EPPENDORF CONSUMABLES: SOLUTIONS FOR FORENSICS

In routine forensic DNA testing laboratories, the demand is still growing for high quality DNA preparations, high reproducibility in testing results, cost-effective methods and probably most importantly, the absence of contamination.

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Reliable sample preparation in forensic science can be a challenge as forensic samples are often very difficult specimens to process. They are typically limited in quality and quantity, can be environmentally exposed, and may require purification from difficult substrates that contain PCR inhibitors.

Consumables for processing and storing forensic samples should therefore be selected very carefully as they can directly influence the quality and reproducibility of the analytical result. Especially when the sample amount is limited or only available in low volumes, which is a routine situation in forensic DNA testing, the choice of the right consumable is of highest importance. Eppendorf consumables are tailored to the needs of forensic scientists as they offer a superior performance in the fields of:

- Purity levels
- Absence of additives that can influence assay results
- Minimised sample-to-surface binding

Eppendorf Consumables – Highest purity

In 1963 Eppendorf invented the first microtube and created a standard which is applied to the present day in all research and diagnostic laboratories throughout the world. The quality of Eppendorf consumables is a result of 50 years of experience in manufacturing consumables. With fully-automated production and continuous quality monitoring of molding tools and production areas, Eppendorf can guarantee minimal production tolerances as well as batch-to-

batch and tube-to-tube consistency.

Clean-room manufacturing from selected raw materials and advanced product handling ensures the highest product purity and no foreign contaminants. As a further commitment to product purity, each batch of sterile, PCR-clean and Eppendorf Biopur® quality products is tested and certified by an independent off-site laboratory to guarantee traceability and purity. Eppendorf purity levels suited for forensic applications are:

- Sterile
- PCR clean: Free of human DNA (Ph.Eur./USP),
- DNase, RNase, PCR inhibitors
- Biopur: Individually packed, sterile, and certified free of pyrogens, RNase, human and bacterial
- DNA (Ph.Eur./USP) and ATP.

Unaffected assay results with Eppendorf Consumables

It has been known for some time that plastic products such as food packaging and beverage bottles can leach chemical substances that lead to contamination of the contents.[1,2] This phenomenon also plays an important role in the laboratory, as plastic consumables are routinely used for sample storage and experiments.

A 2008 *Science* publication and other papers described that slip agents such as oleamide and erucamide, as well as biocides, which were washed from vessels and tips, are able to show inhibition in specific enzyme assays.[3,4,5] As a consequence, false positive or false negative results are produced.

But also, applications routinely performed in forensic laboratories have been shown to be

compromised.[6] UV absorbing substances from plastic containers are leached into samples by laboratory applications requiring temperatures of 37°C or above (DNA preparation, lysis steps, PCR, centrifugation, ultrasound). Since these substances absorb light in the same range as the absorbance maxima of nucleic acids, they can interfere with photometric detection reactions. DNA concentrations are overestimated thus providing a source of error with adverse effects on downstream applications.

Forensic DNA testing often requires very accurate determinations of DNA concentrations in samples. Even small variations in measurements can have an effect on downstream analysis making the choice of highest quality consumable for forensic DNA testing a reasonable decision. Eppendorf tips, tubes and plates are produced without critical additives like slip agents or biocides that have been shown to influence bioassay results (Fig. 1). In addition, it was demonstrated that samples stored or incubated in Eppendorf Tubes did not show compromised values in DNA concentration measurements (Eppendorf Application Note, in press, Fig. 2) thus making them the ideal choice for sensitive detection methods in the forensic laboratory.

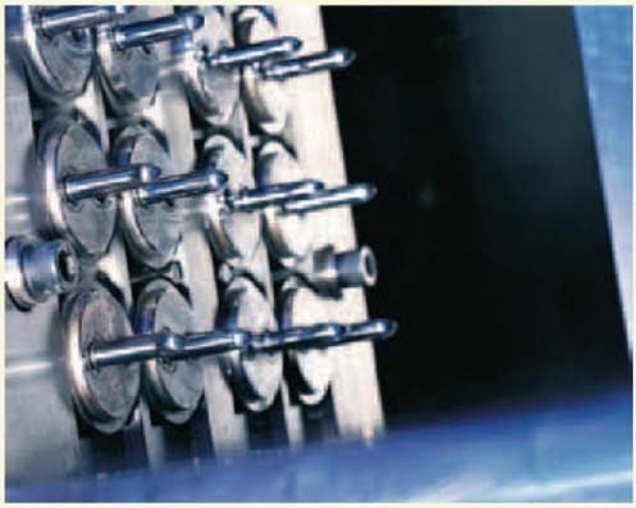

Fig. 1: Highly-polished, optimised moulds for manufacturing original Eppendorf reaction tubes and tips. Slip agents like Oleamide, Erucamide or Stearamide are not used at any time during the production process.

