National Defence Défense nationale

- STATEMENT OF WORK -
Task Authorization (TA) – 45

For Sub Contract with CIMVHR

1. Task 45– TITLE OF TASK AUTHORIZATION

TA 45 – Development of a sensor system for rapid quantification of small (100-300Da) molecules in complex biological fluids

2. VALIDATION OF SCOPE OF CONTRACT

2.1 The following task(s), as written in the SOW of the main contract (W7714-145967/001/SV) apply to this Task Authorization (TA):

a. **Tools and Treatments** – Develop software or hardware tools and pharmacological products related to the diagnosis and treatment of healthcare issues in the target population.

b. **Data Analysis -** Perform state of the art analysis of data from experimental studies, clinical trials, field studies or trials, and existing databases.

c. **Presentations to Government and Health Care System Stakeholders -** Prepare and deliver presentations to Government and Healthcare system stakeholders.

d. **Advice** -Provide recommendations on peer review research proposals, publications, experimental studies, surveys, and scientific evidence.

3. ACRONYMS

CAF	Canadian Armed Forces
DRDC	Defence Research and Development Canada
SA	Scientific Authority
SGHRP	Surgeon General Health Research Program
TA	Task Authorization

4. REQUIREMENT

4.1 The following services of the Sub Contractor are required: investigate chemical modifications to the surface of a thermoplastic for covalent attachment of a protein/peptide, chemical synthesis of molecular units that compete for protein/peptide binding sites with their biological analogue, develop prototype systems for quantification of a target biochemical, perform experiments to compare prototype systems for chemical quantification capabilities with gold-standard methods in a Bland-Altman Limit of Agreement plot, document all procedures in the form of academic publications/manuscripts for experimental confirmation/duplication by DRDC and/or other labs.

5. BACKGROUND

5.1 The objective of the research contract is to complete the development of a biosensor that quantifies the concentration of small (100-300 Da) molecules that are found in low concentrations (<1 nM) in biological fluids, such as saliva. The first proof-of-concept of the device will be targeted towards the quantification of melatonin in saliva for the purpose of ascertaining the timing of an individual’s circadian rhythm for precisely scheduling circadian entrainment treatments.

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5.2 This research contract will specifically look at using well-known enzyme chemistry techniques for analytic quantification integrated into a novel form factor to enhance the sensitivity of the device. Lateral flow immunoassay techniques are reliant on the fluid flow dynamics of aqueous solutions on nitrocellulose membranes. Herein we are contracting a research laboratory to develop a novel form factor for quantification of small molecules in low concentrations in biological fluids. We have chosen Salivary Melatonin as an ideal candidate for this work because of the concentration in which it is physiologically found, and because of its chemical properties (.e.g., molecular size). We have a conceptualized and started development on a probe/piston design for the analytic detection head with each of the enzyme chemistry steps located in their own chamber; however, alternative form factors will be investigated and further developed by the contract laboratory.

5.3 The technology development being proposed herein could be used outside of the realm of Human Health and Performance. The electrochemical detection system may be capable of identifying specific molecular patterns relevant to Intelligence, Surveillance, and Reconnaissance (ISR) as long as the electroactive assay components are compatible with the specific molecular signature being detected. For example the electrochemical test kit could theoretically be useful for detection drugs or agents of bioterrorism in a waterway or pond without the need for bulky and/or expensive equipment. Additive/flexible sensor fabrication will focus on inexpensive electronics or a 'plug-and-play' system that can be used over a long term, with a small (non-bulky) form factor.
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6. OBJECTIVES

6.1 Investigate surface chemistry modifications of 3D printable thermoplastics for covalent attachment of bioactive proteins (e.g., Melatonin Antibody or a Melatonin Receptor unit) in a full/complete monolayer suitable for capturing small-molecule analytes in saliva.

6.2 Develop small molecule competitor units consisting of the binding group (e.g., Melatonin), an oligomer/spacer, and a signaling enzyme (e.g., a peroxidase or other enzyme capable of creating a signal).

6.3 Integrate the products of Objectives 6.2 and 6.3 into a hand-held apparatus that is capable of quantifying low concentrations (i.e., <1 nM) of small-molecule analytes (i.e., 100-300 Da; e.g., Melatonin) in complex biological fluids (e.g., Saliva).

7. SCOPE

7.1. The Sub Contractor must develop full, working prototypes of hand-held systems that are capable of rapidly quantifying small concentrations of analyte (e.g. Melatonin) in complex biological fluids (Saliva).

