Toll-Like Receptor (TLR) Workshop: Opportunities and Challenges

30–31 August 2016, Corporate Office, Ottawa

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IMPORTANT INFORMATIVE STATEMENTS

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Abstract

The two-day Toll-Like Receptor (TLR) workshop was co-organized by Dr Nora Chan, lead scientist of the TLR biosensor project, and Mr Albert Chan, Project Director for the Inform Project within the CBRN Defence Program. Biological detection, identification and monitoring (BioDIM) is the top S&T priority for the Directorate of Chemical, Biological, Radiological and Nuclear Defence (D CBRN D). The TLR biosensor work element within the Inform Project aims at addressing this problem. The purpose of this workshop was to learn about the progress made by the team members so far, and discuss the future ideas. Information presented included work from each of the invited research teams as well as an overview of the current challenges from the lead scientist. The open discussion and face to face meeting format facilitated advancement of the TLR work. The most significant achievement of this two-day workshop was that all participants had an opportunity to learn and understand the results of research conducted by each team in great detail. The outcome of this workshop will lead to faster technological maturation for our sponsor.

Significance to Defence and Security

The TLR biosensor aims at providing rapid detection and classification of pathogens. This capability would be useful for the CAF with respect to their functionality in a biological threat environment. It will be a revolutionary capability in handheld biological detector.

Résumé

L'atelier de deux jours sur les récepteurs de type Toll (TLR) a été coorganisé par Nora Chan, PhD, scientifique en chef du projet de biocapteur TLR, et par Albert Chan, directeur du projet Inform dans le cadre du Programme de défense CBRN. La détection, l'identification et la surveillance biologique (BioDIM) est la priorité absolue en matière de S et T de la Direction - Défense chimique, biologique, radiologique et nucléaire (DDCBRN). L'élément de travail sur le biocapteur TLR dans le cadre du projet Inform porte sur cette question. Le but de l'atelier était de faire connaître l'état d'avancement des travaux des membres de l'équipe et de discuter des idées pour la suite du projet. Chacune des équipes de recherche a présenté son travail, et la scientifique en chef a fait un survol des difficultés actuelles du projet. Le choix de tenir des discussions ouvertes et de réunir les participants en personne a facilité l'avancement des travaux sur le TLR. L'aboutissement le plus important au terme de cet atelier de deux jours est que tous les participants ont pu connaître et comprendre les résultats de la recherche menée par chacune des équipes. Grâce à cet atelier, le projet pourra atteindre sa maturité technologique plus rapidement pour le parrain du projet.

Importance pour la défense et la sécurité

Le biocapteur du TLR a pour fonction de détecter et de classifier rapidement les agents pathogènes, ce qui peut s'avérer utile aux FAC qui évoluent dans un environnement de menace biologique. Il s'agit là d'une capacité révolutionnaire pour un biodétecteur portatif.

Table of Contents

List of Figures

List of Tables

Acknowledgements

The authors would like to thank our military sponsor for their financial support and the Bio Treat Defence Section Head, Ms Susan Rowsell, for her helpful comments on this Reference Document. The assistance of the Informatics Section, Ms Joanne Hodges and Mr Mike Weatherby, at DRDC – Suffield Research Centre to set up the video teleconference (VTC) is acknowledged.

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1 Introduction

Inform Project Work Breakdown Element (WBE) 5.3, is a multidisciplinary collaborative research effort among DRDC – Suffield Research Centre, National Institute of Nanotechnology (NINT), University of Calgary (U of C), University of Toronto (U of T), and Royal Military College of Canada (RMCC).[1] This project is important for the CAF as there is a prompt need for a handheld biodetection system. The outcome of this project will enable them with technology that provides rapid biodetection and classification during their mission. The attendance of three military sponsor in this workshop to listen and participate, is an indication of the importance of this event. The two-day workshop was organized to give each research group the opportunity to present and exchange their data followed by a discussion session. This event aimed to ensure that our individual team work is fully aligned with the needs of the CAF. The workshop commenced by co-organizers welcoming the attendees. The objectives and the expected outcomes were also presented. On the first day the following subjects were presented by our sponsor (CAF) and the individual research groups: (i) D CBRN D research priorities, (ii) intellectual property (IP), (iii) virus detection by TLR[2] 7/8, (iv) nanotechnology-enabled impedimetric sensors for pathogens detection, (v) detection of Gram negative bacteria using TLR4, and (vi) technology readiness levels (TRLs) for TLRs 1–5 for development of TLR biosensor. On the second day, a group discussion on the following areas was held: (i) technological challenges, (ii) IP, (iii) consortium contracting, (iv) work plan, (v) common standard operating procedure (SOP), (vi) using uniform reagents and controls, and (vii) funding. The agenda for this workshop and the attendance list is available in Annexes A and B respectively.

