End user perspectives on using qPCR and next-generation sequencing in the field

Kyle Parker[1], Jonathan Forman[2], George Bonheyo[2], Brittany Knight[1], Rachel Bartholomew[2], Rich Ozanich[2], Kenneth B. Yeh[1]

[1]MRIGlobal - 425 Dr. Martin Luther King Jr. Blvd., Kansas City, MO. 64110; [2] Pacific Northwest National Laboratory - 902 Battelle Blvd, Richland, WA 99354

Abstract

Quantitative real-time PCR and genomic sequencing have become mainstays for performing molecular detection of biological threat agents in the field. There are notional assessments of the benefits, disadvantages, and challenges that each of these technologies offers according to findings in the literature. However, direct comparison between these two technologies in the context of field-forward operations is lacking. Most market surveys, whether published in print form or provided online, are directed to product manufacturers who can address their respective specifications and operations. One method for comparing these technologies is surveying end-users who are best suited for discussing operational capabilities, as they have hands-on experience with state-of-the-art molecular detection platforms and protocols. These end-users include operators in military defense and first response, as well as various research scientists in the public sector such as government and service laboratories, private sector, and civil society such as academia and nonprofit organizations performing method development and executing these protocols in the field. Our objective was to initiate a survey specific to end-users and their feedback. We developed a questionnaire that asked respondents to 1) determine what technologies they currently use, 2) identify the settings where the technologies are used, whether lab-based or field-forward, and 3) rate the technologies according to a set list of criteria. Of particular interest are assessments of sensitivity, specificity, reproducibility, scalability, portability, and discovery power. Our findings summarize end user perspectives and highlight technical and operational challenges.

Introduction

Previously, we discussed a notional qualitative comparison of qPCR and sequencing technologies, based on literature searches (Table 1). From these references and earlier panel feedback[1], we derived evaluation criteria and definitions (Table 2).

Table 1. List of field and first responder biodetection marketing references
Including print and electronic formats available online. Citations counted from 11 September 2021.

Title, (Year Published), and [Reference Number]	Google Scholar Citations (11 September 2021)
An Introduction to Biological Agent Detection Equipment for First Responders (2001) [24]	30
Biological Detectors Market Survey (2007) [25]	4
Chemical, Biological, Radiological Technology Survey (2011) [26]	7
Edgewood Biosensors Test Bed Handheld and Man-portable edition (2013) [27]	1
WMD Detector Selector (2015) [28]	Website only, not available
CBRNE Tech Index (2015) [29]	Website only, not available
Biodetection Technologies for First Responders (2015) [30]	9
Recommendations on the use of diagnostics devices in far-forward military operations (2016) [31]	1
Global CBRN Detector Market Survey (2017) [32]	2

Table 2. Evaluation criteria and definitions

Criteria	Definition
Ease of Use	The ability to be used by operators with limited training.
Time to Results	The ability to quickly produce actionable results.
Sensitivity	Analytical sensitivity; ability to measure a low number of copies, genomic equivalents, etc.
Specificity	Analytical specificity; ability to detect a particular target. Sequencing accuracy
Reproducibility	Ability to generate similar results consistently across sequential runs.
Portability	Ability to move instrument from one location to another without impacting instrument integrity.
Ruggedness	Ability of instrumentation to withstand significant movement, vibrations, environmental impacts.
Discovery Power	Ability to detect novel variants, unknown targets
Scalability	The number of samples able to be processed simultaneously, low (1-8) to high (96 or greater).
Low cost per test	The cost to process a sample, inclusive of reagents.

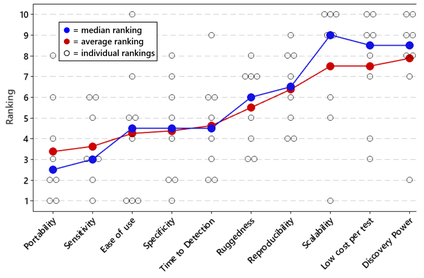
[1] Yeh KB, et al. 2019. Molecular detection of biological agents in the field: then and now. mSphere 4:e00695-19.https://doi.org/10.1128/mSphere.00695-19.

Material and Methods

We sought feedback from end-users on their actual experience with qPCR and sequencing technologies, both in the field and in laboratory settings. We designed a survey that asked respondents to evaluate the technologies using the criteria listed in Table 2. Candidate respondents were solicited by email from the author's network of contacts, seeking input from end-users with a diversity of experience and backgrounds. The survey results were compiled and analyzed to determine trends amongst the responses.

