

Comparison of the Cytotoxicity and Leaching Effects between epT.I.P.S.[®] BioBased and epT.I.P.S. Standard

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Abstract

In this study, the new Eppendorf epT.I.P.S. BioBased pipette tips (made from biobased feedstocks) were compared to epT.I.P.S. Standard (made from fossil fuels).

There were no significant differences noticeable between the performance of the pipette tips in any of the parameters tested. This indicates that the more sustainable, biobased material from renewable feedstocks offers the same properties as materials derived from fossil-based sources.

Introduction

A pipette and a pipette tip comprise a system which ensures precise results in any laboratory procedure involving liquid handling steps. Whereas the pipette can be used for years, the plastic tip belongs to the single-use consumables which contribute to laboratory waste, and which must be taken into consideration with respect to the quest for more sustainability and reduced reliance on fossil raw materials.

Eppendorf Tubes[®] were the first lab consumables manufactured from biobased feedstocks, opening the door for more sustainable labware. They are now followed by the epT.I.P.S. BioBased pipette tips. These are manufactured from a minimum of 90% renewable feedstocks and thus significantly reduce the use of fossil resources required for the production of the products.

This study compares epT.I.P.S. BioBased and epT.I.P.S. Standard in the Biopur[®] purity with respect to the parameters cytotoxicity and leaching in order to examine whether the new source of raw material exhibits comparable properties to those of the standard material. Competitors' tips were also tested for the parameter leaching.

Materials and methods

Materials

- > epT.I.P.S. BioBased Biopur, Reloads, 2–200 µL
- > epT.I.P.S. Standard Biopur, Racks, 2–200 µL
- > Pre-sterilized 200 µL non-filtered pipette tips from other manufacturers

For a complete description of the materials and methods used, see [Application Note 477](#).

Cytotoxicity assay

Preparation of the liquid extract

The pipette tips tested were cut into small pieces, placed in extraction vessels, and covered with complete medium (4 mM MEM glutamine, 100 UI/mL penicillin, 100 µg/mL streptomycin, 10% FBS) in a 3 cm²/mL surface-to-volume ratio. The extraction conditions were 37°C for 72 h, compliant with ISO standards 10993-5:2009 and 10993-12, and additional extracts were generated at 50°C for 24 h and 37°C for 30 min. Following the incubation, the extracts were used for cell culture growth of murine L929 fibroblasts (N = 3).

Cell viability – morphology

L929 cells were cultured in complete medium (ATCC[®], 30-2003; 5% CO₂) and digested by 25% trypsin/EDTA to obtain a single-cell suspension. After inactivation of the trypsin/EDTA, cells were collected and diluted in fresh medium to achieve a cell density of 1 x 10⁵ cells/mL. After 48 h, cell morphology was examined, and parameters including detachment, cell lysis, and vacuolization were assessed in accordance with the ISO 10993 standards.

Cell viability – MTT assay

After the morphology assessment, the medium was replaced with an MTT solution (1 mg/mL; 50 µL/well). The cells were incubated for 2 h (37°C, 5% CO₂). Absorbance of the culture at 570 nm indicated its viability, as only intact, metabolically active cells are capable of converting the yellow MTT dye to the purple metabolite. Cells cultured in untreated medium served as control.

Leaching

One pipette tip was placed inside a glass tube and fully covered with 8 mL ethanol, 99.9% p.a. The glass tube was sealed with aluminum foil and incubated inside a shaker at a 45° angle (60°C, 140 rpm). Following a defined time interval, 200 µL of

the ethanol were transferred directly to an Eppendorf UVette[®] and absorbance was measured at 260 nm and 280 nm. Ethanol 99.9% p.a. incubated in the same manner without a tip served as a blank (N = 3).

Results and discussion

Cytotoxicity

Murine L929 cells were grown in media which had previously been incubated with the respective pipette tips in compliance with ISO standard 10993. Culture morphology was assessed in a qualitative manner (Fig. 1).

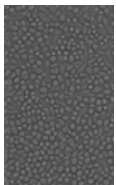
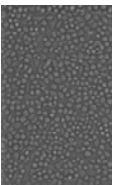
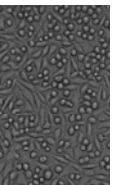
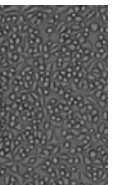
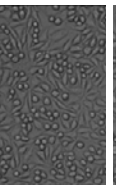
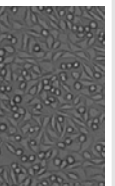
Extraction conditions	37 °C 30 min		37 °C 72 h		50 °C 24 h	
	Standard	BioBased	Standard	BioBased	Standard	BioBased
epT.I.P.S.						
Replicate	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
Morphology						

Fig. 1: Evaluation of the morphology of L929 cells after growth in medium which, in accordance with ISO 10993, had been pre-incubated with pipette tips. A score below 2 is indicative of non-cytotoxic material. Neither epT.I.P.S. BioBased nor epT.I.P.S. Standard show any cytotoxic effects on the murine fibroblasts

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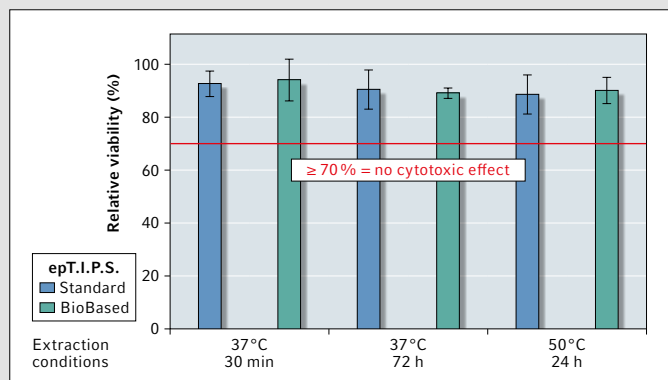


Fig. 2: Relative viability of L929 cells as determined by MTT assay, in accordance with ISO 10993. A value above 70 % shows that the tested material does not elicit cytotoxicity. Neither epT.I.P.S. BioBased nor epT.I.P.S. Standard show any cytotoxic effect

A score of 0 indicates no abnormalities whereas scores greater than 2 are indicative of cytotoxic effects. The MTT assay was used as a quantitative parameter for the viability of the cells (Fig. 2).

