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## ANTI-OXIDANT ADDITION TO CD-CHO MEDIA TO PREVENT DAMAGE INDUCED BY UV DISINFECTION

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Ultraviolet (UV) irradiation is an advantageous sterilization technique in the biopharmaceutical industry as it is capable of targeting non-enveloped viruses and smaller viruses that are difficult to remove via conventional filtration. Unfortunately, cell culture media contains many components that, upon exposure to light, undergo phototransformations that subsequently lead to poor cell growth. We have shown previously Chinese Hamster Ovary (CHO) cell culture performance was affected at fluences above 200 mJ/cm<sup>2</sup> due to the depletion of several key nutritional components (pyruvate, tryptophan, tyrosine, pyridoxine, pyridoxamine, thiamine) and the accumulation of reaction by-products (acetate, formate, sarcosine). Our objective was to determine if the addition of anti-oxidants, prior to irradiation, could prevent observed UV-associated damage to components of chemically defined Chinese hamster ovary cell growth (CD-CHO) media. We assessed the effects of adding several anti-oxidants already present in the formulation (including pyruvate and pyridoxine) up to 3x the initial concentration. After the media was passed through the UV reactor, the composition was assessed using nuclear magnetic resonance (NMR) and liquid chromatography-mass spectroscopy (LCMS), and cell culture was evaluated. We also tested other anti-oxidants in a similar manner, including ascorbic acid. Compounds found to be completely depleted by UV-irradiation (such as thiamine and pyridoxamine) were added to CD-CHO post-irradiation, followed by CHO cell culture to assess the importance on growth. Our results indicate that while the additions of pyruvate or pyridoxine prior to irradiation did not lead to any reduction of UV-induced damage, ascorbic acid did prevent the depletion of several key media components. In addition, pyridoxamine was found to be a necessary nutrient for CHO cell culture; therefore, must be protected from damage during UV disinfection. In conclusion, passage of CD-CHO media through a continuous-flow UV reactor is an effective disinfection method, and harmful photooxidative effects can be mitigated by the pre-irradiation addition of anti-oxidants.