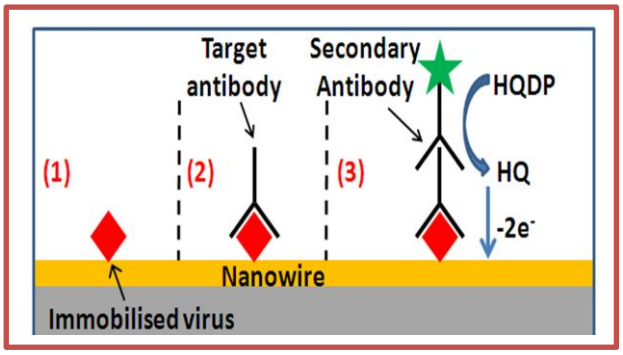
Project number: 6509
Funding source: Dairy Research Ireland (Dairy Levy)
Date: April 2020
Project dates: Jan 2015 – Dec 2018

IBR-Nano: Development of a pen-side biosensor diagnostic device blueprint for detection of IBR in bovines



Key external stakeholders:
Animal Health Ireland (AHI)
Department of Agriculture, Food & the Marine (DAFM)
The Irish Cattle Breeding Federation (ICBF)
Dairy Farmers; Veterinarians
Diagnostic laboratories

Practical implications for stakeholders:
This study has

- developed a blueprint for rapid, point-of-care, nanosensor bovine herpesvirus-! (BHV-1), the causative agent of infectious bovine rhinotracheitis (IBR), detection on-farm
- applied this blueprint to a range of additional analytes including bovine viral diarrhea virus (BVDV) and immunoglobulin G (IgG)
- evaluated the technology against archived samples from AGRIC, Teagasc, Moorepark
- evaluated the technology on a range of samples from research and commercial farms

Main results:

- Diagnostic nanochips have been fabricated, characterised, and are operating in accordance with design specifications.
- Microneedle diagnostic devices have also been designed and developed with the aim of employing continuous on-farm monitoring of disease-related analytes.
- Label-free immune assay have been developed using model antibody–antigen sandwich pairs.
- Label free sensing in serum (undiluted) has been demonstrated without significant non-specific binding/adsorption. This is a key result as no blocking chemistries were used in the assay layer stack. This is the first time that this has been demonstrated.
- BHV-1, BVDV, and IgG field samples were sourced from research and commercial farms and were tested using the label free assay approach. Label free BHV-1, BVDV and IgG assays were undertaken on serum samples of known status. Time to result was 15 minutes and on-chip results were in 100% agreement with ELISA results.
- Most recently, in conjunction with a commercial partner, the electronic 'packaging' for an on-farm device has been developed to allow real time results to be read on-farm.

Opportunity / Benefit:

Ireland does not, at present, employ a coordinated approach to IBR control. In the interest of maintaining live export markets across Europe, discussion are taking place amongst key stakeholder to rectify this situation. It is conceivable that without implementation of a national BHV-1 control programme, transport of live Irish cattle across France, Belgium and the Netherlands would be prohibited. The establishment of Animal Health Ireland provides a vehicle through which a national programme can be rolled out and testing for BHV-1 and viral exposure will play a role in this. Addition of a rapid point-of-care device to the diagnostic technologies currently available would prove of immeasurable benefit to the role out of such an IBR eradication scheme.

Additionally, offering farmers tools with which to gain increased efficiencies and a competitive advantage is of considerable value in increasing farm profitability and viability. Provision of point-of-care diagnostic

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devices to both veterinarians and farmers for a range of analytes will allow rapid treatment interventions to improve livestock health, livestock welfare and farm viability.