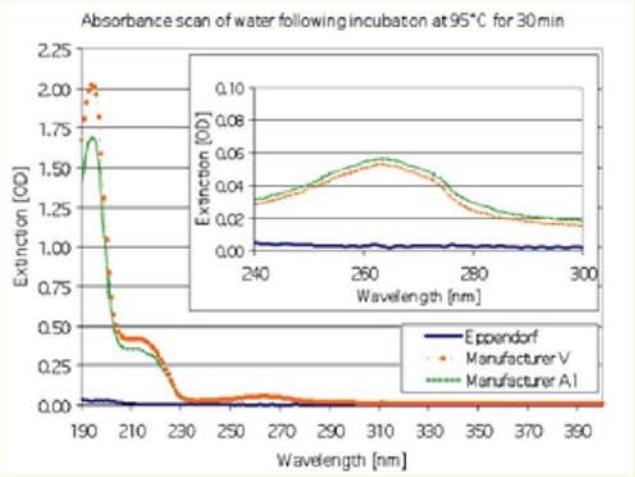

Fig. 2: Leaching of plastic reaction tubes following heat incubation. Following incubation of water in reaction tubes at 95°C, a temperature typically used, for example, in lysis steps for DNA purification or in PCR, UV absorbing substances leached from plastic containers of two manufacturers, can be detected in a photometric range usually used to detect DNA (260 nm). Water examined from Eppendorf Tubes did not show any elevated readings, thus photometric detection of DNA remains uncompromised.

Eppendorf LoBind® Tubes and Plates – Maximum recovery for better results

The reliable preparation and storage of DNA samples is a prerequisite of successful forensic analysis. Apart from the purity of the sample, recovery after preparation becomes important. Most often the amount of available forensic sample is small. Sample losses are therefore critical, leading to faulty or ambiguous analytical results, or none at all. Costs should also not be underestimated when it comes to the use of expensive reagents. Products whose material has been optimised to guarantee low affinity binding of biological samples will provide an obvious advantage, both from a financial and analytical perspective.

When biological samples are stored or incubated in standard reaction vessels, more than 90% of the sample material can be lost within 24 hours due to adsorption to the plastic surface (Fig. 3). Therefore, several approaches have been pursued to minimise the sample-to-surface binding of plastic reaction vessels:

- The use of coated (i.e. siliconised) reaction vessels. This may result in leaching of the coating and interference with the sample, possibly influencing downstream applications.
- The addition of proteins like BSA that will bind primarily to the vessel surface and thus protect the sample. However, the high BSA concentrations necessary will have an adverse effect on the precision of pipetting and may also influence further analyses.

Eppendorf is focusing on manufacturing consumables featuring DNA-repelling surface characteristics without a potentially

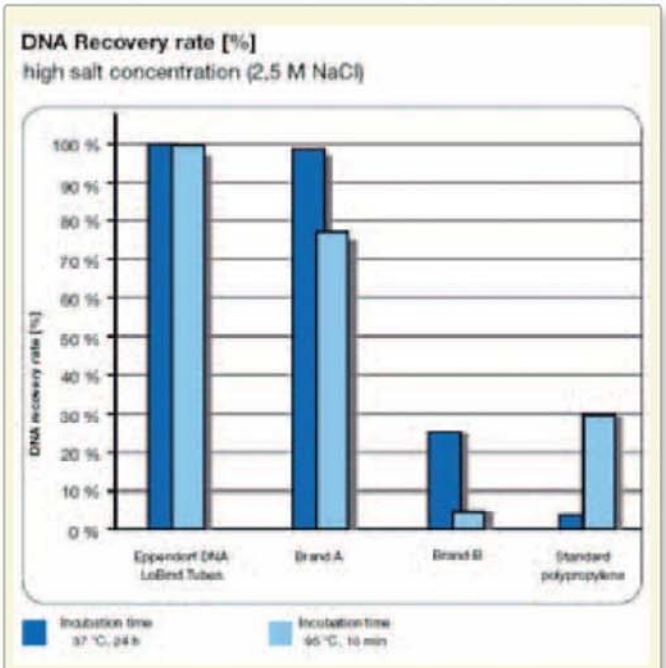

Fig. 3: DNA recovery rate (0.2 ng/µL DNA fragment (130 bp, 32P labeled) in 2.5 M NaCl/TE buffer) in Eppendorf DNA LoBind tubes compared to standard polypropylene and maximum recovery tubes of different suppliers. The Eppendorf DNA LoBind material guarantees up to 99% sample recovery independent of incubation temperature or time. In contrast, sample recovery in standard micro centrifuge tubes is dependent on incubation conditions, as low as 4%.