[1] U of T/RMCC work is ATIR-funded directly by D CBRN D.

[2] Toll-like receptors (TLRs) are a class of proteins that play a key role in the innate immune system.

2 Presentations

The following research groups presented a summary of their research, results, challenges, future direction, and their time frame for deliverables. Please see the List of Attachments for the PowerPoint listing.

2.1 Project Director for the Inform Project within the CBRN Defence Program: Mr Albert Chan

The participants of the workshop included people from academia and National Institute of Nanotechnology, who had limited or little knowledge of the work conducted by DRDC. The following points were introduced as background information for how DRDC supports the CAF with its research.

- Organizationally, DRDC is a civilian organization under DND, conducts research for the CAF, and supports operations when requested with activities conducted in Canada or while deployed abroad; and
- Programmatically, DRDC's research is conducted by domestically leveraging industry, academia and other government departments. As Canada is a small country in terms of Gross Domestic Product and resources, leveraging international efforts is also a key strategy to optimize DRDC's investments.

To set the tone for the workshop, the objectives were emphasized with the intention to ensure a common goal for all participants, both during the workshop and beyond:

- Facilitate communications, build partnerships, and exchange best practices;
- Coordinate the TLR research by devising a work plan so that there won't be duplication of effort; and
- Reach a technological maturity level for military exploitation as fast as possible to respond to client's procurement plan.

2.2 S&T and MedCM Desk Officer: LCol Alexander Natale

The S&T and MedCM desk officer presented the following key points:

- D CBRN D S&T priorities are in the following order: (1) Bio detection, identification, and monitoring (DIM), (2) Rad DIM, (3) Chem nontraditional agents (NTAs), (4) sensitive equipment decon, and (5) low burden individual protection equipment (IPE).
- The intended purpose of a Toll-like receptor biosensor includes: (i) characterizing all TLRs, (ii) having a multiplex biosensor, (iii) creating a library for known pathogen associated molecular patterns[3] (PAMPs), (iv) classifying pathogens, (v) creating a model for a proof of

[3] PAMPs are signature molecules (e.g., lipids, lipoproteins, proteins, and nucleic acids) common to a wide range of pathogens that are recognized by of the innate immune system.

concept, (vi) creating a standard process for obtaining results and whether or not sample is a biohazard.

- The concept of Local Biological Defence System (LBDS) is to identify and monitor biological hazards to protect forces of all sizes, whether static or mobile.

In addition to the above topics, a draft document entitled "Military requirement on the TLR biosensor" was presented by our military sponsor to have the comments and input from each research group. Following the workshop, a video teleconference (VTC) was organized to discuss this document among the scientific groups. The content of this VTC is presented in Annex C.

2.3 DRDC Intellectual Property Expert: Mr Ron Poirier

The rules and regulations for IP in collaborative research projects were presented by a DRDC IP expert and all participants discussed the following items:

- The IP in government institutions belongs to the Crown; however, this is not the case for universities.
- Some parts of the TLR project at U of C and U of T have been completed through the University grants and the cost was not totally covered by the Crown, therefore, a fragmented IP should be considered.

2.4 Suffield Research Centre: Dr Sara Sheibani

Suffield Research Centre is working on TLR7/8 to detect single stranded RNA (ssRNA) viruses.[4] The following subjects were presented and discussed:

- The history of viruses, their classification, the importance of viruses in terms of pathogenicity and emerging infectious diseases. Virus detection and, in particular, ssRNA viruses, is important as most of the biothreat agents on the category "A" of the Centers for Disease Control and Prevention (CDC) are viruses.
- A background on TLR, their unique role in the innate immune system, and the rationale for using TLR7/8 for virus detection in this project.
- Summary of the literature on TLR7/8 for virus detection. Recent data showed there is no actual binding between TLR7/8 and ssRNA. In fact, ssRNA should be fragmented to be recognized by TLR7/8. This could be a challenge for sample processing.
- Methodology for virus detection using TLR7/8 and the time table for deliverables.

[4] A Scientific Letter on the utility of TLR7/8 for detection of viral pathogens has been submitted by the authors (L16-1222-1302).