7.2. To achieve this aim, the Sub Contractor will perform the following:

a. Perform a full chemistry investigation of known and novel surface modification techniques for attaching bioactive proteins/peptides to printable thermoplastics including all necessary confirmation experiments to show chemical modification of the surface and covalent attachment of the biologically-derived binding groups (e.g., X-Ray Photoelectron Spectroscopy, Fourier transform infrared spectroscopy, or similar techniques).
b. Experimentally show that the biological activity (e.g., functional binding) of the attached protein/peptide has remained high (i.e, the protein binding sites have not been impeded/destroyed) following covalent attachment to the plastic surface.
c. Chemically synthesize the molecular units that will compete for protein/peptide binding sites with their biological analogue with Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS), or equivalent techniques, for confirmation experiments.

d. Develop prototype systems with form factor development via 3D modeling and printing
e. Integrate the chemically modified surface with the competitor units into system prototypes for rapid quantification of the target biochemical.
f. Develop electrical and optical components for end-signal detection and requisite analysis.
g. Perform experiments to compare prototype systems for chemical quantification capabilities with gold-standard methods in a Bland-Altman Limit of Agreement plot, document all procedures in the form of academic publications/manuscripts for experimental confirmation/duplication by DRDC and/or other labs

7.3 The Sub Contractor must ensure they have adequate resources for designing and testing the various components of the prototype hand-held handheld sensor systems and are staffed for the experimentation, statistical analysis and publication of the resulting research findings.

8. APPLICABLE DOCUMENTS & REFERENCES

None

9. TASKS TO BE PERFORMED

Ongoing Tasks

9.1 Prepare and submit Quarterly Progress Reports summarizing all results/findings to date, and provide conclusions and recommendations with respect to the requirements. The Quarterly Progress Reports will include a write-up that summarizes and describes results/findings to date in a word processing document, all raw data that was collected over the past 3 months, and a short PowerPoint presentation with graphical representation of the main findings/results.

9.2 Prepare draft scientific manuscripts, in association with the SA and DRDC co-investigators, suitable for publication in the open peer-reviewed literature.

9.3 Complete all data analysis, statistical analyses, and tabulation/presentation of results in accordance with standard scientific publishing guidelines;

Phase 1 – Development of Device Probe and Analyte Competitor Units (Concurrent with Phase 2)

9.4 Purchase all necessary equipment and laboratory reagents/supplies.

9.5 Modify the surface chemistry of a 3D printable thermoplastic for covalent attachment of bioactive protein (Melatonin Antibody or Receptor) in a full/complete monolayer, and perform a confirmation experiment to show covalent attachment (i.e., boil the surface in a sodium dodecyl sulphase and sodium hydroxide solution followed by Fourier transform infrared spectroscopy, or a comparative protocol for confirmation of covalent binding);

9.6 Experimentally show that the biological activity (e.g., functional binding) of the attached protein/peptide has remained high (i.e, the protein binding sites have not been impeded/destroyed) following covalent attachment to the plastic surface.

9.7 Create small molecule competitor units consisting of the binding group (e.g., Melatonin), an oligomer/spacer, and a signaling enzyme (e.g., a peroxidase or other enzyme capable of creating a signal). These synthetic molecular units will compete for protein/peptide binding sites with their biological analogue. Confirmation of the covalent attachment of the oligomer/space to a suitable location on the melatonin molecule, such that protein/peptide binding

is not compromised, will be done with Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS), or equivalent techniques..

9.8 Test the limits of detection by varying the timing that the protein-modified thermoplastic from Requirement 4.1.1 is immersed in a fluid containing the analyte, with subsequent addition of the competitor solution. This will be done with a simple aqueous buffer and with pooled human saliva.

Phase 2 – Form Factor Development and Integration of Chemistry Components (Concurrent with Phase 1)

9.9 Develop the form factor for the sensor device by 3D printing all chambers and components. Fasten multiple chambers together with a thin film separation to prevent fluid transfer between the chambers. The film should not deteriorate in mildly acidic/basic aqueous solutions; however, the film must be capable of being penetrated by a lollipop probe.

9.10 Incorporate chemistry components into the final form factor, such as the surface-modified sensor probe, and chamber components.

Phase 3 – Integration of electrical, optical components and software

9.11 Develop the requisite electrical and optical components for signal detection in the final chamber of the biosensor.

9.12 If required, all software elements will be developed and tested. Software may be required if the optical/electrical components of the signal detection system are external to the biochemistry device, such as employment of a smartphone or other handheld device for signal detection.

9.13 Instructions for use (i.e., a Standard Operating Procedure) for the final system (biochemistry device with signal detection component) will be drafted.

