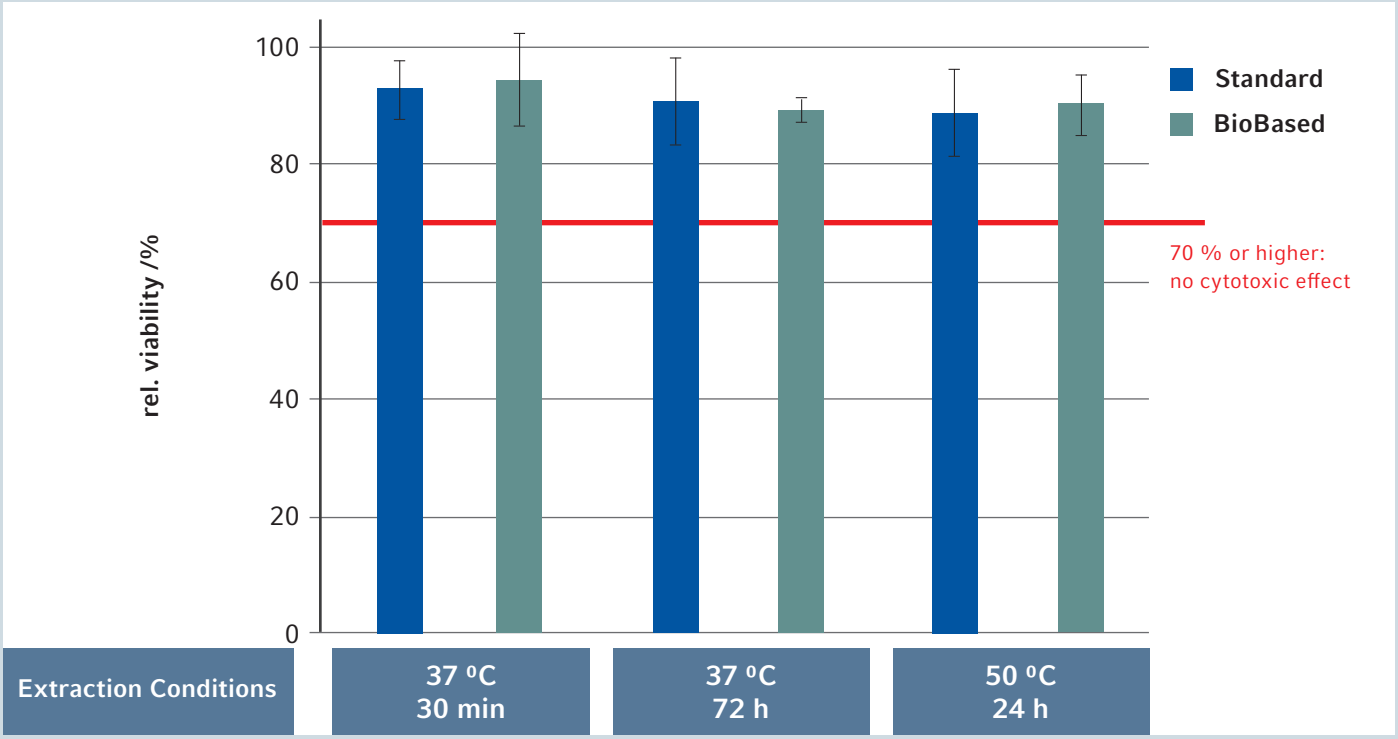


Figure 4: Relative viability of L929 cells grown in liquid pipette tip extracts generated at the indicated conditions.

