Developing Serum Based Infrared Spectroscopic Diagnostics: Optimising Sample Preparation and Sampling Mode

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Recently, many studies have investigated the diagnostic potential of serum spectroscopy. However, the optimum sample preparation, sampling mode and the effect of sample preparation on the serum spectrum are unknown. The most common protocol for analysing biofluids by IR spectroscopy is the drying of drop deposits but it has been shown by optical and spectroscopic assessment that this deposition is not homogenous. Thus, reproducibility and reliability of drop-dried spectroscopic results are still questioned. Moreover, when analysing serum using IR transmission mode, due to saturation of spectral peaks, it is common to dilute the sample with water but the optimum serum dilution has not been previously investigated. This study reports the use of FTIR imaging and synchrotron FTIR spectroscopy to investigate and to better understand the effect of sample preparation upon the serum spectrum.

In addition we report the results of a preliminary study using pre- and post-operative serum samples from patients needing surgery for different clinical reasons. High throughput (HT)-FTIR and attenuated total reflection (ATR)-FTIR techniques enable the acquisition of broadbeam spectra originating from whole surface of a well (silicon plate 384 wells) or a crystal respectively, resulting in a representative spectrum of the entire serum sample contrary to spectra collected using a microscope as serum dried drops spectra have shown spatial but also chemical heterogeneity. This set of samples (n=105) was used to compare the two techniques, understand the influence of the chemical heterogeneity on a spectrum collected from a large sample area and also to enable the profiling of a baseline IR response when considering only surgery.

These fundamental studies provide proof of robust spectral collection that will be required to enable clinical translation of serum spectroscopic diagnostics.