Is That Really DNA in Your Tube? Comparative Analysis of UV-Absorbing Leachables in Micro Test Tubes

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Abstract

Bioactive contaminants leaching out of laboratory consumables (leachables) may significantly affect experiments and may pose a likely source of error in many assay systems. Recent scientific evidence provides numerous examples of biological assays, which have been shown to be affected by leachables, including routine applications such as spectrophotometric measurements of nucleic acids. This study provides a comparative analysis of UV-absorbing leachables and resulting false DNA readings upon incubation of water samples in 1.5 mL micro test tubes from several manufacturers.

A majority of tubes tested (see Fig. 1 and 2) showed high to very high false DNA concentrations, as opposed to tubes from Eppendorf.

Introduction

Increasing scientific evidence shows that chemical substances leaching out of plastic consumables (leachables) may significantly affect experiments and pose a likely source of error in many assay systems [1, 2, 3, 4]. These include not only various enzymatic [1, 2, 3], receptor binding [1, 5], cell culture [3, 6], and high-end analytical assays [7], but also common laboratory procedures such as spectrophotometric measurements of nucleic acids [8].

In the latter case, the UV-absorbing leachables lead to false positive readings and may hamper various downstream applications, which rely on accurate DNA/RNA measurements, such as PCR, qPCR, sequencing, and NGS, as well as forensic and microarray applications. In this study, we performed a comparative analysis of UV-absorbing leachables and resulting false DNA readings of water samples incubated in 1.5 mL micro test tubes from several manufacturers. The majority of tubes tested showed high to very high false DNA concentrations, with the exception of Eppendorf Tubes®.

Materials and methods

Eppendorf Safe-Lock Tubes and standard 1.5 mL micro test tubes of ten other manufacturers (24 tubes for each manufacturer) were filled with 1.5 mL of ultra-pure water and incubated at 95 °C, 30 min or at 40 °C, 24 hours (as indicated in the results section). Incubations were performed with autoclaved (120 °C, 20 min, 10 bar) and non-autoclaved tubes.

Samples for each individual manufacturer were pooled and spectrophotometric measurements were performed using the Eppendorf BioSpectrometer® and UVette®. Absorbance at 260 nm and the factor 50 µg/mL were used to calculate the false DNA concentration derived from UV-absorbing leachables for each sample.

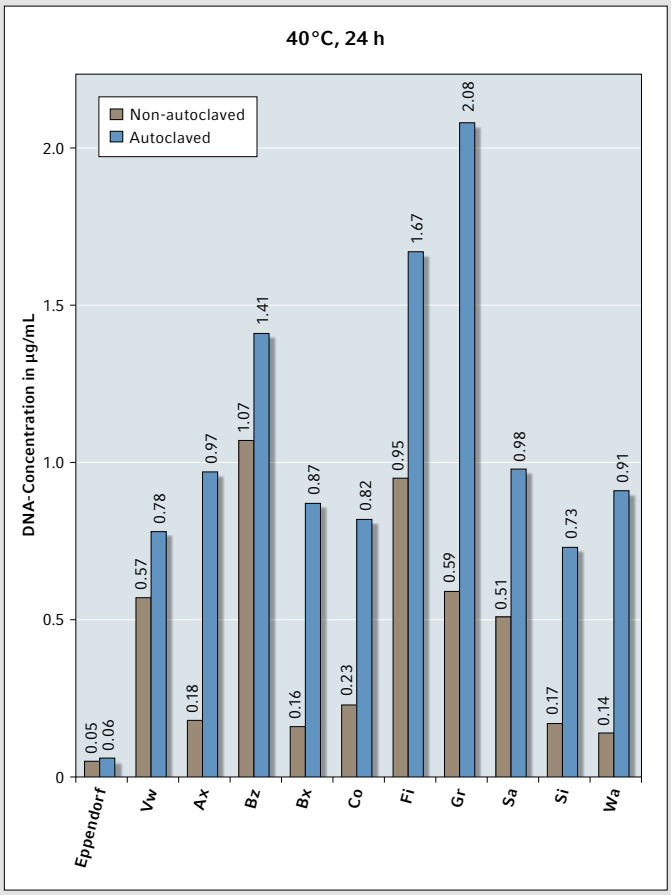

Fig. 1: False DNA concentration (µg/mL) based on UV-absorbing leachables, which had been released from micro test tubes into ultra-pure water samples after incubation at 40 °C, 24 h. Mean values of 24 pooled samples from non-autoclaved (brown bars) and autoclaved (blue bars) tubes are shown.

