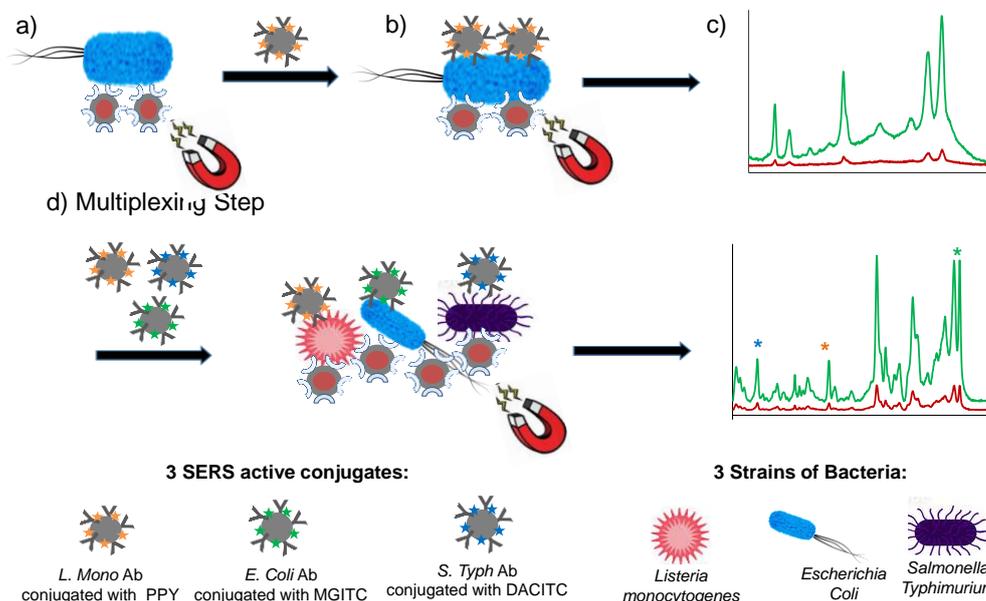


## Rapid, Point of Use Identification of Bacteria using Bionanosensors

**Aim:** The major research aim is to develop a novel, rapid, sensitive and multiplexed optical bionanosensor for the detection of bacteria in different environments using a POU detection strategy for the isolation, concentration and detection of multiple pathogens. A specific assay using enhanced Raman scattering and a portable device, with a simple user interface for data interpretation will enable the detection and identification of specific bacteria. This will be used for sensitive and specific detection of multiple pathogens, without the need for an enrichment culture step, which is rapid and compatible with different sample matrices e.g. liquids, swaps etc.

**Concept:** The approach is based on the use of surface enhanced Raman scattering (SERS) due to its high sensitivity and multiplexing capabilities. SERS active gold coated magnetic nanoparticles will be functionalised with lectins (or antibodies if required) which are capable of recognising and binding to carbohydrate constituents on the surface of bacteria (Figure 1). These lectin functionalised magnetic nanoparticles will be used to selectively capture, isolate and concentrate bacteria from the sample matrix. Gold nanoparticles will then be functionalised with a Raman reporter and a biorecognition molecule (antibodies initially) which is bacterial species specific. The magnetic 'plug' will then be analysed using a portable Raman spectrometer. The device will be fully portable and allow point of use detection. Once the multiplexed quantitative SERS signal is generated it will be analysed such that the high confidence detection of a pathogen is made and/or the concentration of the bacteria predicted. Our unoptimised, preliminary data show that we are able to detect bacteria down to 10 CFU/mL using magnetic concentration with SERS detection.<sup>1</sup> We have also demonstrated proof of concept for the simultaneous detection of 3 bacteria, we believe this can be scaled up by creating many different coded 'flavours' of nanoparticles that are specific to different bacteria species (10-15), however it is only likely that no more than 2 or 3 different bacteria strains are likely to be present in a given sample which will simplify the subsequent data analysis. This preliminary data shows that this approach is very attractive for POU, portable and rapid detection of multiple bacteria in an sensitive and selective manner.



**Figure 1:** Schematic illustrating the single-plex and an example multiplex detection assay. Assay format: a) lectin functionalised Au@MNPs bind to bacteria and allows magnetic separation of the bacteria from the sample matrix b) SERS active AuNPs functionalised with a biorecognition molecule (antibody) and a unique SERS reporter are added. These will bind and be concentrated by the magnet. c) SERS signal obtained (green spectrum). Note, SH-PEG-COOH is used to stabilise NPs and enable conjugation between the NP and Ab via EDC/NHS coupling. When no bacteria is present the functionalised AuNPs will be washed away, so minimum SERS signal obtained (red spectrum). d) Multiplexing step: 3x AuNP conjugates each functionalised with a different Raman reporter and an antibody (which is specific for a bacterial pathogen) are added together with 3 bacterial pathogens and lectin (which binds to all three bacteria) functionalised Au@MNPs. Analysis by SERS will allow identification of which of the three bacteria or combinations thereof are present.

