VALIDATION OF THE ALUQUANT™ KIT FOR FORENSIC EXAMINATIONS

M.S. Adamowicz and H.G. Nelson
Connecticut Department of Public Safety Forensic Science Laboratory

With the advent of the extremely sensitive PCR multiplex kits presently used in forensic DNA analysis, examiners are required to quantify the amount of human genomic DNA in a sample more precisely than ever before. A validation study of Promega's AluQuant™ kit was carried out to determine if this system could meet the unique needs of forensic scientists. This study focused on several salient issues that are often encountered in forensic DNA analysis.

The AluQuant™ kit's ability to generate consistent results using a standard set of known DNA quantities (0.03ng/µl to 4ng/µl) was tested over the course of several independent runs. The data indicated that the system performed well; the largest run-to-run % error was less than 50%, and most variations were significantly below this mark. Independent samples were amplified with the ABI AmpF*l*STR® Profiler Plus® kit using AluQuant™ generated concentration values to determine if the resulting peak heights corroborated with the expected levels for each concentration. All of the results from the amplified samples indicated that the AluQuant™ kit generated accurate data.

The effects of sample support and extraction method on quantification were also examined. Human DNA extracted from liquid blood, blood on blue denim, and blood on leather using the Chelex®, DNA IQ™, Qiagen, and phenol-chloroform methods was quantified. The results indicated that the AluQuant™ kit worked well with all extraction methods tested, with the exception of Chelex®, which gave poor results. Concentration values for blood extracted from blue denim or leather did show that these supports could influence the AluQuant™ system, however acceptable results were obtained without any special clean-up procedures.

Non-human DNA is often present in forensic samples; therefore the AluQuant™ kit's reactivity with various animal DNA extracts, as well as with mixtures of human and non-human DNA was tested. Dog, horse, goat, and deer DNA gave little or no positive results with the AluQuant™ kit. In mixture studies of horse or dog DNA with human DNA, the results indicated only small decreases in quantified yield as compared to human DNA. Similar experiments were carried out with *Escherichia coli*, *Saccharomyces cerevisiae*, and *Candida albicans*. Human DNA mixed with *C. albicans* DNA in several ratios showed no significant difference in quantified yield with that of pure human DNA. Human DNA mixed with *E. coli* or *S. cerevisiae* DNA showed relatively small increases in the quantified yield at some ratios.

Degraded DNA is a common and significant obstacle in forensic DNA analysis; therefore the AluQuant™ kit's ability to accurately quantify degraded DNA was tested. Human DNA was sheared by several methods; known quantities of DNA were boiled and then vigorously vortexed, subjected to passage through a French pressure cell, and sonicated. Regardless of the method used, degraded samples gave quantified yields that differed only slightly from the intact control samples (40% or less).

This study indicated that the AluQuant™ kit is a reliable, sensitive, and accurate technique for the identification and quantification of human genomic DNA. Reliable quantification data was obtained from commonly used DNA extraction methods and samples that were mixed with a variety of non-human DNA species. The kit demonstrated a high degree of specificity for human DNA. It also maintained its accuracy and reproducibility when quantifying degraded DNA samples.