Experimental Design for Bacterial Identification Using Vibrational Spectroscopy

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Vibrational spectroscopy is a rapid, cheap and non-destructive technique that has previously been used to identify bacteria. In contrast, current established identification techniques (cell culture and genetic analysis), are often costly and time consuming processes.

The ability to rapidly identify bacteria offers utility in a number of important areas: for instance the current UK national strategy lists the use of biological warfare agents (BWAs) as a tier 1 threat, the highest of the threat levels making identification of them a major priority. These pathogens could be left in natural environments for prolonged periods of time on various different backgrounds after their initial release. Natural environments can be classified based on ten factors. For this work five of these factors have been down selected to be used with two different environments, these were hot dry and wet warm climates. This project looks at the effect that environmental conditions have on the spectral signature of bacteria including the surface on which they are found. The bacteria chosen for this study include surrogates of warfare agents and bacteria that are commonly found in the environment.

The approach taken during experimental design was to use six bacteria of known identity and identify them using vibrational spectroscopy to prove the techniques' capability before building on previous research that has taken place to develop a methodology that has two main aims. Firstly to identify bacteria found on complex substrates and secondly identify bacteria that have been affected by environmental conditions. This will create a large number of variables that are to be investigated using the optimum spectroscopic technique, down selected from preliminary studies. In order to reduce the number of experiments required, whilst still ensuring that all potentially important parameters are investigated, a fractional factorial design will be performed.

Developing a methodology that can be used on a handheld spectrometer would mean that the technique could be used for rapid, *in situ* identification of bacterial samples in 'real world' scenarios reducing the possible consequences of a natural or deliberate release of bacterial pathogens.