A18 Analysis of Oxidative Damage DNA in Degraded Tissue of Forensic Samples

Maribel E. Funes-Huacca, PhD, University Park 11200 Southwest 8th Street, Miami, FL 33199; and Bruce R. McCord, PhD, Department of Chemistry, Florida International University, University Park, Miami, FL 33199*

The goal of this presentation is to develop a method for evaluate the concentration of 8OHdG in DNA from both degraded and undegraded tissue samples, using HPLC with UV - EC detection.

This presentation will impact the forensic community by comparing the levels of 8OHdG in various tissue samples in order to develop a biomarker useful in assessing sample quality for subsequent PCR amplification.

In postmortem decay, the single most important factor in the degradation of DNA is the rate of which cellular nucleases degrade the endogenous DNA. However, the DNA recovered from tissue in such samples may also be heavily degraded through hydrolytic cleavage and oxidative base damage, limiting its successful retrieval and amplification. The major site of oxidative attack on the DNA bases are the C=C double bonds of pyrimidines, and purines, leading to ring fragmentation and base modifications. A majority of these oxidative base products are replication blocks and this process will affect amplification with standard Taq-DNA polymerases used in PCR.

Modified purine and pyrimidine bases constitute one of the major classes of hydroxyl-radical-mediated DNA damage. Guanine nucleobases are frequently targeted by oxidants due to their lowest oxidation potential among the DNA bases. Among guanine oxidation products, 8-oxo-7,8-dihydro-2´-deoxyguanosine (8-OH-dG) is used widely as a biomarker for guanine oxidation because of its *in vivo* incidence and its facile measurement by HPLC with electrochemical detection. Specifically, 8-OH-dG is know to cause GC →TA transversions and its presence in DNA causes mutations resulting in mispairing and multiple amino acid substitutions.

Levels of 8OHdG in cells, tissue, and whole animal have been reported as an important biomarker for oxidative stress when evaluating disease pathologies. Thus it is likely that this same compound may provide information on the relative amount of oxidative damage to target tissues used in forensic STR and mitochondrial analysis. Such an assay would be useful in situations where it is not clear if the lack of amplification success is the result of PCR inhibition, oxidative damage or fragmentation. The aim of this study will be to develop a method for evaluate the concentration of 8OHdG in DNA from both degraded and undegraded tissue samples, using HPLC with UV-EC detection.

This work will describe the application of enzymatic hydrolysis of DNA and HPLC-UV-EC detection methodology for the determination of 8OHdG in forensic samples. The goal of the study is also to compare the levels of this compounds in various tissue samples in order to develop a biomarker useful in assessing sample quality for subsequent PCR amplification.

Oxidative DNA Damage, HPLC-UV-EC, 8OHdG

 ** Presenting Author*