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Performance comparison of Eppendorf Conical Tubes: cap tightness, centrifugation stability and leachable levels.

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Abstract

Conical Tubes with screw cap (15 mL and 50 mL) are one of the most wide-spread formats of vessels nowadays used in laboratory. Typically, they have to withstand a great variety of conditions and provide good integrity and sample/user safety. Here we investigate the performance of Eppendorf Conical Tubes 15 mL and 50 mL under critical conditions and compare them to other common brands used in laboratory.

The results provided here show that Eppendorf Conical Tubes 15 mL and 50 mL compared to other manufacturers offer best combined performance with respect to the tested characteristics and a well balanced set of features making them optimal vessels for a broad range of demanding applications.

Introduction

Conical tubes with screw cap belong to most commonly used laboratory vessel formats and are universally used in a variety of laboratory procedures. Typical applications include setting up, handling, storage and transport of various samples and reagents, centrifugation and mixing, various incubations, cell culture and many others. Being a versatile laboratory consumable conical tubes therefore have to withstand a great variety of conditions often regarded as extreme: temperatures between - 86 °C and 100 °C, high centrifugal forces (in the range of 20,000 x *g*), aggressive chemicals or solvents and many others.
Under such broad range of conditions the tube has to remain stable and the screw cap tightly sealed ensuring protection of samples, user and equipment.

Furthermore, as recent scientific evidence suggests, plastic consumables can release various substances added during their production process and hamper many experimental procedures [1]. Absence or very low levels of such substances in laboratory tubes may therefore be of pivotal importance for most applications and increase data quality and process safety in general.
In this Application Note we investigate the performance of Eppendorf Conical Tubes 15 mL and 50 mL under application-relevant conditions and compare them to conical tubes of other manufacturers used in the laboratory.
We evaluate in particular the safety of the conical tubes: tightness of the screw cap during storage at -86 °C of ethanol samples and high speed centrifugation of organic solvent samples (phenol-chloroform).
Finally we also provide comparison data on substances released from plastic material (leach) which may have adverse effects on experiments - so called "leachables".
We demonstrate here that Eppendorf Conical Tubes as compared to other common brands provide optimal combined performance in these safety tests and show minimal levels of leachables.

Materials and Methods

Centrifugation stability
Two 15 mL and 50 mL conical tubes of different manufacturers (Eppendorf, Fa, C and G) were respectively filled with 10 mL and 32 mL of mixture of water:phenol:chloroform (2:1:1). All vessels used were in sterile purity grade.
The tubes were centrifuged at 18,000 x *g* for 30 min at 4 °C and 40 °C. For centrifugation an Eppendorf Centrifuge 5810R with a fixed 45° angle rotor was used: FA-45-6-30 (for 15 mL Tubes an adapter was placed). After centrifugation the vessels were visually inspected or in case of broken vessels removed from the rotor.

Screw cap tightness
The tubes were tested according to the following pattern: for 15 mL format 50 vessels from Eppendorf and 38 from other brands (G, Fa, C) were tested. For 50 mL format 50 vessels from Eppendorf and 22 from other brands (G, Fa, C) were tested. All tubes were in sterile purity grade. The vessels were labeled and weighed and subsequently filled with 96 % ethanol to respective nominal volume of 15 mL and 50 mL. The tubes were then placed horizontally on the tray and stored in the Freezer at -86 °C for 24 hours.
After storage the tubes were slowly equilibrated to room temperature until no moisture was visible and surface was dry. The tubes were again weighed using ultra precise scale and sample loss calculated as percentage of starting weight.

Leachables spectrum
Eight 15 mL and four 50 mL conical tubes of different manufacturers (Eppendorf, C, T, Fi and Fa) were filled respectively with 14 mL or 45 mL water (molecular biology grade). The purity grade of all used vessels was sterile. The tubes were incubated in the Eppendorf ThermoMixer® comfort at 90 °C, 600 rpm for 30 min.
Following incubation absorbance spectra were obtained (between 220 nm to 340 nm) using the Eppendorf BioSpectrometer® and Eppendorf UVettes (10 mm optical path). Non-incubated water was used as the blank. The graphs depict average curve of three independent measurements and may be considered proportional to the amount of substances leaching from the vessels.
The dsDNA concentration which could theoretically be derived from the measured values was calculated from the extinction at 260 nm using the factor 50 µg/mL per unit of extinction.