2.5 National Institute for Nanotechnology: Drs Abebaw Jemere and Rajesh Pillai

NINT collaboration with Suffield Research Centre is on the development of an electrochemical biosensor. Their presentation covered the following subjects:

- Part I: Nanowire electrodes. The following topics were presented in this part: (i) fabrication of nanowire electrodes using glancing angle deposition (GLAD)[5] technique and immobilization of TLR4, (ii) steps involved in surface fabrication, characterization, TLR4 deposition, and electrochemical impedance spectroscopy (EIS)[6] detection following TLR4 incubation with lipopolysaccharide (LPS),[7] (iii) generating the calibration curve of *E. coli* O157:H7 and *Salmonella typhimurium* using indium tin oxide (ITO) GLAD biosensor.

  As a summary of Part I, GLAD electrodes have the potential for integration of addressable arrays on the mass production scale and TLRs can be used to provide a rapid pathogen classification tool.

- Part II: Self-assembled gold nanoparticles for EIS detection and regeneration of the sensor. The following subjects were presented in this part: (i) fabrication of self-assembled adenovirus sensor and presenting the results of dose response EIS of adenovirus 5, (ii) regeneration of self-assembled monolayer (SAM)[8] electrodes and X-ray photoelectron spectroscopy (XPS)[9] results before and after regeneration, (iii) seven steps involved in fabrication of self-assembled adenovirus sensors on regenerated gold electrode.

  As a summary of Part II, the SAM multiple layers formation using the immersion technique is time consuming. This issue is addressed in Part III.

- Part III: Voltage assisted self-assembly of monolayers. To address the issue in Part II, a new technique, electrodeposition, can be used. This technique works very well and takes only a few minutes.

- Conclusion: The self-assembly by electrodeposition and immersion have comparable quality and the electrodeposition method is promising for reducing fabrication time. For a better response, the sensors should have an optimum coverage ratio of sensor molecules and antifouling components. Preliminary studies showed poor stability of TLR4 biosensors upon storage in the dry state while their stability may improve with wet storage.

2.6 University of Calgary: Drs Viola Birss, Maggie Renaud-Young, and Mr Robert Mayall

The research group at U of C has extensive experience in electrochemistry and their work is focused on a thiol-based system. Among the TLRs, they are working on TLR4, due to its well characterized interaction with LPS. Their contribution was presented as follows:

[5] GLAD is a single step physical vapor-deposition technique for fabricating nanostructured thin films.
[6] EIS is a sensitive and label-free analyte (target) detection technique that can be utilized to investigate a biorecognition event occurring at an electrode-electrolyte interface.
[7] LPS is the principal component of Gram negative bacteria cell membrane that is specifically recognized by TLR4.
[8] SAMs are organized layers of molecules which form on a solid surface.
[9] XPS is a technique for analyzing the surface chemistry of a material.

- Phase I: In this phase, the initial experiments (2012–13) and preliminary results on 30 min Mercaptoundecanoic acid (MUA) deposition was presented. The results were promising but not reproducible, therefore the SAM formation time was increased (from 30 min to 24 h). One of the achievements of phase I was to keep the TLR4 upright which improves its ability to dimerize with LPS, likely improving selectivity.
- Phase II: This phase was on protocol optimization. By increasing the incubation time with MUA, higher coverage was observed but resistance was very high, so different complexes were tried which resulted in a smaller current. As part of the optimization, the thiol length was changed. Also, to track surface modification the contact angle was measured. One of the issues was on the logarithmic response of TLR4 to LPS. Based on the previous work of U of T, purified LPS has a logarithmic response while the LPS soup response is linear.[10] In summary, the interaction between TLR4 and LPS is 2:2[11] however, it is highly dependent on neighbour molecules being available to sandwich the LPS. The TLR4 sensor has an excellent selectivity for Gram negative bacteria and insensitivity to Gram positive and viral challenges.
- Phase III: The focus of this phase was on the next generation of sensor design. The MUA SAMs had great utility however, the resistance is still high, reproducibility is problematic, and a logarithmic response is still observed. Therefore, the next generation of SAMs was considered. Ferrocene thiol could be a useful approach for lowering the resistance of TLR-based sensors while retaining stability. The results showed a much lower resistance compared to that in Phase II. In addition, the sensor responded to LPS from heat killed Gram negative bacteria.
- Phase IV: In this phase, the future direction for the next three years was presented. In the first year, further work on TLR4-based sensors will be performed and the optimal sampling parameter will be determined. In the second year, (i) the reusability of TLR4 sensor and (ii) development of Gram positive sensor with TLRs 1–2 and 6 will be explored. The initial designs for aerosol collection and assessment will be started. In the third year, the focus will be on multiplexing sensor designs.