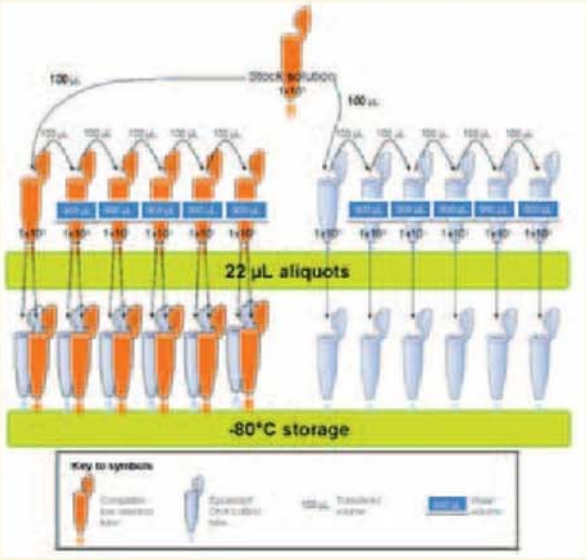

Fig. 4: Adsorption of DNA to tube surfaces effects accuracy of real-time PCR results. Flowchart of genomic DNA standard assay panels preparation. DNA stock solution was serial 10-fold diluted in either competitor's low retention or Eppendorf DNA LoBind tubes. 22 µL aliquots of each dilution were stored in either competitor's low retention or Eppendorf DNA LoBind tubes. Aliquots were stored at -80°C for 24 hours before real-time PCR assay (Results of PCR in Figure 5).

contaminating coating. Eppendorf LoBind Plates are made of a special two component polymer mix that creates a hydrophilic surface, thus significantly reducing sample-to-surface binding – without the use of any type of coating. Recovery rates of almost 100% can be obtained even after several days of sample storage. This technology is especially helpful in sample processing for demanding forensic applications, like DNA profiling and quantitative PCR.

Scientists of the University of

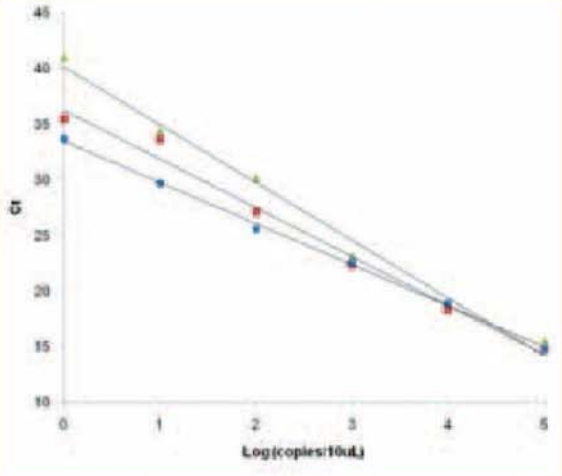

Fig. 5: Adsorption of DNA to tube surfaces effects accuracy of real-time PCR results. DNA templates (DNA standard panels) prepared in Eppendorf DNA LoBind tubes show an amplification efficiency of 86% compared to 56% for samples prepared and stored in competitor low retention tubes. Improved standard curves lead to more accurate interpretation of DNA quantity in samples assayed. Standard curves are obtained for each panel. Ct versus log10 of expected DNA concentration was plotted and slopes were obtained after linear regression. PCR efficiencies are derived from slope values.

Low bind tubes used for	Panel 1	Panel 2	Panel 3
Serial dilution performed in	Competitor	Competitor	Eppendorf DNA LoBind
-80°C sample storage in	Competitor	Eppendorf DNA LoBind	Eppendorf DNA LoBind
Slope	-5.20	-4.40	-3.70
Efficiency	56%	69%	86%
r2	-0.9939	-0.9931	-0.9993

Paris[7] have shown that apparent amplification efficiencies were up to 30 percentage points higher, when for dilution, a series of DNA Eppendorf DNA LoBind tubes where used, opposed to competitor low retention tubes (Fig. 4). In qPCR assays, where dilution series of DNA are usually made to assess sample concentration, it's especially the low concentrated dilutions which are critical for correct standard curves. Incorrect standard curves obtained from panels prepared in such a competitor's tube can lead to misinterpretation of PCR efficiency values and to significant overestimation of DNA quantity in samples assayed (Fig. 5).

In the case of forensic DNA testing, it can mean that DNA profiles can even not be generated anymore. Improvements in results with Eppendorf LoBind® technology have been shown in numerous peer-reviewed scientific publications.[8] The use of Eppendorf LoBind Tubes and Plates can help to obtain more reliable and better results, especially when sample amounts are limited.

References:

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