Field-Forward Priorities

As part of our survey, respondents were asked to rank the importance of the 10 performance metrics for field-forward applications of qPCR and sequencing (Figures 1 and 2). According to both median and average ranking, **portability** was the highest ranked metric for both qPCR and sequencing applications. After that, **ease of use** and **time to detection** were the next most important metrics for sequencing. For qPCR, **sensitivity** was the second most important metric, followed by **ease of use**, **specificity**, and **time to detection**. These three metrics all had similar median and average rankings.



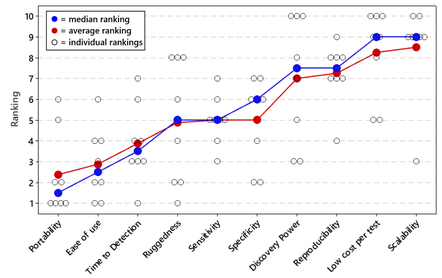


Figure 1 and 2: Priorities for Field-Forward qPCR (left) and Sequencing (right) Applications. Results are based on responses from n= 14 respondents.

Next, respondents were asked to provide feedback on the overall challenges of performing qPCR and sequencing in field-forward settings. First, we asked the respondents to rank common issues with field-forward applications to determine the most common challenges that need to be addressed (Figure 3). Access to power supply, ease of sample preparation, and the availability of ambient-stable reagents were found to be the most important and had similar average and median rankings.

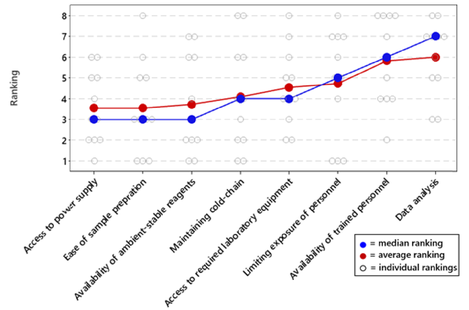
Respondents were also asked to specifically address data analysis challenges in the field (Figure 4). Here, the challenges were all evenly ranked, with computing power reliable power supply and access to the internet all having the same median ranking. Access to reference databases had a significantly lower median ranking.

Contact Information

Kyle Parker
T: 816-326-5932
E: kparker@mriglobal.org

MRIGlobal
425 Volker Blvd.
Kansas City, MO 64110

Field-Forward Challenges



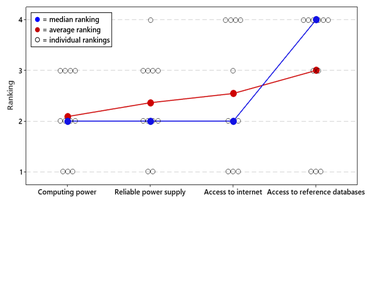


Figure 3 and 4: Challenges for Field-Forward Biodetection Applications (left) and Challenges for Field-Forward Biodetection Analysis (right)

Results

The survey results confirmed that qPCR and sequencing applications have different benefits and challenges in the field for biosurveillance applications. While qPCR methods are typically lower-cost and provide rapid turnaround detection with high sensitivity, sequencing methods are more reliable for discovery power. Both field-forward and portable qPCR and sequencing platforms show a shift toward increasing **ease of use**, **portability**, **ruggedness**, and **time to results**. On the other hand, both technologies show a decrease in **sensitivity** and **reproducibility** when used in the field compared use in the laboratory.

- For field-forward applications, both **portability** and **ease of use** were determined to be amongst the top three priorities for both qPCR and sequencing technologies. It is noteworthy that **sensitivity** was ranked in the top three for qPCR, whereas **turnaround time** was similarly ranked for sequencing. Surprisingly, **specificity** was not listed as a top priority for field-forward sequencing (ranked as number 5 with sensitivity). **Specificity** is a key concern for the use and interpretation of sequencing data, but the lower ranking may simply reflect an acknowledgement that field-forward sequencing is an emerging capability and a willingness of researchers to work within existing limitations and to sacrifice specificity for other features.

- Continuing challenges besides having a reliable power supply, the top four were all related to sample preparation in the field. The ability to quickly and accurately prepare samples for analysis is an important topic in the conversation of field-forward biodetection methods.

Conclusions

Survey findings reinforce presumptive published performance metrics of qPCR and sequencing technologies with feedback from current end-users.

- Currently, qPCR and NGS methods are complementary and interdependent-there are a limited number of field-forward-capable next-generation sequencing options.
- Findings also provide guidance for future studies on what sample preparation factors to consider when developing field-forward applications and the important challenges to overcome.

Ideally, these technologies would increase ease of use and minimize traditional laboratory equipment or infrastructure needs while maintaining high-performance metrics observed with qPCR and sequencing instruments. As new applications become available and adopted in the field, future assessments should consider these methods while inviting larger audience of end-users to participate.