A relative viability of more than 70 % indicates a non-cytotoxic material. Neither case showed any cytotoxic effects of the pipette tips, and no differences between the two types of pipette tip could be detected.

Leaching effects

Compared to reaction vessels, pipette tips are in contact with the sample for a short time only. Nevertheless, concerns arise with respect to substances leaching from the plastic (“leachables”), especially during work with organic solvents. For this reason, the tips, as well as comparable products by competitors, were incubated in ethanol, and the ethanol extract was subsequently measured photometrically to test for leachables. In general, leachables show absorbance in the UV-range. Since biochemical analytic assays are also performed at 260 nm and 280 nm (quantification of DNA and protein, respectively), these leaching effects have significant potential for interference.

Even after incubation for 24 h, very little leaching was detectable from both variants of the epT.I.P.S. Comparable pipette tips by competitors showed noticeably elevated leachable effects in both wavelengths after only 1 h of incubation (Fig. 3).

Conclusion

In compliance with ISO 10993-5:2009 (“Testing for in vitro Cytotoxicity”) and ISO 10993-12 (“Sample Preparation and Reference Materials”), the material cytotoxicity of the Eppendorf epT.I.P.S. BioBased, in comparison with epT.I.P.S. Standard, was assessed. Neither the fossil-based nor the biobased materials induced morphological changes, and neither material compromised cell viability. As well, the assessment of leaching effects after contact with an organic solvent showed comparable, low values for both types of pipette tip, whereas competitors’ products showed noticeably higher effects.

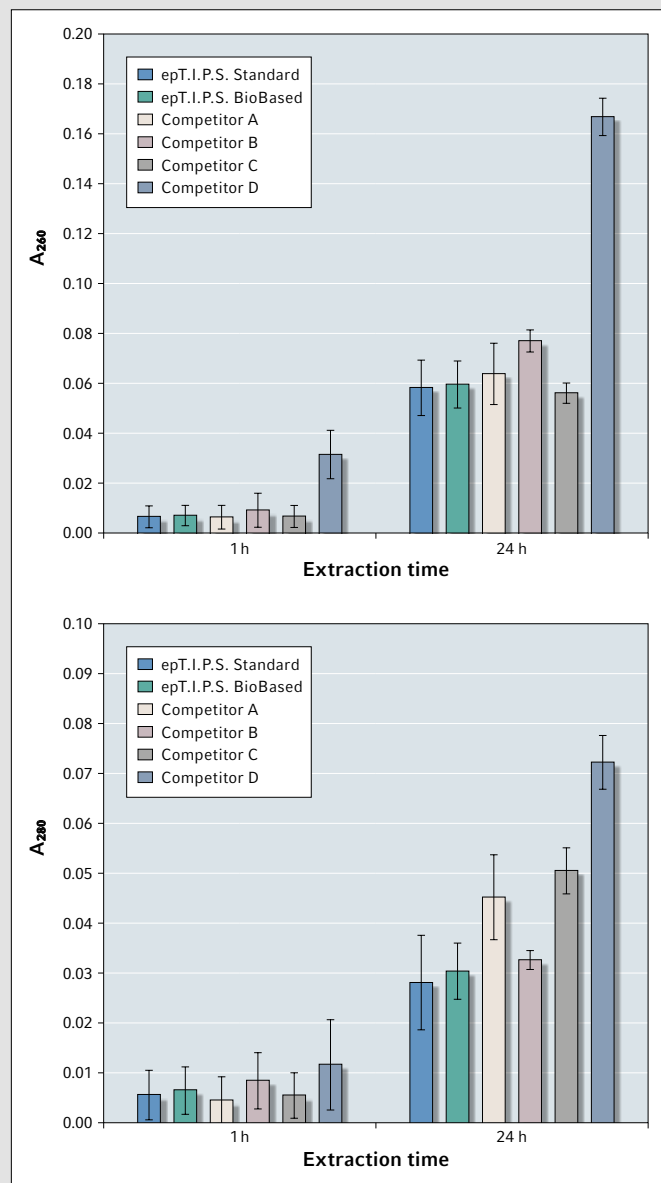


Fig. 3: Absorbance of pipette tip extracts in ethanol at 260 nm (above) and 280 nm (below) for the purpose of non-specific detection of leachables. epT.I.P.S. show the lowest absorbance values. Competitors A–D show noticeably higher absorbance values

The outstanding characteristics and suitability for biochemical applications of both Eppendorf materials – standard and biobased – could thus be verified, as well as the fact that these materials are superior to those used by competitors.

Download the complete [Application Note 477](#)

Literature

- [1] www.iscc-system.org
- [2] Grzeskowiak *et al.*, [Eppendorf Application Note 470](#)

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