RCloud Tasking Form – Part B: Statement of Requirement (SoR)

Title of Requirement	**Bio-SERS -** *working prototype for single bacteria detection*
Requisition No.	1000165609
SoR Version	**0.1**

1.	**Statement of Requirements**
1.1	**Summary and Background Information**
	Strathclyde University is a world leader in detection of biomolecules using surface enhanced Raman scattering techniques. This series of tasks is intended to develop techniques which will provide an in-field rapid detection methodology for detection of Redacted due to FOI exemption simultaneously to reduce the number of test required to identify any particular material. The overall aim is to align the detection methodology with an equipment capability that is already in-service.
1.2	**Requirement**
	The programme of work is anticipated to initially take three years with suitable break-point at the end of each year. The programme of work will run in parallel with a hardware development support programme with Redacted due to FOI exemption *The overall aim is 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 working prototype for single bacteria detection (include testing of sensitivity, selectivity, etc using one lead target bacteria)* The following parameters will be investigated: • Initially we will develop the proof of concept assay using one bacterial strain chosen by Dstl e.g. Redacted due to FOI exemption • 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)*

- The bacterial species for the initial multiplex will be informed by Dstl and will include surrogates for Redacted due to FOI exemption.
- 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*

- 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)*

- 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*

- 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 – *Final proof of concept testing with real samples* - Proof of concept of SERS assay and sample handling that can be integrated with portable Raman detection.

1.3	**Options or follow on work** *(if none, write 'Not applicable')*
	Whilst the initial work plan is for 3 years it is anticipated that successful completion of the above task would lead to further developments, which could extend the programme by a further two years.
1.4	**Contract Management Activities**
	Contract shall be managed as per the Dstl Management System particularly relating to Project Management Governance.
1.5	**Health & Safety, Environmental, Social, Ethical, Regulatory or Legislative aspects of the requirement**
	None specific

1.6	Deliverables & Intellectual Property Rights (IPR)					
Ref.	**Title**	**Due by**	**Format**	**Expected classification (subject to change)**	**What information is required in the deliverable**	**IPR Condition**
D – 1	*Example of the detection of one bacterial species with limit of detection and dynamic range.*	*31/03/2022*	*Live demonstration or by video call and short progress report*	Redacted due to FOI exemption	*Deliverable to include but not limited to:* *• Update on technical progress* *• Progress report against project schedule.* *• Risks/issues.*	*Default RCloud Agreement Terms and Conditions shall apply*
D - 2	*Example of the multiplexing capabilities of the assay with 2 bacteria from a library of 5-10.*	*31/03/2022*	*Live demonstration or by video call and short progress report*	Redacted due to FOI exemption	*Deliverable to include but not limited to:* *• Update on technical progress* *• Progress report against project schedule.* *• Risks/issues.*	*Default RCloud Agreement Terms and Conditions shall apply*
D - 3	*Example of proof of concept for the determination of a triplex of targets.*	*31/03/2023*	*Live demonstration or by video call and short progress*	Redacted due to FOI exemption	*Deliverable to include but not limited to:* *• Update on technical progress* *• Progress report against project schedule.* *• Risks/issues.*	*Default RCloud Agreement Terms and Conditions shall apply*
D – 4	*Increase number of Raman codes for increased multiplexing (n=10).*	*31/03/2023*	*Short progress*	Redacted due to FOI exemption	*Deliverable to include but not limited to:* *• Update on technical progress* *• Progress report against project schedule.* *• Risks/issues.*	*Default RCloud Agreement Terms and Conditions shall apply*

D - 5	*Finalisation of the specifications for a proof of concept assay using a portable Raman spectrometer*	*31/03/2024*	*Final Report*	Redacted due to FOI exemption	*See D-8 below.*	*Default RCloud Agreement Terms and Conditions shall apply*
D - 6	*Example of proof of concept for 2 different sample formats (swab, direct from culture etc).*	*31/03/2024*	*Final Report*	Redacted due to FOI exemption	*See D-8 below.*	*Default RCloud Agreement Terms and Conditions shall apply*
D - 7	*Optimisation and stability testing of assay under different environmental conditions.*	*31/03/2024*	*Final Report*	Redacted due to FOI exemption	*See D-8 below.*	*Default RCloud Agreement Terms and Conditions shall apply*
D - 8	*Proof of concept testing with real samples.*	*31/03/2024*	*Full final report*	Redacted due to FOI exemption	*Report detailing the work undertaken with performance and specification of the device and any user training instructions as appropriate.*	*Default RCloud Agreement Terms and Conditions shall apply*

1.7	**Deliverable Acceptance Criteria**
	Delivery of assay technique.

2	**Evaluation Criteria**
2.1	Method Explanation
	Review of techniques as stated in final report along with demonstration of technique as per milestones, progress reports and regular meetings
2.2	Technical Evaluation Criteria
	Review of techniques as stated in final report along with demonstration of technique as per milestones, progress and regular meetings
2.3	Commercial Evaluation Criteria
	NA