Results and discussion

Centrifugation stability

Higher speed centrifugation of samples with organic solvents poses certain challenges for bigger laboratory vessels. Particularly at lower temperatures conical tubes may show deformations/cracks of the tube wall or tightness decrease of the screw cap, which results in sample loss and contamination. In the centrifugation test performed here a typical application of nucleic acid extraction was simulated by filling the tubes up to two thirds of nominal volume with water:phenol:chloroform (2:1:1) and centrifuging the tubes at 18,000 x *g*. The results shown in figure 1A confirm high centrifugal stability of Eppendorf Conical Tubes as well as tubes from manufacturer G. All other vessels exhibited major wall/lid deformations and cracks (figure 1B) and all or at least the majority of the content was released to the rotor.

Table 1: shows Centrifugation stability of conical tubes with organic solvent samples at 18,000 x *g* and if sample has been retained (blue) or lost (white) during centrifugation.

Manufacturer	Conical Tubes 15 mL	Conical Tubes 50 mL
Eppendorf	no damage	no damage
Fa	tube wall/lid damage	tube wall/lid damage
C	tube wall/lid damage	tube wall/lid damage
G	no damage	no damage

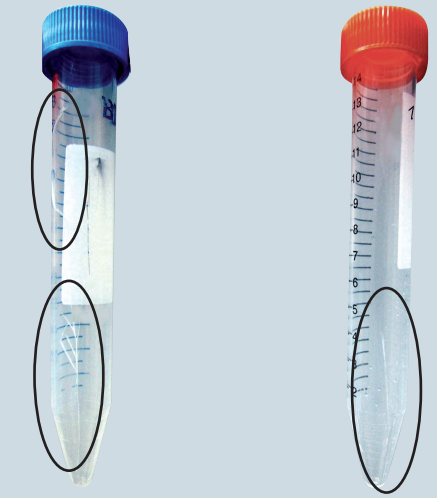
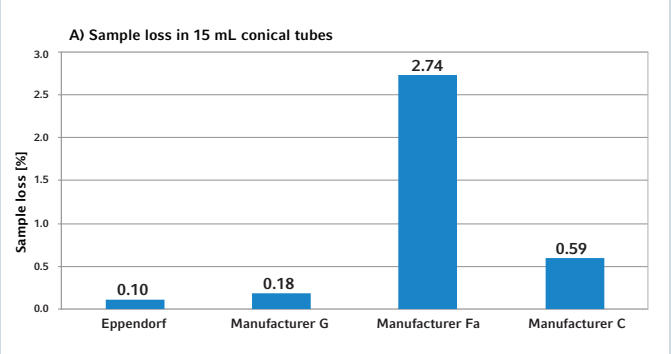


Figure 1: shows examples of typical damage of tested conical tubes after centrifugation, referring to table 1. Trademarks have been masked. Damage area marked with black ellipse.



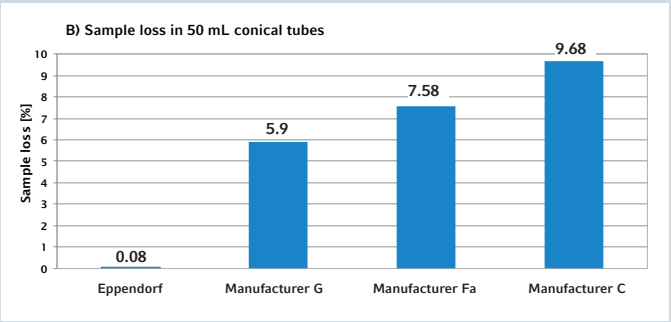


Figure 2: Sample loss after horizontal storage of ethanol in different conical tubes at -86 °C for 24 hours. Sample loss is expressed as percentage of initial weight before storage.
A) 15 mL conical tubes.
B) 50 mL conical tubes.