2.7 University of Toronto: Drs Bernie Kraatz, Zhe She, and Maj Kristin Topping

U of T is working on 5 TLRs (TLRs 1–5). Their research activities and their key expertise over 17 years include: (i) development of a DNA sensor for biomedical application, (ii) anti-microbial peptide for detection of *E. coli* O157:H7 and *Salmonella*, and (iii) RNA/DNA detection for *Enterococcus faecalis*. Their recent and future activities on the TLR project are as follows:

- Construction of the TLR recognition layer using a new surface technology by Queen`s University and related published work by their lab.

[10] The soup is a heat killed Gram negative bacteria, boiled before delivered to the lab. This would lyse the bacteria and release the LPS. This LPS called LPS soup as opposed to purified LPS which is commercially available.
[11] It means that 2 LPS molecules are bound by 2 TLR4 when it forms a dimer.

- Using hybridized TLRs (TLRs 1–2). The results showed that the detection response of Pam3CSK4[12] is enhanced when a mix of TLR1 and TLR2 is used compared to when each of them used individually.
- TLR3 sensor for double-stranded RNA (dsRNA) virus detection. There was a technical challenge as dsRNA tends to be fragmented, therefore the pH had to be optimized (optimum pH: 6.8, but changed to 6.5 for surface plasmon resonance (SPR) analysis).
- TLR4 and LPS binding. The data for detection of LPS with TLR4, using *E. coli* and *Salmonella* were presented.
- TLR5 for detecting flagella.[13] Their data showed that the sensor was able to detect and differentiate flagella extracted from *Salmonella typhimurium* and *Bacillus subtilis*. Also, an electrochemical response was observed when detecting the whole cell (*E. coli* K12) using TLR5. At higher concentrations of the *E. coli* K12, TLR5 sensor showed reproducible data.
- Sensor shelf-life. Although the preliminary data showed that the intensity obtained from electrochemical imaging diminishes after 14 days, the sensor had a reliable classification of *Salmonella typhimurium*.
- Development of multiplex TLRs 1–5 sensor. This will be evaluated using two different approaches, including scanning electrochemical microscopy (SECM) and electrochemistry (ECHEM).
- The multiplex TLRs 1–5 biosensor was assess at a technology readiness level of 4–5 (see Annex D for technology readiness levels).
- Feasibility of TLR multiplexing. The preliminary results showed the pattern recognition capability of a TLR multiplex by differentiating a whole-cell species from a PAMP stimulus. Currently, the direction is to obtain more patterns for other targets such as Gram positive bacteria.
- The ATIR-funded project milestones for years 1–3. In the first year, the milestones met successfully and the reports submitted to D CBRN D. In the second year, the bio-recognition capability of all TLRs against their corresponding PAMPs was verified. Finally, in the third (and current) year, work on whole cell pattern recognition has been initiated and some preliminary results obtained.

[12] Pam3CSK4 is a synthetic triacylated lipopeptide (LP). Recognition of Pam3CSK4 is mediated by TLR2 which cooperates with TLR1.

[13] Flagella are slender threadlike structures that many bacteria use for motility which is an important process in many stages of a pathogen's life cycle.

3 Group Discussion

During the workshop discussion sessions, several views on the current and future work were presented. The key points of the discussion include:

3.1 Technological Challenges

The proposed multiplex handheld biosensor is a small device that can be carried in pocket and includes all TLRs (TLRs 1–9). The sensor chip will have two necessary elements: (i) target detection with TLRs and (ii) signal transduction with electrochemistry. Some of the current technological challenges are:

- **Sample processing:** The TLR biosensor works as an offline or online system. There is a drawback in sample processing using an online system and the sample is transferred manually in offline system. All data obtained so far within the groups involved in this project are from liquid samples. Therefore, analysis of non-liquid samples needs to be developed. It has been discussed with the military sponsor that initial work should focus on liquid samples, with later emphasis on aerosols.
- **Aerosol samples**: It is desirable that TLR biosensor be used to analyze aerosol samples; however, none of the involved research groups have aerosol expertise. Therefore, an aerosol expert needs to be included in our team to close the gap of expertise in this field. Two aerosol experts from Laval University and McGill University were proposed by Drs Chan and Sheibani and their biography has been submitted to all research groups for review. A decision will be made to select one of the scientists in the next meeting.

3.2 IP Agreement

The TLR project is a joint project among universities and government organizations. While the IP in government institutions (i.e., DRDC and NINT) belongs to the Crown, this is not the case for universities. Considering that some parts of the TLR project have been conducted at U of T and U of C and the cost has been partially covered by University grants and the Crown, a fragmented IP needs to be considered. Currently, Mr Chan continues conversation with DRDC IP expert, Mr Ron Poirier, on this topic. According to the latest update from Mr Chan (March 2017), the IP issue expected to be re-evaluated sometime in April 2017.

