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Plastic Contaminant Masquerades as DNA in Mutation Detection by Denaturing HPLC

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Denaturing high-performance liquid chromatography (DHPLC) has quickly become popular as a tool for the automated discovery of single base substitutions as well as small insertions and deletions (1,2). Under partially denaturing conditions, heteroduplexes are retained for a shorter time than their corresponding homoduplexes on a DNA separation matrix. We have identified a contaminant emanating from polypropylene PCR tubes that masquerades as DNA in DHPLC assays. While small amounts of plasticizer contaminants are well known anecdo-

tally in DHPLC analyses, the contaminant detected here had a peak area 20–50 times that of the level of PCR products required for analysis.

The tube contaminant was identified when using 8 Tube Strips from Axygen Scientific (Union City, CA, USA) while amplifying for 40 cycles a 475-bp fragment of the Paraoxonase gene, PON-1. DHPLC was conducted on a Varian Helix System fitted with a 75-mm Helix Analysis Column packed with C18 alkylated silica (Varian, Walnut Creek, CA, USA). Standard operating procedures were used, utilizing 100 mM triethylammonium acetate (TEAA), pH 7.0, 0.1 mM EDTA as buffer A and 100 mM TEAA, pH 7.0, 0.1 mM EDTA, 25% (v/v) acetonitrile as buffer B (both from Varian), with a gradient of 50%–68% buffer B over 5.5 min. A similar contaminating peak has been observed using Axygen Scientific 96-Well PCR Plates (personal communication, Me-

Benchmarks

Figure 1. Identification of PCR tube contaminant in DHPLC traces of positive and negative control reactions. The PON-1 PCR product peak is indicated by an arrow.

Figure 2. Comparison of the amount of contaminant released from different brands of PCR tubes. Oxygen Scientific tubes (A) produced substantially more of the contaminant than tubes from Scientific Specialities (B), Sarstedt (C), and Porex Bio Products (D). Almost no contaminant was released from tubes from Eppendorf and Applied Biosystems (E). Insert shows full-scale chromatograms.

lissa Barker, Queensland Institute of Medical Research, Brisbane, Australia). The presence of the contaminant peak does not appear to greatly affect PCR product yield.

The contaminant peak had a retention time of 4.9 min under non-denaturing conditions at 50°C (Figure 1). The retention time corresponded to approximately 180 bp, but the retention time and its relationship to DNA base pairs changed with column age. The plastic contaminant had a reduced retention time with increased DHPLC temperature (about 1 min less from 50°C to 62°C), but the change was much less than for PCR products. The change in retention time with temperature led to an obscuring of the 475-bp PCR product peak when DHPLC was conducted under partially denaturing conditions at 57°C (data not shown). Gel electrophoresis confirmed the presence of a single PCR product in positive control reactions and no DNA bands in negative controls.

Incubation was required to obtain the contaminant, as when freshly purified water (Milli-Q® Ultrapure Water System; Millipore, Bedford, MA, USA) was dispensed into the polypropylene tubes, almost no peak was detected on the DHPLC run at 50°C compared to water that had been incubated at 94°C for 1 h. Seven different brands of tubes were tested in quadruplicate by incubation of water at 94°C for 1 h and DHPLC at 50°C. Axygen

Scientific tubes were compared to 0.5-mL GeneAmp® Thin-Walled Reaction Tubes (Applied Biosystems, Foster City, CA, USA), Eppendorf® 0.2-mL PCR Tubes with hinged lid (Eppendorf, Hamburg, Germany), QSP® 0.6-mL Thin Wall PCR Tubes (Porex Bio Products, San Francisco, CA, USA), 8-Strip PCR Tubes (200 µL; Scientific Specialities, Lodi, CA, USA), Multiply®-Pro 0.2 mL PCR Cup (Sarstedt, Numbrecht, Germany) (Figure 2), and 0.2-mL Strip Tubes (eight thin walled tubes/strip; MJ Research, Waltham, MA, USA) (data not shown). The Axygen Scientific strip tubes produced far more plastic contaminant (approximately 15 times) than any other type of tube tested. The Applied Biosystems, Eppendorf, and MJ Research tubes showed very little of the plasticizer.

Subsequent experience with the MJ Research tubes has indicated that the relatively low levels of contaminant are still a concern when analyzing lower yielding reactions. When chromatogram normalization and heteroduplex detection is conducted by Star Reviewer Software (Varian), chromatograms of lower yielding reactions are not always grouped with other traces, as the contaminant peak features significantly in the normalization. The identification of polypropylene contaminants by the Varian Helix System emphasizes the importance of running PCR negative control reactions along with sample re-

actions at 50°C, as well as at partially denaturing temperatures, particularly in the developmental phase of DHPLC assays. PCR tubes and plates need to be chosen carefully and used consistently for each assay.

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