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New Approaches for Virus Detection through Multidisciplinary Partnerships

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ABSTRACT: A critical requirement for controlling outbreaks of viral infection is sensitive and accurate diagnostics, which can be expensive and are frequently located in resource-intensive clinical laboratories. Outbreaks of many viral infections occur in countries where healthcare resources are limited and clinical laboratories scarce. This creates a fulfillment gap, one that could be filled through the development of inexpensive, sensitive, easy to use, and portable diagnostics. Here we describe our efforts to develop a diagnostic technology that detects viruses without needing to label the particle directly. Our approach has the advantage of speed and assay simplicity while maintaining high sensitivity. Essential in this approach has been the assembly of an integrated, diverse, and interdisciplinary team that worked together to evaluate technologies, spin-out a company, and produce a product for infectious disease diagnostics. The synergy of different individuals with complementary skills has been critical for the development of our transformative technology.

THE UNSOLVED CHALLENGE OF INFECTIOUS DISEASE DIAGNOSIS

As the Ebola epidemic ripped through West Africa in 2014, it became clear that a major early challenge in the outbreak was effectively diagnosing infection. The outbreak occurred in a region where multiple infectious agents circulate, meaning that someone presenting with flu-like symptoms could be infected with any of a variety of pathogens. Initial symptoms following infection with many of these diseases (e.g., Ebola and Lassa) are nonspecific, making definitive diagnostic tests a critical part of any effective response strategy.

A challenge of implementing definitive ebolavirus diagnostic tests during the 2014 outbreak was that the few existing Ebola virus diagnostics were difficult to implement in Liberia, Sierra Leone, and Guinea. PCR, which became the most implemented assay in the outbreak, required centralized facilities, sample handling containment, and lots of electrical power. These were largely absent in the affected countries. Significant effort from many nations combined to build new diagnostic facilities, fly/drive/carry the necessary reagents, and provide experts to run the critical assays. In the end this approach was successful, but the tremendous cost and effort expended underscore the need for a better approach. Regions where virus outbreaks occur often lack the resources and expertise for PCR-based diagnostics, so a diagnostic that is not only simple to use but reduces potential exposure to high-consequence pathogens to the technician is essential.

There is not yet a single diagnostic approach that addresses all diagnostic needs. Nucleic acid based diagnostics offer incredible sensitivity and specificity but portability, instrumentation, and usage expense are challenges. ELISAs offer high sensitivity at low expense and high throughput but are often restricted to clinical laboratories with trained personnel. Lateral flow tests excel at the point of care, where ease of use dominates, but have often been cited for sensitivity issues.[1] Thus, although there are many tools available, each has drawbacks that prevent it from meeting all needs.

THE DIAGNOSTICS DEVELOPMENT CHALLENGE

The limitations of current diagnostic technologies argue strongly that new technologies be investigated for their abilities to provide high-sensitivity diagnosis at low cost and with ease of use and point-of-care applicability[2−4] We have approached this problem by investigating how different optical detection technologies might improve the diagnosis of Ebola, Marburg, and Lassa viruses through the development of a multicenter partnership involving Boston University (BU), Becton-Dickinson (BD), and University of Texas Medical Branch (UTMB).

To do this, we asked the following question: Can we use the size of an intact virus particle as an asset? Most clinical diagnostic approaches destroy an intact virus particle so that internal components such as genomes can be identified. Although this approach has many advantages, it also destroys information about intact virion shape and size, which can be important and unique features. We also reasoned that the lysis and identification strategy adds processing steps, including the need to amplify signals through a labeling process. We sought

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to avoid these problems by directly identifying intact virus particles while they were in complex solutions such as serum.

The goal of our investigation was to use label-free virus identification to create a diagnostic platform that provided ease of use and sensitivity. On the basis of the knowledge that PCR-based diagnostic assays often take 3−4 h to complete from start to finish, we sought to develop an assay capable of moving from sample to answer in approximately 30 min. We reasoned that this time frame would allow diagnosis within a relatively short wait period, thereby speeding any necessary treatment. A core principle of the development was that the assay should be simple to use, and so we sought a technology that would work in serum without needing significant follow-on sample preparation or handling. We further sought to develop an end product made of inexpensive materials and use simple workflows that would enable tests to be done inexpensively at the point of need.

