Te Niwha

Research Project Impact Case Study

Development of non-invasive "liquid-biopsy" methods for Infectious disease using microbial cell-free DNA

Short Research Title

Creating simple blood and urine tests to detect infections using microbial DNA

Key researchers

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Introduction

Infectious diseases are traditionally diagnosed by culturing the microorganism causing disease. However, in many cases a diagnosis can be difficult to achieve because of inability to obtain the appropriate invasive specimen, non-viability of the micro-organism (for example due to antibiotic pre-treatment) or inability to reliably grow the organism in a laboratory environment. When micro-organisms cause disease in the body they release bits of their own DNA, and this can be detected in the blood and urine of patients. Our project set out to determine if two assays (one for blood and one for urine) can detect the microorganism DNA released during infection by quantitative polymerase chain reaction (qPCR). The two assays rely on non-invasive samples that are easy to obtain and would allow for quick infection diagnosis and treatment. Using either of these tests in the clinical setting will benefit all New Zealander's including our Māori and Pasifika communities.

We are investigating two different methods to detect microbial DNA in patient blood and urine. One approach uses magnetic beads coated with special DNA probes to capture pathogen DNA for detection by qPCR. The other uses CRISPR technology to detect microbial DNA in patient samples after qPCR amplification. The initial application of both assays has been for the detection of Legionella bacteria which causes a type of pneumonia called Legionnaires' disease. While the initial work is on the improved detection of Legionnaires' disease these two assays can be modified to detect any infectious disease as long as the microorganisms sequence is known.

This work has been made possible by collaborations with Canterbury Health Laboratories, Canterbury Respiratory Research Group and the Te Whatu Ora Waitaha Canterbury Paediatrics Department. These collaborations align with the Te Niwha Investment Objectives by strengthening collaborations between researchers, clinicians and health agencies while building on New Zealand's capability to demonstrate research excellence for infectious disease research and research translation. Since both platforms can be used for any infectious disease detection this project contributes to increasing New Zealand's preparedness and readiness for future infectious disease outbreaks. Likewise, the CRISPR assay has the potential to be developed into a point of care system that can contribute to improving health outcomes and increasing equality in low resource settings.

Results

Platform 1: Hybridisation assay

The qPCR we developed can detect as little as 10 copies of bacterial DNA (Figure 1A), this was then coupled to the hybridisation assay. Our assay out-performed a commercial spin column and phenol-chloroform extraction however it was less sensitive in comparison to spiking the same concentration of cfDNA directly into the qPCR. Specificity was 100% once a probe was inserted into the qPCR to remove non-specific amplification and potential primer-dimer formation (Figure 1B). A sub-set of patient samples were chosen for analysis with the hybridisation assay. Unfortunately, no Legionella cfDNA could be detected in the urine of patients diagnosed with Legionnaires' disease. The next step is to trial blood and sputum in this system using the hybridisation assay, a new commercial extraction kit and a whole blood polymerase.

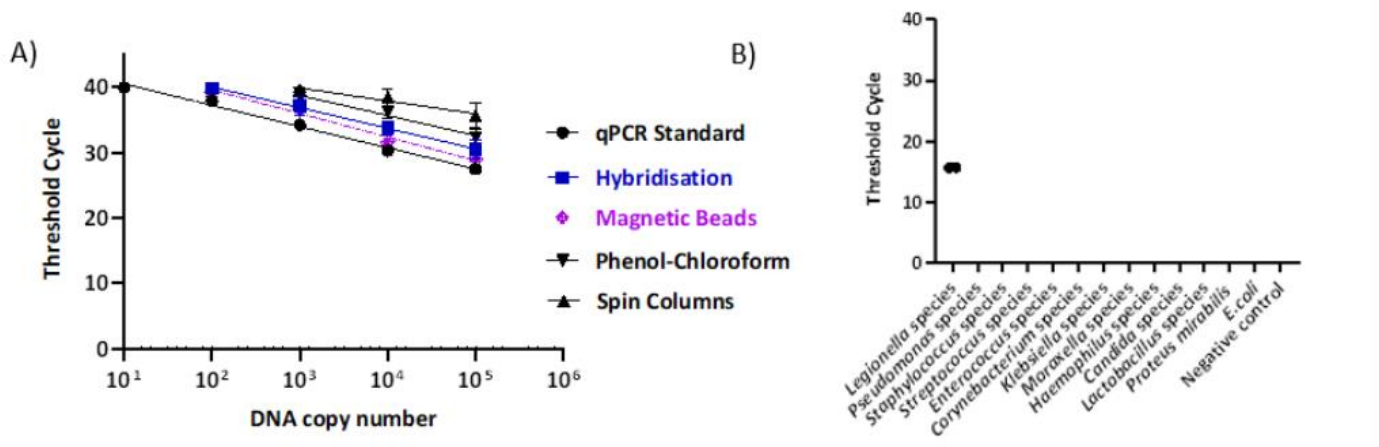

Figure 1. Legionella hybridisation assay sensitivity (A) and specificity (B).