Collaborating Institutions:
Tyndall National Institute (TNI) based at University College Cork (UCC)
Queen's University, Belfast (QUB)
The Irish Equine Centre (IEC)

Teagasc project team:	Ríona Sayers (PI); Robert Prendiville
External collaborators:	Alan O'Riordan (TNI) Mark Mooney (QUB) Ann Cullinane (IEC)

1. Project background:

The top seven animal diseases prioritized as important by both experts and farmers in the Animal Health Ireland Delphi study included Infectious Bovine Rhinotracheitis (IBR). Bovine herpesvirus-1, the causative agent of IBR, is endemic in Ireland. Similar to other herpesviruses, IBR is maintained in a herd through reactivation of a latent state within infected carrier animals, usually multiparous individuals in the herd. Once reactivated, individuals become viraemic and shed the virus resulting in infection of in-contact animals. In addition, once IBR enters a herd, viral spread is rapid and full herd-infection can result within weeks. It is therefore of enormous benefit if both shedding and carrier animals can be identified at an early stage to allow removal from the herd or application of an appropriate vaccination programme. Laboratory based tests can result in a window of opportunity being missed in that results may not be available for over 7 days allowing uncontrolled viral spread to continue in the interim. Application of nanosensor technology, as proposed in this project, to allow rapid generation of results on-farm when immediate action can be taken would be hugely beneficial and would ultimately reduce requirements for antibiotic administration as fewer animals would succumb to the clinical signs of IBR before intervention can take place.

Ireland must become a consumer responsive sector and FoodWise 2025 has highlighted the need for roadmaps to guide optimal production at all stages of the production process in order to increase efficiencies but also improve product quality. The primary producer must operate with, i) reduced costs, ii) higher productivity, and iii) produce higher value outputs. *IBR-Nano* has the capability to impact on primary producers in all three areas. It will provide a diagnostic strategy to improve on-farm IBR status, thereby increasing productivity and improving cow health and welfare. This will ultimately lead to production of higher value outputs. The nanosensor blueprint developed for BHV-1 also has the potential to be applied to a range of additional analytes. In that regard, the current project was expanded to include work on BVDV in cattle and IgG in calves.

2. Questions addressed by the project:

Partnering nanoelectodes with biorecongnition tools such as antibodies and enzymes yields highly specific and sensitive biosensors. Development of nanoelectrochemical biosensors has been largely driven by the demand for point-of-care devices for monitoring health and as nanoelectrodes have a small footprint and the potential for high sensitivity, they are ideal for this application. Such devices are already employed in human medicine for the detection of glucose, dopamine, and pharmaceutical drugs, amongst others. This project sought to address the need for point-of-care devices in veterinary medicine by developing a nanosensor device for detection exposure to BHV-1. As additional biological resources were available, the opportunity was also taken to develop sensors for BVDV and IgG.

3. The experimental studies:

- Study 1: The aim of study 1 was the identification and sourcing of suitable IBR paired antibodies (Ab) and antigens (Ag). This work was undertaken by Teagasc, Moorepark and QU, Belfast.
- Study 2: The aim of study 2 was to develop a label-free assay using surface plasmon resonance (SPR) technology.
- Study 3: The aim of study 3 was to design and develop a nanowire array sensor chip with improved signal to noise ratios and to fabricate silicon chips capable of antigen binding. This was subsequently expanded to include microneede design and fabrication.
- Study 4: The aim of study 4 was to immobilize antigen/antibody to the nanowire/mcironeedle.
- Study 5: The aim of study 5 was to evaluate the nanosensor device on archived and field samples including BHV-1, BVDV, and IgG.

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4. Main results:

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- Label-free immune assay have been developed using model antibody–antigen sandwich pairs.
- Label free sensing in serum (undiluted) has been demonstrated without significant non-specific binding/adsorption. This is a key result as no blocking chemistries were used in the assay layer stack. This is the first time that this has been demonstrated.
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- Most recently, in conjunction with a commercial partner, the electronic 'packaging' for an on-farm device has been developed to allow real time results to be read on-farm.

5. Opportunity/Benefit:

Infectious Bovine Rhinotracheitis leads to sub-optimal performance in both sub-clinical and clinical phases of the disease, and a number of studies have highlighted the biological and economic consequences of IBR infection. IBR is a viral infection of bovines caused by Bovine Herpesvirus-1 (BHV-1). On entering the host, following a brief viraemia, which may or may not be associated with clinical signs, BHV-1 (in common with all herpesviruses) maintains a latent infection within the host. During periods of stress, the virus has the capability to re-activate resulting in an additional period of viral shedding and infection of herd cohorts. In addition, once IBR enters a herd, viral spread is rapid and full herd-infection can result within weeks. It is, therefore, of enormous benefit if both shedding and carrier animals can be identified at an early stage to allow removal from the herd or application of an appropriate vaccination programme. Laboratory based tests can result in a window of opportunity being missed in that results may not be available for days allowing uncontrolled viral spread to continue in the interim. Application of nanosensor technology, as developed in this project, to allow rapid generation of results on-farm when immediate action can be taken would be hugely beneficial and would ultimately reduce requirements for antibiotic administration as fewer animals would succumb to the clinical signs of IBR before intervention can take place.

