FROM BENCH TO PATIENT
TACKLING HURDLES TO IMPLEMENTING NGS-BASED AGNOSTIC PATHOGEN DETECTION

Messages from the symposium on "Tackling Hurdles to Implementing NGS-based Agnostic Pathogen Detection"

Symposium website hosted by DRIVe: https://drive.hhs.gov/ngs_sym.html

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Summary

On January 19-20, 2023, the Biomedical Advanced Research and Development Authority's (BARDA) Division of Research Innovation and Ventures (DRIVe), within the Administration for Strategic Preparedness and Response (ASPR) under the U.S. Department of Health and Human Services, co-sponsored an in-person summit on the clinical implementation of metagenomic next-generation sequencing (mNGS) in collaboration with the Chan Zuckerberg (CZ) Biohub. More than 90 people representing 40 leading industry, non-profit, academic, clinical, investment, and government (Centers for Disease Control and Prevention, National Institutes of Health (NIH), National Institute of Standards and Technology, Defense Threat Reduction Agency, and the Joint Program Executive Office for Chemical, Biological, Radiological and Nuclear Defense) organizations attended this invitation-only meeting. The objectives of the meeting were to identify barriers to

the widespread clinical use of this agnostic diagnostic technology and agree on a plan of action to address those challenges. Discussions among key opinion leaders using mNGS for diagnosing infectious diseases in the U.S. and developing nations validated sequencing technology's strength and utility as an agnostic diagnostic, with clear opportunities to benefit individuals and populations from a pandemic preparedness standpoint. Meeting participants agreed that additional regulatory guidance is needed to enable further clinical development of mNGS technology, among other technical and financial challenges in hardware, software, reagents, and assay development. They agreed that closer collaboration, knowledge sharing, and engagement with the U.S. Food and Drug Administration (FDA) would be critical to the further development of mNGS technology and applicable regulatory guidance. DRIVe catalyzed BARDA's investment in mNGS-based agnostic diagnostics for pandemic preparedness in 2022 with five partners funded through an Easy Broad Agency Announcement (EZ-BAA) program. BARDA's Detection, Diagnostics and Device Infrastructure Division is continuing its leadership in the field with a Broad Agency Announcement (BAA) program designed to take such technologies through commercialization and regulatory clearance.

Need for mNGS-based Agnostic Diagnostics

The future of pathogen detection and disease diagnosis lies in using mNGS technology. mNGS allows for the comprehensive detection of all microorganisms, including bacteria, viruses, fungi, and parasites, in a single test. This technology has revolutionized the field of infectious disease diagnostics by overcoming the limitations of traditional molecular diagnostic assays, which rely on prior knowledge of the pathogen genome. Moreover, the COVID-19 pandemic highlighted the importance of an agnostic diagnostic capability, like mNGS technology, especially for use in a pandemic situation when targeted molecular diagnostics have not been developed and a novel pathogen can only be detected by unbiased sequencing. Furthermore, mNGS as a pathogen-agnostic diagnostic has already demonstrated clinical utility at CLIA-certified labs, including the University of California, San Francisco (UCSF), where cerebrospinal fluid samples are sequenced and analyzed through mNGS, uncovering new and unexpected pathogens in complex in-patient neurology cases. Laboratories like UCSF have received breakthrough designation device from the FDA and continue to work with the FDA to develop industry guidance. Ultimately, mNGS has the potential to not only detect pathogens but also provide a glimpse into host response, including signatures that may indicate malignancies or autoimmune disorders. Although barriers like cost, assay complexity, and bioinformatic challenges exist, there is also a potential for mNGS to move from centralized laboratories to point-of-care settings. To work towards this vision, a conference was organized to address the challenges of commercializing and regulating mNGS technology. Attendees from academia, industry, and government came together to brainstorm solutions and move towards realizing this future. The following section summarizes the talks and panel discussions at the conference.

