CREATING A PIPELINE FOR EMERGING WATER TESTING TECHNOLOGIES

JUDITH ISAAC-RENTON AND NATALIE PRYSTAJECKY, UNIVERSITY OF BRITISH COLUMBIA,
BRITISH COLUMBIA PUBLIC HEALTH MICROBIOLOGY AND REFERENCE LABORATORY

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WHY DID WE DO THIS RESEARCH?

Waterborne pathogens can be dangerous to environmental, aquatic and human health. Currently, water quality monitoring practices assess fecal contamination by looking for indicator bacteria such as *Escherichia coli* (*E. coli*). Indicator organisms are preferred over the direct detection of pathogens because they are simpler to detect given the large number of possible waterborne pathogens. However, the presence of *E. coli* indicates recent contamination, but doesn't necessarily indicate the presence of viral, bacterial or protozoan pathogens or further information about the source of contamination. It is also important to note that water that is free of these surrogate markers may still harbor pathogens, as was the case for an outbreak of *Cryptosporidium parvum* in Nevada in 1994. Furthermore, these culture-based methods have slow turn-around-times and issues with false-positive and false-negative results.

In comparison, molecular tests are a rapid and sensitive alternative method of directly detecting pathogens. In the field of clinical diagnostic testing, the detection of nucleic acids (i.e., DNA or RNA) has shown great promise, as these methods are a rapid and sensitive method of detecting the pathogen of interest. Where culture-based methods can take up to several days to produce a result, these rapid molecular methods can generate results in hours. Molecular tests (assays) have the added benefit of targeting multiple pathogens at the same time, with greater accuracy.

The same features that are rapidly improving the diagnosis of infectious diseases could also be applied to water. The literature is rich in innovative molecular tests that can detect indicator organisms or pathogens, and can also determine the source of contamination with great accuracy. These tests have the potential to revolutionize the water quality industry, yet there has been limited uptake of molecular tests in water testing.

In order for a new test to be taken up by a laboratory, it must perform equal to, if not better than current methodology. The reporting laboratory must be confident that the new assay improves overall work flow, while providing the highest of quality and standards expected of them. Water testing requires extensive and rigorous validation within an intense regulatory environment.

The limited uptake of molecular tests is partly due to the absence of guidelines to evaluate the performance of these assays under real world situations. This knowledge is critical so that a laboratory has sufficient evidence to confidently use the new method. However, there are no clear guidelines in the literature on how to validate molecular water quality tests. The goal of this project was to narrow the gap between assay developers and end users through a microbial test translation pipeline (MTTP).

HOW WAS THE RESEARCH CONDUCTED?

The project evaluated the appropriateness of promising novel tests, conducted a laboratory study of the test method using standardized validation criteria, and developed a report summarizing the test performance to facilitate communication between test developers and knowledge end-users.

The key element in an MTPP is the validation data, which allows developers to ensure that they have rigorously checked the performance of their assay, and show that the test is equal to or exceeds current testing methods.

While the United States Environmental Protection Agency publishes their Alternative Test Procedure (ATP) guidelines, these are only suitable for the evaluation of culture-based methods against a culture-based gold standard. Guidelines do not exist for the validation of a molecular diagnostic test for water samples. The clinical validation literature was adapted for water quality testing, which pointed to seven parameters that are critical for test validation:

Table 1: Molecular Test Validation Criteria

CRITERIA	DEFINITION
Reportable Range	The range of values that the method is allowed to report
Limit of Detection	The lowest level of target that can be detected by a method
Precision	The closeness of replicate measurements to one another
Specificity	How well a method measures only the target it intends to measure
Accuracy	How close the measured value is to the known value
Reference Interval	The range of normal amounts of target found in safe water samples
Field Trial	How the test performs in real life, typically in parallel with a gold standard method

The draft validation guidelines were shared with four academic and government partner laboratories from across Canada, who were asked to trial the guidelines by applying them to a molecular test of their choice. The laboratories validated *E. coli*, *Enterococci* and *Bacteroides* semi-quantitative and quantitative polymerase chain assays (qPCR) that were published in the literature, using surface water as the water matrix.

The laboratories provided feedback throughout the process, by responding to the following questions:

- Is the guidance document clear and easy to read/interpret?
- Can the experimental design be followed?
- Can statistical tests be performed?
- Does the validation study generate sufficient evidence to consider adoption of the new method?

WHAT WERE THE RESULTS?

The partner laboratories indicated that while the validation guidelines themselves and the statistical analyses were easy to follow, the experimental design for some validation criteria was cumbersome. The number of validation samples and replicate tests required was too high, given the challenges associated with collecting and processing water samples.

One of the issues identified through the review of publications of molecular water tests is that they were validated on improper materials. For example, the limit of detection was based on pure culture of an organism, rather than on water samples. The experimental design for some of the validation criteria are written to address this challenge (e.g., spiking known quantities of organism into surface water samples, rather than laboratory grade water). However, the partner laboratories noted that this is significantly challenging to carry out, because the amount of background nucleic acid in real water samples make interpretation of the data quite difficult.

Based on this information, changes were made to the validation guidelines so that they are still rigorous but entail a more reasonable workload. The revised validation guidelines satisfy the test generators, yet also generate sufficient data to provide evidence of the test's performance for end users.

It should be noted that for all the tested assays, the assays underperformed in the hands of the partner laboratories compared to the peer-reviewed paper, highlighting the challenge of translating published methods to real-life settings.