**Need for rapid bacteria detection:** Rapid detection of bacteria is crucial for the health of the general public as the threat of infectious disease is dramatically increasing as a result of bacteria developing resistance to antimicrobial drugs. Major threats to human health from bacterial infections have led to urgent demands to

develop highly efficient strategies for isolating and detecting microorganisms in clinical samples, the environment or for food safety. Bacteria are commonly detected using traditional culture techniques followed by microscopy, luminescence, enzyme-linked immunosorbent assay (ELISA) or the polymerase chain reaction (PCR) to allow definitive identification and speciation of the bacteria. These tests generally take over 24 h due to the culture step followed by sample preparation and analysis. Lateral flow tests using antibody functionalised gold nanoparticles exist for some pathogens, however high concentrations of bacteria are needed and they tend not to perform well with complex sample types. Hence, there is an urgent requirement for the pathogen to be identified quickly and with high confidence levels and a clear need for a point of use (POU) multiplexed sensor. Extension of use of this new bionanosensor into other areas where there is a requirement for rapid bacterial detection that is simple enough to be carried out by non-scientists is a further benefit of this approach.

**Background to Proposed Research:** In recent years a number of ingenious optical methods for detecting biomolecules have been developed. The majority use fluorescence or colorimetric detection coupled with various assay formats such as QPCR or lateral flow immunoassays. An alternative spectroscopic technique to fluorescence or spectrophotometry is Raman scattering which has the advantage that a pattern of *sharp* bands are produced due to specific molecular vibrations producing a signal with superior molecular specificity. The sensitivity can be greatly improved by adsorbing the target molecule onto a roughened metal surface such as silver or gold known as surface enhanced Raman scattering (SERS)<sup>2</sup> leading to single molecule detection and reported enhancement factors of up to  $10^{14}$ .<sup>3,4</sup> Whilst SERS has been shown to be a more sensitive technique than fluorescence for the detection of labelled DNA and antibodies when using standard lab based equipment,<sup>5</sup> the main advantage of SERS over fluorescence is that the sharp bands are molecularly specific therefore it can be used to easily discriminate between analytes in a mixture, without recourse to chromatographic separation. We have previously demonstrated this by the identification of 6 labelled DNA sequences in a mixture,<sup>6</sup> as well as for the simultaneous detection and quantification of 3 pathogens involved in meningitis.<sup>7</sup> Raman and SERS have also previously been used for the detection of bacterial pathogens using label free approaches, in the case of Raman a culture step is usually required to detect the bacteria and these methods are not suitable for the capture and detection of trace bacteria.<sup>8,9</sup> In addition, the Raman spectra of different bacterial species result in only minute changes in the Raman spectra that require powerful mathematical algorithms to separate spectral features, usually using chemometrics, and these techniques do not lend themselves to POU detection where capture and isolation of very low bacteria numbers is required and culture is not possible for rapid detection.<sup>10</sup>

Multiple SERS approaches have been developed for the detection of bacterial pathogens in food, some examples include using nanorod arrays for the detection of different bacterial species in food down to 100 CFU/mL in 4 h<sup>11</sup>, and detection of *E. Coli* from beef samples was achieved in 3 h using a complex filtration assay after labelling with gold nanoparticles followed by a silver development step.<sup>12</sup> SERS immunoassays have previously been used for the specific detection of bacteria through selective antibody-bacteria binding demonstrating detection of *E. Coli* down to 100 CFU/mL in tap water using an immunoassay<sup>13</sup>; however, these approaches tend to require a surface based detection with a microscope set up and lower detection limits have been achieved by incorporating antibody functionalised nanoparticles directly into the assay.<sup>14,15</sup>

Nanoparticle-based biosensors have been developed by using SERS combined with magnetic nanoparticles; *E. Coli* has been detected in apple juice to 100 CFU/mL<sup>16</sup>, *S. Typhimurium* and *S. aureus* to 1000 CFU/mL in spiked spinach and peanut butter<sup>17</sup>, *S. Typhimurium* and *S. aureus* in pork to 35 CFU/ml using aptamer functionalised nanoparticles<sup>18</sup>, magnetic nanoparticles and gold nanorods for the detection of *S. aureus* down to 10 cells/mL and detection using polymer coated gold nanoparticles for the physi-sorption of *E. Coli* and *S. aureus* leading to detection of 1000 CU/mL<sup>19</sup>. However many of these approaches required complex separation steps or required dropping the sample onto a surface before analysis resulting in extra handling steps. *Listeria* detection has been reported using a SERS based lateral flow approach down to  $10^4$  CFU/mL in 8 h after carrying out bacteriophage amplification (although it should be noted that no SERS spectra are shown in this paper)<sup>20</sup> and from SERS active PDMS substrates down to  $10^4$  CFU/mL.