Screw cap tightness

Tight sealing of the screw cap in the conical tube is the critical prerequisite for sample integrity and necessary to prevent sample loss. Particularly long term storage at very low temperatures and/or use of volatile samples with organic solvents may lead to sample loss. In this Application Note the tightness of the lid has been tested to extreme: ethanol samples were stored in horizontal position at -86 °C to increase vapor pressure and lid strain under very low temperature conditions. Figure 2 shows sample loss after 24 hr storage in different conical tubes. Noteworthy is larger sample loss when 50 mL vessels were used as compared to 15 mL. This may be explained by larger sealing circumference of the cap where imprecisions of the mold/cap can lead to disclosures and sample loss. Both 15 ml and 50 mL Conical Tubes from Eppendorf showed lowest relative sample loss under the conditions tested.

Leachables spectrum

Recent scientific evidence indisputably shows that various additives (leachables) used during production process of consumables may hamper various experimental systems and falsify the results [2]. To date many such substances have been identified and their effect on various assay systems has been demonstrated – also in routine methods such as absorption spectra or photometric detection of nucleic acids/proteins [3]. Based on this a simple test system to evaluate general level of leachables has been established: pure water samples are incubated in given consumable and absorption spectrum measured indicating general amount of UV-absorbing substances leached during the incubation.
In Figure 3 we show such absorption spectra of water samples incubated at 90 °C for 30 min in various conical tubes. All of the vessels were sterile which in general has been connected with increased leachable levels. In summary the data show the lowest levels of UV-absorbing substances from Eppendorf conical tubes (both 15 mL and 50 mL). Absorption spectra of tubes from manufacturers Fa, Fi and C were highest in the experimental conditions used. The absorbance values obtained at 260 nm translate to dsDNA concentrations and this may yield false elevated results during photometric analyses of molecules such as nucleic acids and proteins which are primarily conducted at 260 nm – 280 nm. Respective values of theoretical dsDNA concentrations derived from absorbance at 260 nm are shown in Figure 4. The lowest values were observed for samples incubated in Eppendorf conical tubes: 0.75-0.80 µg/mL (values for 15 mL and 50 mL tubes). These values are in accordance to previous findings for other Eppendorf vessels [4, 5] and below the recommended limit of DNA quantification for the Eppendorf BioSpectrometer® (1-1.5 µg/mL) [6]. Manufacturers Fa, Fi and C showed highest values. Noteworthy are large concentrations differences (up to 5 fold) between 15 mL and 50 mL tubes of manufacturers C and Fa. These may indicate higher material inhomogeneity and different rates of leaching in these vessels.

