

## The Methods and Limitations in Measurements of Oxidative DNA Damage

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## DESCRIPTION

Using the majority of currently known techniques, DNA must first be isolated in order to measure oxidative Deoxyribonucleic Acid (DNA) damage. The hydrolysate from the hydroxylation of the isolated DNA is then ready for examination of oxidized bases. The analytical techniques often use Gas Chromatography-Mass Spectrometry (GC-MS) or High Performance Liquid Chromatography (HPLC). Guanine is the base of DNA that is most vulnerable to chemical damage. DNA and its bases are exposed to transition metal ions as well as ambient oxygen concentrations (hyperoxia compared to nuclear oxygen concentrations) during isolation and preparation for analysis. These metals can be contaminants in laboratory reagents and equipment, such as dialysis membranes, and are strong catalysts of free radical damage. When tissues are homogenized before DNA extraction, metal ions are released from intracellular sites of sequestration (such as lysosomes). In various processes, DNA is exposed to high temperatures and oxidizing agents like phenol, such as during acidic hydrolysis and derivatization for GC-MS. Hence, all of the processes of isolation, hydrolysis, and analysis run the risk of causing additional art factual oxidation of DNA (particularly of guanine residues), which would increase the apparent level of base oxidation products and render the measurement useless. It is simple to understand how the oxidation of just 0.01% of DNA bases that have not been damaged can invalidate a measurement if the steady state level of oxidized bases in cellular DNA is 1/105 bases. Due to the exposure to 21% oxygen when mitochondria are separated, they continue to produce reactive oxygen species and may even do so more quickly.

Contrary to earlier research in this subject, Beckman and Ames

recently asserted that "it is impossible to assume that mitochondrial DNA suffers higher oxidation than nuclear DNA" for the reasons mentioned above and others. A lot of labs are heavily researching more effective ways to isolate, hydrolyze, and analyses cellular DNA. The unspoken standard by which the outcomes of such efforts are assessed appears to be that the results are more likely to be accurate the lower the level of oxidized bases in cellular DNA that is obtained. This is apparently logical because one could anticipate that organisms would "perceive" oxidative DNA damage as a danger to the integrity of the genome and take steps to use their extensive DNA repair systems to reduce steady state concentrations of oxidized DNA bases. The discovery that human tissues may be exposed to potentially DNA-damaging reactive oxygen species to a considerably greater extent than previously thought is one of many surprises in biology, thus one must be cautious. For instance, the quantities of 80HG detected by GC-MS methods in acid-hydrolyzed calf thymus DNA are frequently, but not always, higher than those detected (as 8OHdG) by HPLC following enzymatic DNA hydrolysis. The disparity is typically attributed with good reason to artefactual guanine oxidation during DNA preparation for GC-MS analysis. Studies using artificial oligonucleotides containing known levels of 8OHdG, however, revealed that the conventional techniques for enzymatic digestion do not fully release 8OHdG from double-stranded DNA, meaning that HPLC detection of 8OHdG following enzymatic hydrolysis of DNA may generate. Protocols involving sodium iodide instead of phenol for DNA extraction were found to decrease measured levels of 8OHdG in isolated DNA, but it has been claimed that sodium iodide can destroy 80HdG in DNA.

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**Received:** 27-Jan-2023, Manuscript No. JCM-23-20053; **Editor assigned:** 30-Jan-2023, Pre QC No. JCM-23-20053(PQ); **Reviewed:** 14-Feb-2023, QC No. JCM-23-20053; **Revised:** 21-Feb-2023, Manuscript No. JCM-23-20053(R); **Published:** 03-Mar-2023, DOI: 10.35248/2157-2518.23.S36.004.

Citation: Halli B (2023) The Methods and Limitations in Measurements of Oxidative DNA Damage. J Carcinog Mutagen. S36:004.

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