3.3 Consortium Contracting

A contracting vehicle is required to enable the research groups at U of C and U of T to continue their work on the TLR biosensor project. According to the most recent update from Mr Chan, the contracting officer prepared a document (March 2017).

3.4 Task Related Work Plan

In order to collect the information on tasks that each research group has done or could do, a task-related work plan needs to be developed. This will provide a picture of the current progress and a proposed plan for future. Following the TLR workshop, a work plan was prepared by Dr Chan and was sent to all research groups to provide input. The information will be gathered and the document will be discussed in our next group meeting.

3.5 Common Standard Operating Procedure (SOP)

Considering that different research groups are involved in this project, it is very important to have common SOP to form a basis for their methods. Following the workshop, Drs Jemere and Pillai prepared and distributed the SOP for review by each research group.

3.6 Reagents and Controls

During the workshop, it became apparent that different sources of reagents and materials would lead to different results. As a specific example, it was observed that the results from the binding assay with TLR4 and two different sources of LPS (see p.10) were different. In order to have reproducible data, all research groups agreed to use uniform reagents and the same number of tested controls for future studies. Following the workshop, a reagents listing was prepared by Dr Sheibani and was sent to all involved research groups to be completed. The collected information will be discussed during a future meeting.

3.7 Funding

Funding was also one of the issues that was discussed; the involved research groups expressed their concern for the future funding of the project. LCol Natale and Mr Chan will update us in our next meeting on funding.

4 Conclusion

The two-day workshop was a productive event and we were able to accomplish our objectives. As team work requires a multidisciplinary effort, this workshop was useful as it enabled leveraging of each other's strengths, recognized the gap in expertise, and indicated how to avoid duplication of experimental approaches. In addition, the general knowledge of the participants increased which will lead to advanced scientific expertise and faster technological maturation for our client. Since it was the first time the workshop was held, a lot of work remains to be done and the client representatives were asking for another workshop. The next event is being considered pending common availabilities amongst the participants.

As of March 2017, no update is available on the progress of any lab work from the involved research groups. As discussed in our meeting, all research groups had a common agreement that having meetings and discussing the results will be helpful to make further progress in the project. Although face to face meetings are more effective, it would be difficult to organize a meeting at one location on a specific date, owing to the fact that each research group is at different location and have various commitments. As an alternative, VTC could be considered for every 2 months to update on the research progress among the groups.

To further progress in the project, another possible approach is to engage more scientists with the complementary expertise. At this stage, the gap in expertise for aerosol has been recognized. However, we still require more dynamic in our team to recruit difference expertise. Engaging trained postdoctoral fellows and research associates, instead of graduate students would be more beneficial for the progress of the project.

With the effort of the Project director, Mr Chan, the IP and the consortium contracting issues are moving forward and it is expected that these two items will be resolved by summer 2017. For the consortium contracting, the help of a contracting expert has been secured and the documents are ready for evaluation and feedback.

5 List of Attachments

1. DND Directorate of CBRN Defence (Maj Alexi Natale) “Research Priorities” (8 slides, ppt)

2. DRDC (Sara Sheibani) “Viral Detection by Toll-Like Receptors 7 and 8” (26 slides, ppt)

3. National Institute for Nanotechnology (Abebaw Jemere and Rajesh Pillai) “Nanotechnology-Enabled Impedimetric Sensors for Pathogens Detection” (55 slides, ppt)

4. University of Calgary (Viola Birss (PI), Robert Mayall & Maggie Renaud-Young) “U of Calgary TLR-4 Sensor Project” (44 slides, ppt)

5. a) DND Directorate of CBRN Defence (Dr. Zhe She, Maj. Kristin Topping, Dr. Emily C. Corcoran, Dr. David Kelly, Dr. Bernie Kraatz) “Development of A Pathogen Detection Technology Toll-like Receptors & Electrochemical Sensing Techniques” (42 slides, ppt); b) DND Directorate of CBRN Defence “Current Status of TLR Project” (5 slides, ppt)

6. DRDC (Nora Chan) “Development of a handheld aerosol biodetector: technical challenges” (13 slides)

Annex A Agenda

Table A.1: *Toll-like receptor workshop agenda.*

Tuesday, 30-Aug-16			
1	08:30–08:45	Welcome-Introductions	Albert Chan-Nora Chan
2	08:45–09:15	Introduction to DRDC's CBRN Defence Program	Albert Chan
3	09:15–09:45	D CBRN D's priorities and related procurement project	Wayne Willmott-Alexi Natale
	09:45–10:00	Break	
4	10:00–11:00	Discussion on Intellectual Property	Ron Poirier
5	11:00–12:00	DRDC's presentation of TLR research including maturity level and methodology	Sara Sheibani
	12:00–13:30	Lunch	
6	13:30–14:30	NINT's presentation of TLR research including maturity level and methodology	Abebaw Jemere-Rajesh Pillai
7	14:30–15:30	U of Calgary's presentation of TLR research including maturity level and methodology	Viola Birss
	15:30–15:45	Break	
8	15:45–16:45	U of T presentation of TLR research including maturity level and methodology	Bernie Kraat-Zhe She
9	16:45–17:00	Closing Remarks	Albert Chan-Nora Chan