Phase 4 – Testing

9.14 Final testing of the device will involve the use of the complete biochemistry device on human participants in a late evening (2100-hrs to 2400-hrs) data collection in which saliva is collected/tested in a dark (<5 Lux) environment. Participants will be required to provide a saliva sample with passive drool, and then immediately use the final biochemistry device to quantify salivary melatonin content. The passive drool samples will be subsequently tested with a reputable Immunoassay kit, such that the results of the two quantification techniques can be compared in a Bland-Altman Limit of Agreement plot. The contractor will be required to recruit participants for the data collection, execute the data collection, analyze the samples collected, and create a Bland-Altman Limit of Agreement plot.

Phase 5 – Concluding Tasks

9.15 Prepare and submit a Draft Study Report and a Final Study Report detailing all evidence-based data captured over the course of the entire TA; including executive summary, background, objectives, methods, results, conclusions, and recommendations for future research directions in this domain;

9.16 Prepare and submit no less than 10 prototype biochemistry sensor systems. The sensor systems may share one signal detection component.

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10. DELIVERABLES (DESCRIPTION AND SCHEDULES)

All deliverables must be submitted and completed by March 2019. The Sub Contractor must prepare and submit the following deliverables to the CIMVHR:

Deliverable Number	Task reference	Description (Quantity and Format) and Schedule
10.1	9.1	Quarterly Progress Reports to be submitted every 3 months. The Quarterly Progress Reports will include a write-up that summarizes and describes results/findings to date in a word processing document, all raw data that was collected over the past 3 months, and a short PowerPoint presentation with graphical representation of the main findings/results.
10.2	9.3	Submit Draft Scientific Manuscripts suitable for publication in open literature - submitted to the CIMVHR no later than 35 days prior to the end of the end of the contract period
10.3	9.8	Prepare and Submit Research Ethics Protocol for collection of human biological fluids. REB Clearance to be forwarded to CIMVHR upon receipt.
10.4	9.13	Written, detailed standard operating procedures (i.e., instructions for use) of the final system
10.5	9.14	Prepare and Submit Research Ethics Protocol for final data collection with human participants. REB Clearance to be forwarded to SA upon receipt.
10.6	9.15	Submit a Draft Study Report no later than 30 days prior to the end of the contract period. The SA will require no more than 5 business days to provide feedback to the Contractor.
10.7	9.15	Submit a Final Study Report addressing issues and concerns identified by the SA on the Draft Study Report. The Final Study Report is to be submitted within 15 days of receipt of feedback from the SA.
10.8	9.16	Ten (10) prototype biochemistry sensor systems with a shared signal detection component

11. LANGUAGE OF WORK

11.1 Documentation and deliverables must be submitted in the English language.

12. LOCATION OF WORK

The work must be performed on the Sub Contractor's site.

13. TRAVEL

13.1 This task authorization may include the following domestic travel requirements:

a. Sub Contractor travel to present research findings at scientific meetings; and
b. Subjects travel for data collection.

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13.2 All travel must have the prior written authorization of the Scientific Authority and the Technical Authority, and must be undertaken in accordance with the *National Joint Council Travel Directive* and with the other provisions of the directive referring to "travellers", rather than those referring to "employees".

14. MEETINGS

None

15. GOVERNMENT SUPPLIED MATERIAL (GSM)

None

16. GOVERNMENT FURNISHED EQUIPMENT (GFE)

None

17. SPECIAL CONSIDERATIONS OR CONSTRAINTS

None

18. SECURITY

The Sub Contractor will not require access to PROTECTED and/or CLASSIFIED information or asset, nor to restricted access areas.

X Not applicable ☐ RELIABILITY STATUS ☐ PROTECTED A ☐ PROTECTED B

19. INTELLECTUAL PROPERTY (IP) OWNERSHIP

The Sub Contractor will own any Foreground IP created by virtue of the main contract (W7714-145967/001/SV).

20. CONTROLLED GOODS

X Not applicable
☐ Applicable

21. BUDGET

The Sub Contractor will be paid by CIMVHR as per the terms of Contract # W7714-145967 between Defence Research and Development Canada and CIMVHR. The amount of funding available is allocated by fiscal year (April 1 - March 31st) and is approximately $146,000 for FY 2018/19, plus applicable overhead. Details TBD upon award.

A draft budget must be submitted with the proposal along with a budget justification. A detailed budget will be developed post award in consultation with CIMVHR. Interested parties should request budget documents and information on creating their budget from Jocelyne Halladay.