

P043 Modification of a microplate assay to detect advanced oxidation protein products

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A microplate assay for advanced oxidation protein products (AOPP) in human plasma has become established within the field of oxidative stress research, with over 130 published primary research papers which incorporate this method. The assay is based on the ability of oxidatively-modified proteins to convert iodide (I^-) to triiodide (I_3^-), which absorbs at 340nm.

However, addition of potassium iodide and glacial acetic acid to plasma samples is problematic as precipitation of plasma proteins may occur in an unpredictable and variable manner. This leads to elevated optical density (OD) and overestimation of AOPP, and unreliable results as the coefficient of variation (CV) of triplicate measurements is unacceptably large (up to 80%).

We aimed to modify this method so that precipitated proteins do not act as interferants. Mathematical approaches, in which we attempted to correct OD readings for turbidity caused by precipitated proteins were unsuccessful. Attempts to prevent precipitation were moderately successful but did not achieve the required level of reproducibility. Ultimately, centrifugation of the microplate to remove precipitated proteins improved the CV of triplicate measurements to an acceptable level (<10%). This assay is now more suitable for application to studies of the oxidative modification of proteins in ageing and disease.