Wednesday, 31-Aug-16			
1	08:30–08:45	Welcome-Introductions-Recap	Albert Chan-Nora Chan
2	08:45–10:00	Discussion on technological challenges and best practices	Nora Chan
	10:00–10:30	Break	
3	10:30–12:00	Discussion on work plan (20 min) presentation to start off	Nora Chan
	12:00–13:30	Lunch	
4	13:30–15:00	Discussion on work plan (con't)	Nora Chan
	15:00–15:30	Break	
5	15:30–16:00	Work plan back brief to plenary	Nora Chan-Albert Chan-Sara Sheibani
6	16:00–16:15	Closing	Albert Chan-Nora Chan

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Annex B Attendance List

Toll-Like Receptor Workshop
DRDC Corporate Office
400 Cumberland St., Ottawa ON
30–31 August 2016

Toll-Like Receptor Handheld Biodetector Research Project

The information disclosed during this briefing is proprietary to Her Majesty the Queen in right of Canada as represented by the Minister of National Defence through the Agency, Defence Research and Development Canada and the Royal Military College of Canada. This information is provided to attendees on the understanding that it is communicated in confidence; will be used for information and evaluation purposes only; and will not be disclosed (unless specifically agreed to by Her Majesty's representative) to any third party outside of the organizations represented by the attendees.

Table B.1: *The list of attendees and the corresponding organizations.*

Attendee	Organization	Coordinates
Birss, Viola Ingrid	University of Calgary	birss@ucalgary.ca
Chan, Albert	Department of National Defence	Albert.chan@forces.gc.ca
Chan, Nora	Department of National Defence	Nora.chan@forces.gc.ca
Jemere, Abebaw	National Research Council	Abebaw.jemere@nrc.ca
Kelly, David (absent)	Royal Military College	
Kraatz, Bernie	University of Toronto	Bernie.kraatz@utoronto.ca
Mayall, Robert	University of Calgary	rmmayall@ucalgary.ca
Natale, Alexander (Alexi) (L Col)	Department of National Defence	Alexander.natele@forces.gc.ca
Pillai, Rajesh	National Research Council	Rajesh.pillai@nrc-cnrc.gc.ca
Ramaniah, Arun	Promaxis Systems Inc. (Working for Department of National Defence)	
Renaud-Young, Margaret	University of Calgary	Maggie.renaudyoung@gmail.com
She, Zhe	Calian (Working for Royal Military College)	Zhe.she@utoronto.ca
Sheibani, Sara	Department of National Defence	Sara.sheibani@drdc-rddc.gc.ca
Willmott, Wayne (Maj)	Department of National Defence	Wayne.willmott@forces.gc.ca
Topping, Kristin (Maj)	Department of National Defence	Kristin.topping@forces.gc.ca
Ron Poirer	Department of National Defence	Ron.poirier@forces.gc.ca
Nicole Sabourin	Department of National Defence	Nicole.sabourin@forces.gc.ca

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Annex C VTC on the draft of "Military Requirment on the TLR Biosensor"—23 Nov. 2016

Following the TLR workshop in Ottawa, Dr Chan, requested the scientific groups involved in this project (U of C, U of T, and NINT) to provide her with their comments, suggestion, and inputs on the draft of the military requirement on the TLR biosensor, in order for her to present the collective view on this subject to our military sponsor. From the responses received from the scientific community, it was evident that there was no consensus on the intended purpose and the use of the TLR biosensor. Therefore, for all the scientists involved in this project a discussion session through VTC was co-organized by Drs Sheibani and Chan. All invited groups attended the VTC and justified their comments on the draft of the military requirement as follows:

C.1 Suffield Research Centre: Dr Sara Sheibani (12:00–12:05)

The military requirement on the TLR biosensor drafted by Arun Ramaniah included two parts:

- **The "intended purpose" of the TLR biosensor:** "The intended purpose of the TLR sensor is to recognise bio-hazards whether known or unknown to protect the CAF against new and modified bio-hazards. Though TLR technology cannot definitively recognize biological organisms as hazardous, it can create a library of conserved pathogen-associated molecular patterns (PAMPs) of known biohazards."