With regard to the additional analytes investigated in this project, on-going detection of BVDV exposure will be important in on-farm surveillance post completion of the Irish national BVD eradication scheme, while detection of IgG levels in calf serum are essential in assessing whether successful passive transfer of immunity has been achieved in neonatal calves.

6. Dissemination:

International conferences

- Robinson C, Creedon C, Sayers R G,O'Riordan A. Rapid Serological Detection of Bovine Herpesvirus-1 using Nanosensors. Presented at: 29th World Buiatrics Congress; 2016 July 3-8th; Dublin, Ireland
- Robinson C, Creedon N, Kennedy E, O'Riordan A. Label-free Electrochemical Immunosensor for the Detection of IgG in Calf Serum. Presented at: 25th Electrochem; 2019 August 26-28th; Glasgow, UK.
- Creedon, N.; Robinson, C.; Kennedy, E.; Riordan, A. O. Agriculture 4.0: Development of Seriological on-Farm Immunosensor for Animal Health Applications. 2019 IEEE SENSORS.
- Robinson C, Creedon N, Sayers R G, Kennedy E, O'Riordan A. Electrochemical Detection of Bovine IgG in Calf Serum. Poster presented at: Analytical Research Forum; 2019 June 25th; London, United Kingdom.
- O'Flynn, B., De Donno, M., Barrett, C., Robinson, O' Riordan, A. Smart microneedle sensing systems for security in agriculture, food and the environment (SAFE). 29 Oct.-1 Nov. 2017 IEEE SENSORS

National Conference

- Robinson C, Creedon C, Sayers R G,O'Riordan A. Rapid Serological Detection of Bovine Herpesvirus-1 using Nanosensors. Presented at: 70th Association for Veterinary Teaching and Research Work Annual Conference; 2016 Dublin, Ireland.
- Robinson C, Barrett C, O'Mahony C, Sayers R G, O'Riordan A. Microneedle Electrodes for Nitrate Detection in Water. Presented at:8th Conference on Analytical Sciences Ireland; 2016 April 14-15th; Dublin, Ireland

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Open Days & seminars

- Robinson C, Creedon C, Sayers R G, O'Riordan A. Electrochemical Detection of Bovine IgG in Serum. Presented at: 2nd ISE Electrochemistry in Graduate Research Student Meeting; 2018, Limerick, Ireland.
- Robinson C, Barrett C, Bocchino A, O'Mahony C, Sayers R G, O'Riordan A. Implementation of Microneedle Devices as an Electrochemical Sensing Platform. Presented at: 1st ISE Electrochemistry in Graduate Research Student Meeting; 2017 Cork, Ireland.

Theses

This project is currently being written up as a PhD thesis including three experimental chapters (C. Robinson). It also contributed two PhD chapters to another PhD student (N., Creedon).

Main publications:

- Robinson, C., Creedon, N., Sayers, R., Kennedy, E., O'Riordan, A. (2020). Electrochemical-Based Serological Detection of Bovine Immunoglobulin G in Calves. Analytical Methods, *In Press*.
- Tarasov, A., Gray, D.W., Tsai, M.-Y., Shields, N., Montrose, A., Creedon, N., Lovera, P., O'Riordan, A., Mooney, M.H., Vogel, E.M. (2016). A potentiometric biosensor for rapid on-site disease diagnostics. Biosensors and Bioelectronics, 79, 669-678.
- Montrose, A., Creedon, N., Sayers, R., Barry, S., O'Riordan, A. (2015). Novel Single Gold Nanowire-based Electrochemical Immunosensor for Rapid Detection of Bovine Viral Diarrhoea Antibodies in Serum. Journal of Biosensors & Bioelectronics 2015, 6:3

7. Compiled by: Dr. Riona Sayers

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