Leaching

Pipette tips are in contact with the transferred sample for a very short time, especially compared to the reaction vessel or storage container that holds the sample. Nevertheless, concerns arise that additives or other components of the consumable used during the production process could infiltrate the sample ("leaching"), especially when pipetting organic solvents [9].
Thus, we tested for leachables of pipette tips, by incubating them in ethanol. The absorbance of the ethanolic extract was then measured at 260 and 280 nm, where leachables and extractables from plastics can be detected unpacifically [10].
A typical application of UV-absorbance measurement at 260 nm is the quantification of double-stranded DNA. In general, an absorbance of 0.02 indicates 1 ng/µL of DNA. The absorbance at 280 nm is linear to the protein concentration of the sample. An absorbance of 0.02 indicates e.g. 14 µg/mL IgG or 30 µg/mL BSA in the sample. Thus, a consumable leaching into the sample that absorbs UV light in this range, would falsify the results of the DNA- and protein-quantification [10].

The absorbance for both standard, fossil-based epT.I.P.S. and epT.I.P.S., BioBased in the UV-light spectrum was below 0.01 after an extraction period of 1 h (Figure 5). Even after extreme incubation conditions of 24 h, only very little leaching was detectable from both, standard and epT.I.P.S. BioBased, indicating two things:

> the high quality and thus low leaching from epT.I.P.S.
> both materials show the identical results and that the products are indeed, of the same quality.

Alongside the Eppendorf pipette tips, we tested comparable 200 µL pre-sterilized pipette tips from other manufacturers. After 1 h of extraction, competitor D already showed high differences to all other tested pipette tips with more than four times higher absorbance values (hence leachable levels) at 260 nm. Its leaching levels increased further after 24 h incubation to OD_{260} > 0.16, corresponding to a "fake" DNA-concentration of more than 8 ng/µL. Interestingly, after 24 h incubation, Competitor B also showed higher absorbance than both epT.I.P.S. at 260 nm. Competitor D also showed highest leching levels at 280 nm, almost three times higher than epT.I.P.S. and epT.I.P.S., BioBased (Figure 6). At this wavelength, competitor A and C also showed much higher leaching levels after 24 h incubation, which would lead to false protein concentrations of e.g. 75 µg/mL for BSA.