Based on the responses received from all research groups, two points of view were presented: (i) scientists at U of C and NINT were suggesting that no library is required while, (ii) scientists at U of T were making the case that it is critical to keep the idea of a library.

- **The "use" of the TLR biosensor:** "The TLR sensor is expected to analyse a biological sample and derive its PAMP. When the PAMP of the sample is found to bear a close resemblance to a PAMP in the library, it could be assumed that the sample might be hazardous and should be analysed further. If, on the other hand, there is little or no resemblance to the PAMPs in the library, the sample could safely be assumed as benign."

Based on the comments received from all research groups, two different points of view existed: (i) research groups at U of C and NINT indicated that there is no need for comparing the results against a library. It was argued that an increase in the concentration detected by biosensor will indicate presence of hazardous material in the sample. In contrast, (ii) research group at U of T were stating that recognition library with pre-calibrated species can be helpful to have finger print recognition.

From the responses received, it is presumed that there is no consensus among research groups on both parts therefore, this VTC was organized to discuss this issue face to face in more details.

C.2 Suffield Research Centre: Dr Nora Chan (12:05–12:10)

In the proposed biosensor technology, in the presence of the PAMPs, a binding event occurs. The acquired results will not fulfill the requirement for creating library as there is no difference

between the conserved PAMPs in a known biohazard as oppose to an unknown biohazard. Electrochemical signals cannot be used for pattern recognition therefore, creating a library will not be useful in this case.

C.3 CBRN Defence Manager: Mr Albert Chan (12:10–12:15)

TLR biosensor is a promising technology for biodetection and classification. Our client needs however, to be briefed and educated on this technology. It is important therefore, to come up with a unified definition of "library" among our scientific groups, prior to the next meeting with our military sponsor. Our next meeting is being considered for near future pending on the availabilities amongst the participants.

C.4 University of Calgary: Drs Viola Birss, Maggie Renaud-Young, and Mr Robert Mayall (12:15–12:30)

The purpose of the TLR biosensor is to acquire knowledge of the biohazard that the CAF personnel exposed to it, whether the biological agent is live or dead. Also, the biosensor will not provide information whether the sample is harmful or not. The acquired results need to be further confirmed by other techniques (e.g., PCR).[14] Therefore, having a system of systems approach that might be useful. The TLR biosensor is a classification/characterization system based on electrochemistry, in which a library cannot be created.

C.5 University of Toronto: Drs Bernie Kraatz and Zhe She (12:30–12:45)

Creating a library is not the same as creating a conserved PAMPs library or deployable library. In order to examine the robustness of the system, different known samples should be tested with the biosensor and the collected results will reflect the definition of library. Creating this kind of library will be advantageous, as it will be helpful to distinguish the biohazard from the background so, it can add value to the system.

C.6 National Institute for Nanotechnology: Drs Abebaw Jemere and Rajesh Pillai (12:45–13:00)

The interpretation of library is the main issue among all research groups. In this type of biosensor, a library cannot be created. However, as U of T mentioned, different pathogens and samples should be tested with the biosensor. These results will be useful in the evaluation of the robustness of the TLR biosensor.

[14] Polymerase chain reaction (PCR) is a technique used in molecular biology to rapidly amplify sequences of DNA.

C.7 Group Discussion (13:00–13:45)

During the group discussion, some other topics were discussed. Below, are the highlights of some of the discussed points.

- **Background**: This is a critical issue in the TLR biosensor which can make a difference in the results. Therefore, it is important to know where the biosensor will be located and in what type of environment it will be used.

  Action by our military sponsor: determine the type of environment the TLR biosensor will be used.

  Action by Drs Chan and Sheibani: invite the bioaerosol experts from Suffield Research Centre to our next meeting as they are working on the FLAPS system.

- **Level of Understanding (LoU):** The LoU for each TLR needs to be defined.

  Action by Dr Chan: prepare the LoU for our next meeting.

- **Robustness of the system:** This should be verified by using previously characterized pathogens with the system. The collected results will serve to determine the robustness of the system.

  Action by all research groups involved in this project.

C.8 Closing (13:45–14:00)

This VTC was very helpful to understand everybody's point of view on the concept of the library. The collective view on the draft of the military requirement on the intended purpose and use of the TLR biosensor will be prepared by Dr Chan.

C.9 Conclusion

A consensus has been reached among the scientific community on the intended purpose and the use of the TLR personal biosensor. A Scientific Letter is submitted to our military sponsor (DRDC-RDDC-2017-L051) to present our collective view on the purpose, intended use, and design of the Toll-like receptor electrochemical biosensor.