TECHNICAL CHALLENGES

Although the focus of this research was developing validation guidelines for molecular methods in water, some key technical challenges were identified in the process:

1. While molecular tests are often described as simple or straightforward, the upfront work to process water samples to obtain the nucleic acid (DNA/RNA) needed for the test is much more cumbersome than traditional culture-based methods. Just like high amounts of background microorganisms can impact the culture-based tests, high amounts of certain chemicals like humic acids found in decomposing plant material can impact molecular tests. The methods to reduce the presence of these chemicals can be time consuming, making the molecular tests more challenging to run than the comparable culture-based method.

   Nucleic acid extraction is an area of molecular testing of water that still requires further refinement by test developers.

2. Unlike clinical samples, environmental samples are extremely complex and dynamic. This can complicate the validation process, since the validation materials themselves are dynamic, with differing amounts of target material or even organic materials that might prevent these molecular assays from working.

   The dynamic nature of water can make using molecular test for routine use challenging, and both test developers and test end users must be aware of these challenges in their interpretation of results.

3. Test validation always requires comparison of a new method to a gold standard, which creates two challenges:

   For a test method such as the detection of *E. coli*, typically the molecular method is compared to the culture result; this is akin to comparing apples and oranges. As such, this proved to be difficult during the research, as the quantities of *E. coli* detected by qPCR were quite different than the quantities determined by culture. This reflects the differences between culturable bacteria and amounts of DNA in a sample. This may require the development of a calibrator calculation, but this is not available for all target microorganisms/molecular assays yet.

   Some methods, such as microbial source tracking, have no gold standard, and thus this comparison is not possible and can impact the extent of test validation possible.

WHAT ARE THE IMPLICATIONS FOR TEST DEVELOPERS AND LABORATORIES?

There are many potential applications for molecular water quality tests, such as the detection of pathogens, surrogates and microbial source tracking methods, but these cannot be put into routine use without sufficient evidence of test performance.

Moving forward, there are many barriers and challenges to overcome in order to move molecular tests into testing laboratories. There is a need for better filtering and nucleic acid extraction strategies, so that the highest quality target material can be obtained for robust test performance. Incorporating best test practice and a stringent set of performance guidelines will help molecular test developers and end users work together in designing an assay that meets the needs of the laboratory. Although extensive training and expensive machinery will be required, the use of molecular techniques in a routine laboratory will result in more rapid testing, better pathogen detection, source-attribution of contamination and better risk management of our water sources.

CONSIDERATIONS FOR TEST DEVELOPERS AND END USERS

Validation data provided in peer-reviewed publications often provide insufficient evidence for test adoption, in part because there are no established guidelines for validation.

The rigor of the peer review process ensures the publication of scientifically sound research. However, it does not ensure that new test methods stand up to the rigor needed for implementation of molecular tests for water testing. Often the data presented are the best-case scenario, or may be specific to a given water matrix.

Standardized validation guidelines are needed to robustly evaluate new test methods.

Given the range in the quality of validation data in the peer-reviewed literature, there is a clear need to standardize not only which test validation parameters are evaluated for new tests, but also how the data is collected and analyzed. The validation guidelines developed in this study will aid in this standardized validation.

The evidence generated by validation guidelines is needed by end users to feel confident that the test can perform as promised.

Consultation with stakeholders in a related project, *Applied Metagenomics of the Watershed Microbiome* (www.watersheddiscovery.ca), emphasized the importance of evidence in showing that a test is equal to or performs better than the current method. Appropriate communication on what a test can and cannot do — and how to interpret the test results — is required.

Validation data can bridge the gap between developers and end users by allowing developers to rigorously test their newly developed assay against strict criteria, as well as demonstrating to end users that the test will meet their needs.

While molecular water quality tests have come a long way, they are still not perfect, and ongoing research is needed to develop better molecular water quality tests.

Most of the assays performed more poorly than described in the literature. This reflects how dynamic and complex water samples truly are. Molecular techniques often do not work as well when there are components mixed in with the nucleic acids, which is commonplace in environmental samples.

The DNA extraction methods commonly used in water testing are likely too inefficient for recovery of nucleic acids from the sample, thus impacting the limit of detection in real world samples compared to the laboratory.

Research is still needed to better understand the dynamic nature of water matrices to optimize sampling protocols, as well as improvements to sample processing to improve not only the limit of detection, but also the workflow for testing.

TO CONTACT THE RESEARCHER, EMAIL RESEARCHSPOTLIGHT@CWN-RCE.CA
VISIT OUR REPORT LIBRARY AT WWW.CWN-RCE.CA

REPORT AUTHORED BY MATTHEW CROXEN, NATALIE PRYSTAJECKY AND JUDITH ISAAC-RENTON

RESEARCH TEAM – UNIVERSITY OF BRITISH COLUMBIA; BRITISH COLUMBIA PUBLIC HEALTH MICROBIOLOGY AND REFERENCE LABORATORY

JUDITH ISAAC-RENTON
NATALIE PRYSTAJECKY
CHRISTINA COOK
MATTHEW CROXEN
BRIDGET LEE
JUSTIN DIRK
PATRICK TANG

PARTNERS

BRITISH COLUMBIA PUBLIC HEALTH MICROBIOLOGY AND REFERENCE LABORATORY
ENVIRONMENT CANADA
PUBLIC HEALTH AGENCY OF ONTARIO
ÉCOLE POLYTECHNIQUE DE MONTRÉAL

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