■ ASSEMBLY OF TEAM AND TECHNOLOGIES

Meeting our goals required the assembly of a multidisciplinary team. This included virologists able to contribute knowledge regarding how to target/capture virus-specific material, to provide an appropriate biosafety-level graded development path, and to identify handling and containment issues. The team also included industry partners with knowledge of assay development and manufacturing constraints. The team was completed with the addition of engineers from various disciplines who were able to help test and develop different promising detection technologies.

Once the team was assembled, we began by evaluating two different technologies, plasmonic nanohole array (PNA) optical transmission and interferometric reflectance imaging sensor (IRIS). Both technologies offered the prospect of label-free detection of virus particles and could function in an assay format like a one-step ELISA. Outside these similarities, there were a number of implementation differences. PNA offered sufficient sensitivity for relatively easy-to-use in-liquid assay in the target sample-to-answer time of 30 min,[5,6] but reproducibility and sensor surface functionalization were challenges to sensitivity. IRIS as an imaging technique allowed for visualization of virions captured on an inexpensive sensor with robust surface functionalization acquiring information about each individual intact virus and thus meeting sensitivity and specificity requirements.[7] However, IRIS was more difficult to use and did not initially work in liquid, and the assay took longer to run. Both technologies required significant development, and it was unclear at an early stage which would be the most readily adaptable for virus detection. The results of the head-to-head comparison are visually summarized in Table 1.

Accordingly, the two technologies were vetted in a rigorous head-to-head comparison. After 1.5 years of development efforts, the IRIS technology was identified as the most promising technology, benefiting from a well-developed surface chemistry and reproducibility record; IRIS technology also enabled imaging of virions, which was deemed an important asset. This led to a coalescence of effort around the IRIS technology to address the remaining weaknesses, including an inability to be used in liquid and a significant assay time.

Together the team worked to transition the open-to-air IRIS technology[7] to one that functions in plasma and serum within a closed, biocontained cartridge.[8] This transition is simple to conceptualize but was highly difficult to implement. It required input from virology, optics, and microfluidics, while coordinating critical input from industry partners. This multidiscipline input continues as stepwise improvements are integrated, with a constant eye on the development of simpler sample-to-answer functionality with minimal sample processing and operator handling. All groups have to participate heavily, with repeated strategic changes that included abandoning some aspects of microfluidic sample handling approaches because of assay biocontainment constraints. This participation has followed the product development cycle loosely illustrated in Figure 1, which highlights that there are many steps along the road to the development of a diagnostic technology.

Table 1. Estimation of Strengths and Weaknesses of Plasmonic Nanohole Array (PNA) and Interferometric (IRIS)-Based Virus Detection Systems Following Early-Stage Development[a]

category	PNA	IRIS
ease of use	++	−
cost of manufacturing	−	+
30 min sample to answer	++	−
in-liquid testing	+	−
sensitivity	++	++
specificity	−	+
reproducibility	−	+
surface chemistry/antibody attachment	−	++

[a]Plus signs indicate relative strengths; minus signs illustrate liabilities at the time of testing.

The advances we have made in this project have also led to important expansions in the partnership. The technology advancements spurred by our project have spawned a spin-out company dedicated to the development of interference technologies for nanoparticle detection. The addition of an entrepreneurial component to the project has been a strong positive, enabling the development of assay readers that create an automated workflow that can easily integrate into electronic surveillance and reporting systems.

Work within this project has also opened new opportunities for use of the technology. The IRIS-based imaging platform not only acts to provide simple ELISA-type readouts, it is at its core a microscopy technology, so pictures of captured virus particles are also obtained. These pictures allow the automated and digital discrete counting of virus particles and the recognition of unique virus characteristics. The most obvious use of this comes through our analysis of Ebola and Marburg virus assays. Using IRIS, not only do you get a virus-capture signal, you also obtain an image that can show the filamentous shape of the virus. This is a very strong orthogonal confirmation of infection.