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Annex D Technology Readiness Levels

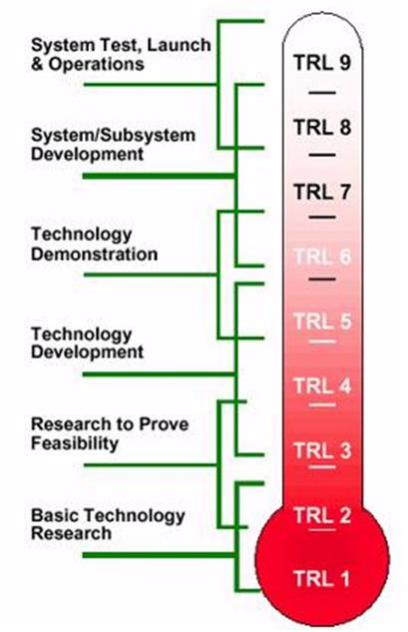

Figure D.1: *Department of Defense (DoD) technology readiness levels.*

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List of Symbols/Abbreviations/Acronyms/Initialisms

CAF	Canadian Armed Forces
CBRN	Chemical, Biological, Radiological, and Nuclear
CDC	Centers for Disease Control and Prevention
DARPA	Defense Advanced Research Projects Agency
DIM	Detection, Identification, and Monitoring
DoD	Department of Defence
DRDC	Defence Research and Development Canada
dsRNA	Double-Stranded RNA
ECHEM	Electrochemistry
EIS	Electrochemical Impedance Spectroscopy
GLAD	Glancing Angle Deposition
IP	Intellectual Property
IPE	Individual Protection Equipment
ITO	Indium Tin Oxide
LBDS	Local Biodefence System
LoU	Levels of Understanding
LPS	lipopolysaccharide
MedCM	Medical Countermeasures
MUA	Mercaptoundecanoic Acid
NINT	National Institute of Nanotechnology
NTAs	Non Traditional Agents
PAMPs	Pathogen Associated Molecular Patterns
RMCC	Royal Military College of Canada
S&T	Science and Technology
SAM	Self-Assembled Monolayers
SECM	Scanning Electrochemical Microscope
SOP	Standard Operating Procedure
SPR	Surface Plasmon Resonance
ssRNA	Single-Stranded RNA
TLR(s)	Toll-Like Receptor(s)

TRL(s)	Technology Readiness Level(s)
U of C	University of Calgary
U of T	University of Toronto
VTC	Video Teleconference
XPS	X-Ray Photoelectron Spectroscopy

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The two-day Toll-Like Receptor (TLR) workshop was co-organized by Dr Nora Chan, lead scientist of the TLR biosensor project, and Mr Albert Chan, Project Director for the Inform Project within the CBRN Defence Program. Biological detection, identification and monitoring (BioDIM) is the top S&T priority for the Directorate of Chemical, Biological, Radiological and Nuclear Defence (D CBRN D). The TLR biosensor work element within the Inform Project aims at addressing this problem. The purpose of this workshop was to learn about the progress made by the team members so far, and discuss the future ideas. Information presented included work from each of the invited research teams as well as an overview of the current challenges from the lead scientist. The open discussion and face to face meeting format facilitated advancement of the TLR work. The most significant achievement of this two-day workshop was that all participants had an opportunity to learn and understand the results of research conducted by each team in great detail. The outcome of this workshop will lead to faster technological maturation for our sponsor.

L'atelier de deux jours sur les récepteurs de type Toll (TLR) a été coorganisé par Nora Chan, PhD, scientifique en chef du projet de biocapteur TLR, et par Albert Chan, directeur du projet Inform dans le cadre du Programme de défense CBRN. La détection, l'identification et la surveillance biologique (BioDIM) est la priorité absolue en matière de S et T de la Direction - Défense chimique, biologique, radiologique et nucléaire (DDCBRN). L'élément de travail sur le biocapteur TLR dans le cadre du projet Inform porte sur cette question. Le but de l'atelier était de faire connaître l'état d'avancement des travaux des membres de l'équipe et de discuter des idées pour la suite du projet. Chacune des équipes de recherche a présenté son travail, et la scientifique en chef a fait un survol des difficultés actuelles du projet. Le choix de tenir des discussions ouvertes et de réunir les participants en personne a facilité l'avancement des travaux sur le TLR. L'aboutissement le plus important au terme de cet atelier de deux jours est que tous les participants ont pu connaître et comprendre les résultats de la recherche menée par chacune des équipes. Grâce à cet atelier, le projet pourra atteindre sa maturité technologique plus rapidement pour le parrain du projet.

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Toll-like receptor; Pathogen; biodetection; biosensor