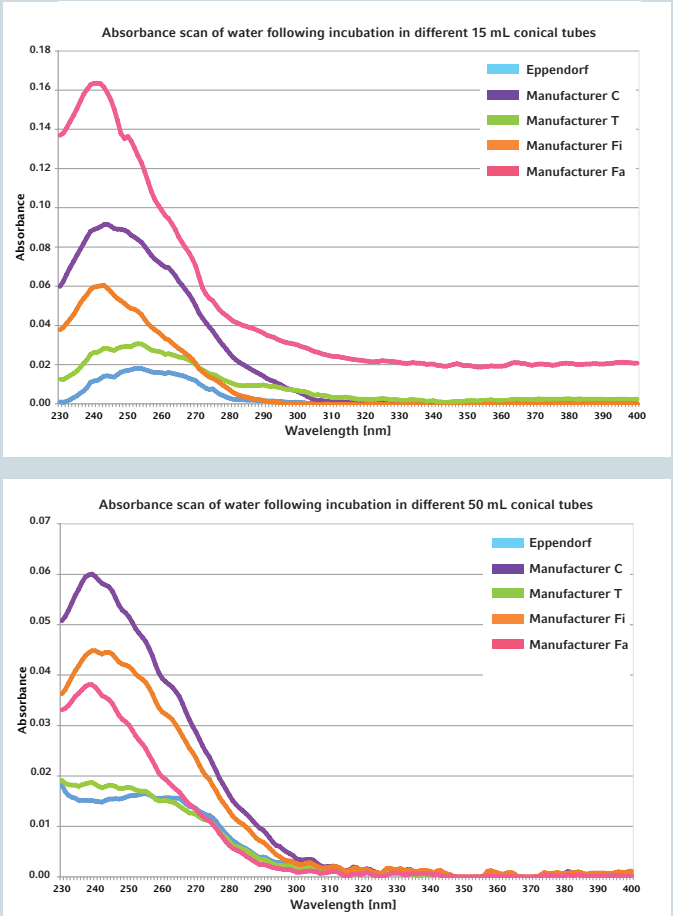


Figure 3: Absorbance spectra of pure water following incubation in different conical tubes at 95 °C for 30 min.
A) 15 mL conical tubes.
B) 50 mL conical tubes.

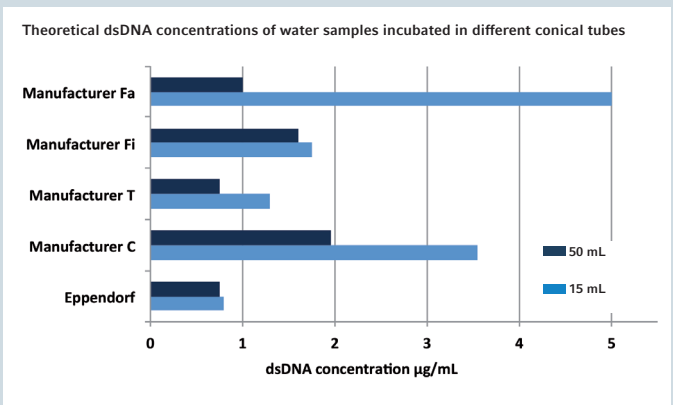


Figure 4: Theoretical dsDNA concentration in water samples after incubation in different 15 mL and 50 mL conical tubes for 30 min at 95 °C. The concentrations were calculated from the extinction at 260 nm using the factor 50 µg/mL per unit of extinction.

Conclusion

In this Application Note we investigated key parameters of Eppendorf Conical Tubes 15 mL and 50 mL under application-relevant conditions and compared to conical tubes of other manufacturers used in laboratory. The examinations focused primarily on parameters which have a high significance for many applications in laboratories with respect to reliability, reproducibility and safety: centrifugation stability, screw cap tightness and leachable levels.

The comparative evaluation of the Eppendorf Conical Tubes 15 mL and 50 mL demonstrates highest combined performance in all performed tests and show minimal levels of leachables making them ideal vessels for a wide array of demanding applications.

Literature

[1] McDonald GR, Hudson AL, Dunn SM, You H, Baker GB, Whittal RM, Martin JW, Jha A, Edmondson DE, Holt A. Bioactive contaminants leach from disposable laboratory plasticware. *Science* 2008; 322:917.
[2] McDonald GR, Kozuska JL, Holt A. Bioactive Leachates from Lab Plastics. *G.I.T. Laboratory Journal Europe* 2009; 13:24–26.
[3] Lewis LK, Robson M, Vecherkina Y, Ji C, Beall G. Interference with spectrophotometric analysis of nucleic acids and proteins by leaching of chemicals from plastic tubes. *BioTechniques* 2010; 48(4) 297–302.
[4] Application Note 235: The influence of UV-absorbing substances released from plastic containers (leachables) on photometric analyses (www.eppendorf.com)
[5] Application Note 264: Comparison of Eppendorf Tubes® 5.0 mL to conical 15 mL tubes with a focus on releasable UV- absorbing substances (leachables) (www.eppendorf.com)
[6] Operating manual Eppendorf BioSpectrometer® (www.eppendorf.com)

Ordering information

Description	Order no. international	Order no. North America
Eppendorf Conical Tubes 15 mL sterile, pyrogen-, DNase, RNase and DNA-free 500 tubes (10 bags of 50 each)	0030 122.151	0030122151
Eppendorf Conical Tubes 50 mL sterile, pyrogen-, DNase, RNase and DNA-free 500 tubes (20 bags of 25 each)	0030 122.178	0030122178

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