Results and discussion

Fig. 1 shows that even under relatively mild temperature conditions, the majority of micro test tubes may release considerable amounts of UV-absorbing contaminants, which in turn will lead to false DNA readings (up to 1.1 µg/mL of false dsDNA), and that this process can be dramatically enhanced by autoclaving (up to 2.1 µg/mL of false dsDNA). The increase of leaching after autoclaving was highest for the following manufacturers: Wa (6.4 fold), Bx (5.6 fold), Ax (5.3 fold), Co (3.6 fold), Si (4.4 fold), and Gr (3.5 fold). In contrast, measurements derived from samples incubated in Eppendorf Safe-Lock Tubes, non-autoclaved as well as autoclaved, resulted in very low values: 0.05 µg/mL and 0.06 µg/mL, respectively.

This considerable increase of leaching after autoclaving in tubes of most manufacturers may be due to different molecular/chemical compositions of polymers used, which may be more prone to alteration or damage during the autoclaving process, and lead to increased release of the polymerization by-products and/or additives used during the production process.

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Eppendorf Tubes are manufactured from highly pure polypropylene and neither plasticizers, slip agents, nor biocides are added during their production.

Thus far there have been no comprehensive studies published on influence of autoclaving on leaching. Since autoclaving is a very commonly used procedure, its effects on experiments should be therefore considered more cautiously in the general lab routine.

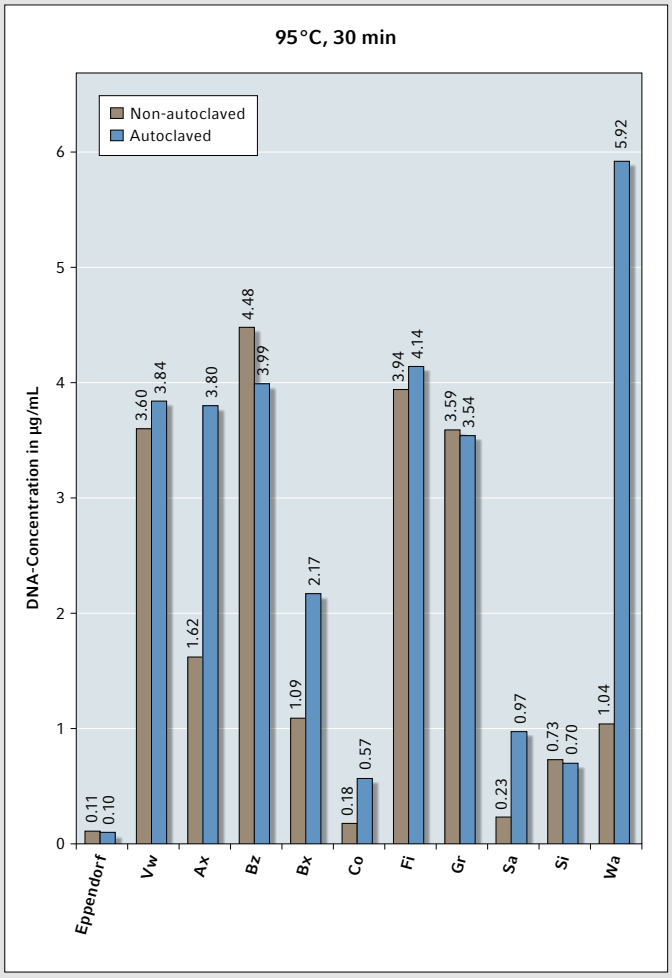

Fig. 2: False DNA concentration (µg/mL) based on UV-absorbing leachables, which had been released from micro test tubes into ultra-pure water samples after incubation at 95°C, 30 min. Mean values of 24 pooled samples from non-autoclaved (brown bars) and autoclaved (blue bars) tubes are shown.

As shown in Fig. 2, general levels of detected UV-absorbing leachables were further increased when water samples were incubated at 95 °C for 30 min. Even during this short time period, the average level of leaching (average for both autoclaved and non-autoclaved tubes) was considerable and reached up to 2.51 µg/mL of false dsDNA as compared to the average of 0.79 µg/mL of false dsDNA obtained after incubation at 40 °C for 24 h (3.17 fold increase).

This indicates that temperature is a critical factor, which influences the process of leaching and is in agreement with previous reports [8].

Similar to incubations at lower temperatures, by far the lowest values were obtained for water samples incubated in Eppendorf Safe-Lock Tubes (Fig. 2), thus significantly reducing the risk of leachable artifacts and false readings of nucleic acids.

Conclusion

In summary, the majority of tubes tested showed high to very high levels of UV-absorbing leachables in water samples incubated at 40 °C and 95 °C. The leachable levels were further considerably increased by the autoclaving process. UV-absorbing leachables were shown to cause false positive readings of nucleic acid and may therefore hamper various downstream applications, which rely on accurate DNA, RNA or nucleotide measurements, such as PCR, qPCR, sequencing, and NGS, as well as forensic and microarray applications. The Eppendorf Tubes showed consistently very low leaching values under all experimental conditions, and irrespective of the autoclaving process and therefore significantly reduce the risk of false spectrophotometric measurements.

Literature

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