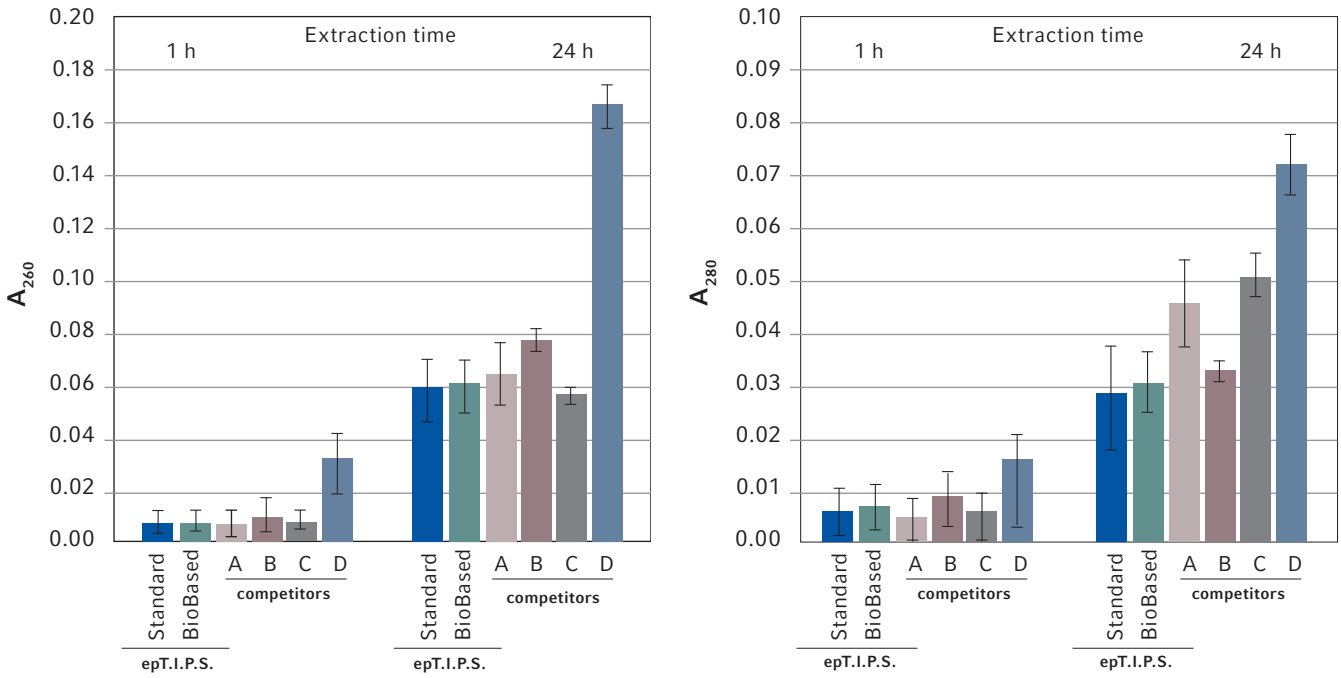


Figure 5: Absorbance of pipette tip extracts in ethanol after the indicated time at 260 (left) and 280 nm (right).

Conclusion

In this Application Note we compared Eppendorf epT.I.P.S., Biopur from the standard, fossil-based material and the more sustainable epT.I.P.S. BioBased, Biopur (figure 1). There were no differences in any of the parameters tested, demonstrating the comparable quality of the two materials. This shows that bio-based polypropylene can be used to form epT.I.P.S. without compromising on performance. Interestingly, we saw a higher levels of leachables for competitor presterilized tips when in contact with ethanol. This also indicates the high-quality of epT.I.P.S. and epT.I.P.S. BioBased compared to pipette tips from other manufacturers.

Literature

[1] Urbina, M. A. *et al*. Labs should cut plastic waste too. Nature 528, 479–479 (2015).
[2] 2nd Generation Feedstock – 1st Class Consumables. https://www.eppendorf.com/product-media/doc/en/5238955/Consumables_Brochure_Tubes-BioBased_2nd-Generation-Feedstock-1st-Class-Consumables.pdf
[3] For more information on the ISCC system please visit: www.iscc-system.org.
[4] ISO 10993-5:2009: Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity. in ICS : 11.100.20 Biological evaluation of medical devices.
[5] ISO 10993-12:2012: Biological evaluation of medical devices — Part 12: Sample preparation and reference materials. In ICS : 11.100.20 Biological evaluation of medical devices.
[6] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63 (1983).
[7] Art, M. *et al*. The Tip of the Iceberg: How Pipette Tips Influence Results. (2016). www.eppendorf.com/appnote354
[8] Technical Data Sheet epT.I.P.S. - Dimensions. (2022). https://www.eppendorf.com/product-media/doc/en/154459_Technical-Data/Eppendorf_Consumables_Technical-data_epTIPS_epTIPS-Dimensional-Drawings.pdf
[9] Olivieri, A. *et al.* On the disruption of biochemical and biological assays by chemicals leaching from disposable laboratory plasticware. Can. J. Physiol. Pharmacol. 90, 697–703 (2012).
[10] Grzeskowiak, R., *et.al*. E. Fake DNA in Your qPCR? – Comparative Evaluation of Leaching Levels in PCR Plates. www.eppendorf.link/appnote459

Ordering information

Description	Order no. international
epT.I.P.S.® BioBased, Biopur®, Reloads, 2-200 µL	0030 075 439
epT.I.P.S.®, Biopur®, Racks, 2-200 µL	0030 075 234
ep Dualfilter T.I.P.S.® BioBased, PCR clean/Sterile, Reloads, 50-1000 µL	0030 081 099
ep Dualfilter T.I.P.S.®, PCR clean/Sterile, Racks, 50-1000 µL	0030 078 578

Your local distributor: www.eppendorf.com/contact
Eppendorf SE · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com