COMPARISON OF DNA STABILITY ON BOTH TREATED AND UNTREATED MATRICES

Michael DeGuglielmo, Stephen Welsh, Martin Smith, Michael A. Harvey, and Stephanie Lowe*,
Whatman, Sanford, Maine, USA
*Midwest Research Institute, Palm Bay, FL, USA

With the rapid expansion of convicted offender and arrestee databases throughout the world, millions of specimens have been collected utilizing a variety of collection and storage methods. Very little data exist to evaluate the effectiveness of long term storage using these varied methodologies. This study was designed to compare the relative rates of degradation on both treated (FTA) and untreated (903) matrices by employing exposure to a variety of environmental factors as a mechanism of accelerating the effects of aging. FTA is a chemically modified paper-matrix used for sample collection that protects DNA contained in biological specimens from degradation and microbial contamination. When a biological sample is applied to FTA, the chemistry lyses the cells, dissociates proteins from nucleic acids and destroys nucleolytic enzymes. Whatman 903 paper is an untreated paper-matrix widely utilized for storage of non-nucleic acid biological specimens.

We approached this study by employing Real Time PCR on the premise that damage to DNA will adversely affect the concentration of amplifiable material resulting in a change in the cycle threshold (C_T). The higher the C_T value, the lower the concentration of available amplifiable DNA, while the converse is likewise true. Initially we developed a model system to study degradation of DNA stored on paper matrices. Our model utilizes a 1 kb segment within the *lac Z* gene on pCH110, as well as four smaller amplicons (ranging in size from 143 to 408 base pairs) located within the 1 kb fragment.

A titration of DNA concentrations ranging from 1 pg to100 pg were spotted onto both treated (FTA) and untreated (903) cards and dried. Initial experiments were conducted to evaluate several potential environmental factors which could be utilized to accelerate the effects of long term storage, including exposure to either 9.9×10^5 μJoules, direct sunlight for 14 days, or high heat & humidity. Subsequent experiments focused on exposure to UV radiation as the most effective mechanism of accelerating nucleic acid degradation. After exposure, punches (1.2 mm) were treated with FTA Purification Reagent, washed with TE, and dried. Punches were placed into a PCR reaction and the subsequent amplification was monitored using real time PCR.

Using this approach, we found that DNA spotted onto FTA cards contained more amplifiable DNA than the 903 paper-only matrix. The assays showed an increase in cycle threshold (C_T) indicating a decrease in amplifiable DNA that was greater with the paper-only experiment versus the FTA storage matrix. This held true for both direct UV and indirect UV light sources. For example, DNA spotted on FTA and subjected to 14 days of direct Florida sunlight showed a C_T shift of only 1.7, while paper-only showed a C_T shift of 3.312 fold indicating that FTA provided greater protection from UV light than

storing DNA on paper alone. No significant change was observed for DNA stored on FTA or paper-only when exposed to high heat and humidity. The data suggests that treated matrices (like FTA) can significantly reduce the amount of degradation observed from exposure to environmental factors including UV radiation.