■ LESSONS LEARNED

As the project has moved from initial proof of principle (i.e., benchtop) demonstration to alpha prototype testing to instrumentation construction and advanced prototype validation in a high-containment facility, a number of hurdles have been traversed. As we transitioned through different stages in a product life cycle, an integrated team and cooperative input were requirements that cannot be understated. The synergy of different individuals with complementary skills makes it possible to generate transformative technology.

Of critical importance in such a multidisciplinary team is maintaining alignment with core objectives of the program. Gate reviews, tasks, and milestone updates are critical to assuring successful completion of the program by the end date

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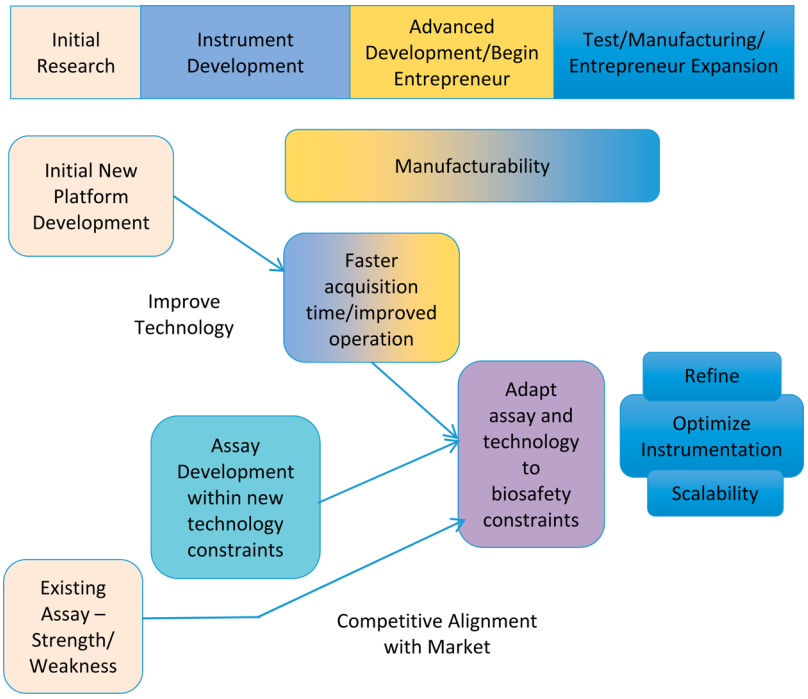

Figure 1. Flow chart paralleling a standard commercial product development program where an initial proof-of-concept is demonstrated during initial research phase and then identification of strengths and weaknesses of the technology factor in to next-life cycle processes of instrument development to advanced development, testing, and exploration of manufacturing and scalability.

of the award. At 6-month intervals, the original proposed milestones are revisited in assurance of delivery of the proposed instrument and what the final end customer required for advancement of technology in the diagnostics field. In addition, each year, the team reviews technology advancement and makes difficult decisions of advances that are in alignment with the product being developed versus the science or engineering. Those that do not align with the tasks and milestones are tabled for future programs with the focus of the team being a deliverable high-biocontainment laboratory where handling in multilayer personal protective equipment (PPE) is not often the first thought of the engineer or scientist.

CONCLUDING THOUGHTS

Our approach to developing a new path to viral diagnosis required a multidisciplinary team. The team was grounded within a university development setting with a goal of moving through research and development and into technology transfer/translation and commercialization. This was a level of product development that required proof of concept, validation studies, and reproducibility in a short time frame and was only viable because of a tightly integrated team that included industry experience, entrepreneurial components, and basic research moving together. This required and continues to require constant communication and oversight to remain on task. This type of integrated partnership is an important experience for training both current and future innovators. Many discoveries are made at the basic research level and then languish because there is no integrated team to help move a smart concept through a development process that requires expertise in different disciplines. The partnership approach helps bridge this divide. Each individual in the team in these types of projects develops a basic understanding of the complexity of transferring idea to product, making overall progress to success faster.

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Notes

The authors declare no competing financial interest.

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