Platform 2: CRISPR-Cas assay

The *Legionella* CRISPR-based test can identify just one bacterial genome per test (Figure 2A) and sensitivity is 100% for all Legionella species (Figure 2B) reporting negative results for over 26 non-Legionella species tested. Initial results of the assay using retrieved blood samples showed the importance of collecting and processing the blood, on ice, immediately after being drawn. As such we introduced an extra blood collection bedside, and these samples have been processed within 15-30 minutes of being drawn. The next step is to test these samples in the assay and compare the results to the clinical and microbiological diagnosis to determine the accuracy of the test.

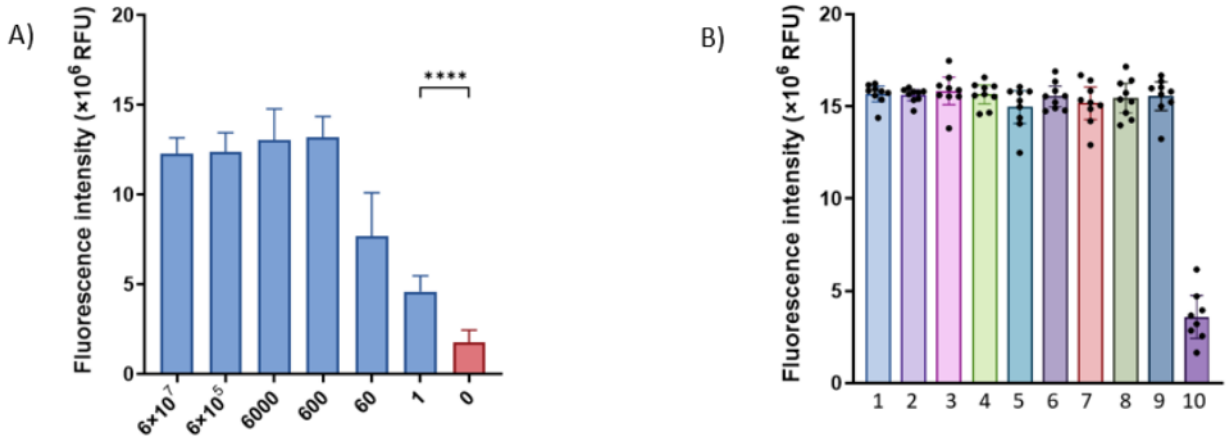

Figure 2. Legionella hybridisation assay sensitivity (A) and specificity (B) for 1. L. longbeachae ATCC, 2. L. longbeachae clinical, 3. L. pneumophila ATCC, 4. L. pneumophila clinical, 5. L. pneumophila clinical, 6. L. anisa clinical, 7. L. bozemanii clinical, 8. L. micdadei clinical, 9. positive control (synthetic oligo) and 10. negative control (sterile water).

Impact

Early and accurate detection of infectious diseases is critical for improving patient outcomes, reducing healthcare costs, and supporting public health, particularly for high-risk and underserved populations such as Māori and Pasifika communities. Our project developed two complementary diagnostic platforms that detect microbial cell-free DNA (cfDNA) in non-invasive samples.

While the hybridisation platform outperformed traditional extraction methods, urine-based testing in patient samples did not detect Legionella cfDNA, highlighting the need to explore alternative specimen types such as blood and sputum.

The CRISPR-Cas platform is highly sensitive and positions the assay for early detection and potential point-of-care applications, particularly in remote or underserved communities. Preliminary blood-based testing has demonstrated that timely collection and processing are critical for reliable detection. Samples processed within 15–30 minutes of collection on ice show promise for accurate cfDNA detection. Testing these blood samples, alongside retrieved bloods samples, is the next step, allowing comparison with clinical and microbiological diagnoses to validate the diagnostic performance.

The practical impact of these platforms is significant, non-invasive and rapid testing enables earlier diagnosis, reduces hospitalisation and invasive procedures. They will also help towards improving antimicrobial stewardship, supporting outbreak detection and subsequent epidemiology. By improving accessibility to reliable diagnostics, this work directly contributes to closing health equity gaps for Māori and Pasifika communities. These platforms are adaptable to a wide range of infectious diseases, increasing New Zealand's preparedness for emerging pathogens. Collectively, these assays have the ability to enhance the reliability and sensitivity of New Zealand's infectious disease diagnostics, supporting better health outcomes and equitable access.