#### **Objectives:**

*We propose to develop a rapid, sensitive and multiplexed optical bionanosensor for the detection and identification of bacteria. This will involve using the inherent sensitivity of enhanced Raman scattering combined with portable Raman instrumentation for optical readout.*

**1 – Creation of a proof of concept assay for single bacteria detection (include testing of sensitivity, selectivity, etc using one lead target bacteria)- T0 + 6 Months, £ [REDACTED]**

The following parameters will be investigated:

- Initially we will develop the proof of concept assay using one bacterial strain chosen by Dstl e.g. anthrax or plague surrogate (ie ACDP 2 bacteria)
- The assay will be initially optimised in buffered solutions to develop the proof of concept assay before moving to more complex samples.
- The assay will integrate an internal control which may consist of a gold nanoparticle functionalised with a Raman reporter and an IgG antibody that will inform if the components of the assay are working correctly.
- The sensitivity of the assay will be determined as well as the dynamic range that can be measured. Our preliminary work on food related bacteria (reference [1]) has allowed detection down to 10 CFU/ml and we believe this can be lowered by optimisation of nanoparticle brightness and the optical sampling arrangement.

**2 – Investigation into nanosensors for the detection of multiple target bacteria (include creation of the nanotags and performance criteria to normalise the SERS per NP for each flavour)- T0 + 7 Months, £ [REDACTED]**

- The bacterial species for the initial multiplex will be informed by Dstl and will include surrogates for anthrax, plague and tularemia.
- We will develop multiple 'flavours' of nanotags specific to the different bacterial strains. To do this we will synthesis different gold nanoparticles, each with a unique Raman reporter molecule and a strain specific antibody.
- The different flavours of nanotags will be optimized to give a similar level of SERS response per unit of nanotag used to balance the signal output.
- Initially we will aim to develop a triplex but with a future aim to increase this to between 5-10 different codes and bacteria, with the realisation that only 1 or 2 bacterial strains are actually likely to be present in any given sample. However, all codes for the different bacterial strains will be present in the assay to allow screening for the one strain that is present.

**3 – Evaluation of the multiplex capability of the assay- T0 + 19 Months, £ [REDACTED]**

- We will optimise the sensitivity of the assay for each individual bacterial strain within the triplex.
- Data analysis algorithms will be developed to allow us to pick out specific codes (bacteria) from the background signals and this will be developed such that we can detect, and potentially quantify, if all three strains are present initially.
- This will then be expanded to a larger multiplex up to 10 codes.

**4 – Evaluation with different sampling formats (swabs, direct from culture etc)- T0 + 19 Months, £ [REDACTED]**

- We will look at the effect of different sample matrices to investigate, and mitigate against, any potential interferences from sample components by extraction and dilution
- We will carry out initial work using direct from culture samples and we will investigate the effect that different culture growth media have on the assay
- We will then increase the complexity of the sampling by moving to swabs from surface deposited bacteria and then add further complexity by looking at spiked synthetic samples e.g. blood, soil etc to ascertain the potential effects of interferants.

**5- Optimisation and Stability testing of assay- T0 + 31 Months, £ [REDACTED]**

- Once the final assay has been developed it will then be optimized to ensure stability of each component of the assay. In particular the storage of the nanotags at different temperatures for

different periods of time to ascertain the required storage conditions and their use for in-field applications.

- The format of the assay will also be investigated to develop an approach that require no, or very limited, sample handling and for integration of the assay and sample format into the Raman detection module.
- At this point the time for analysis will be reduced due to the limited sample handling steps and we will aim to reduce the time for the assay to read out to be under 10 minutes.
- Nanoparticle design for complex, harsher environments to ensure stability of the nanoparticles

#### 6 – Proof of concept testing with real samples *T0 + 31 Months*, £ [REDACTED]

- Proof of concept of SERS assay and sample handling that can be integrated with portable Raman detection.

#### Deliverables:

- 1- Example of the detection of one bacterial species with limit of detection and dynamic range. *T0 + 6 Months*, £ [REDACTED]
- 2- Example of the multiplexing capabilities of the assay with 2 bacteria from a library of 5-10. *T0 + 7 Months*, £ [REDACTED]
- 3- Example of proof of concept for the determination of a triplex of targets. *T0 + 19 Months*, £150,000
- 4- Increase number of Raman codes for increased multiplexing (n=10). *T0 + 19 Months*, £55,250
- 5- Finalisation of the specifications for a proof of concept assay using a portable Raman spectrometer. *T0 + 31 Months*, £ [REDACTED]
- 6- Example of proof of concept for 2 different sample formats (swab, direct from culture etc). *T0 + 31 Months*, £ [REDACTED]
- 7- Optimisation and stability testing of assay under different environmental conditions. *T0 + 31 Months*, £ [REDACTED]
- 8- Proof of concept testing with real samples. *T0 + 31 Months*, £ [REDACTED]

#### Financials:

Total price per annum to provide one full time PDRA, time and contributions from KF and DG and all Strathclyde associated costs for 3 years including annual inflationary rises:

Year 1 – £ [REDACTED]  
Year 2 – £ [REDACTED]  
Year 3 – £ [REDACTED]

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