"Health Security through Agnostic Diagnostics" by Sandeep Patel, PhD; Director, HHS/ASPR/BARDA/DRIVe

Sandeep Patel, Director of BARDA DRIVe, discussed the need for developing agnostic diagnostics to improve health security in the U.S. Specifically, developing scalable technology for public health preparedness requires collaboration between government, academia, and industry to create useful tools for preventing massive outbreaks and staying ahead of the curve. The COVID-19 pandemic has highlighted the need to overcome four

main challenges: preparing for the unknown, proving and scaling technology, ensuring commercial viability, and demonstrating market acceptance, usability, and impact. The core of the DRIVe program has been tackling these challenges through early innovation into the development of medical countermeasures. This pathogen-agnostic diagnostic initiative is focused on de-risking the use of NGS technology in the clinical field via the development of clinically relevant assays, technology for short- and long-read sequencers, and clear regulatory strategies toward commercialization. By addressing these challenges, we have the potential to create tools that provide results within 24 hours, have sensitivity like molecular diagnostic assays, and have a clear pathway to market. This collaborative effort is essential to staying ahead of future outbreaks and ensuring public health preparedness.

“Technology to Clinic: Using mNGS in a Clinical Setting” by Michael Wilson, MD; UCSF

Dr. Michael Wilson studies several conditions related to brain and spinal cord inflammation, such as meningitis, encephalitis, and myelitis. It is estimated that encephalitis affects about 20,000 people annually in the U.S. costing $2 billion and causing a 10% mortality rate, with survivors often experiencing long-term neurological disabilities (Hansen et al., 2020). Additionally, over 50% of recent cases are undiagnosed, and many cases of encephalitis cannot be explained by the pathogens tested for, as many autoimmune conditions can also cause encephalitis, and prevalence and incidence are similar between autoimmune and infectious causes. Although encephalitis is not uncommon, the individual causes of encephalitis are uncommon and challenging to diagnose. Dr. Wilson also helped to identify key roles that mNGS can fill including the discovery of new pathogens that were highly divergent, the identification of pathogens not previously linked with a clinical phenotype or were rare and not on the differential, or the ability to rule out infection in suspected autoimmune cases. His research has demonstrated that mNGS can identify rare and unusual pathogens, ranging from bacteria, amoebas, and fungi. Limitations of mNGS were also discussed including insensitivity to detecting pathogens that are compartmentalized (i.e., in cysts), serologically diagnosed, or have very low abundance in the cerebrospinal fluid. Finally, Dr. Wilson discussed the potential for directed host immune modulators to treat infectious disease causes of encephalitis, where host response can be more damaging than the microbe, and the opportunity for qPCR assay development leveraging signatures discovered from sequencing.

Session 1 | Promise and Impact of Metagenomics

Metagenomics has revolutionized the field of microbiology by enabling the analysis of entire microbial communities rather than just individual organisms. This approach has unlocked a wealth of information about the diversity and function of microorganisms in diverse ecosystems, from soil to human gut. Metagenomics has also shown promise in clinical applications, such as identifying pathogens causing infections, tracking the spread of antibiotic resistance, and exploring the complex interactions between the microbiome and human health. As technology advances and costs decrease, the promise and impact of metagenomics continue to grow, with potential applications in fields ranging from environmental science to personalized medicine.

"Scaling metagenomics for febrile illness in Cambodia" by Christina Yek, MD; National Institute of Allergy and Infectious Disease (NIAID)

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Dr. Christina Yek, representing the International Center for Excellence in Research (ICER) Cambodia of the U.S. NIAID, led studies scaling metagenomics to diagnose febrile illness in Cambodia. In 2019, a historic outbreak of the Dengue virus occurred in Cambodia. Using mNGS, the ICER team discovered that the outbreak consisted of a mixture of DENV1 and DENV2 serotypes, with the latter represented by the Cosmopolitan genotype (Brook et al., 2022). This genotype of DENV2 was highly divergent from previously circulating strains in Cambodia, and modeling suggested that its increased pathogenicity in a relatively immune-naïve population was a pivotal contribution to the unprecedented epidemic. The ICER team also used mNGS to reveal a diverse pathogen landscape, including pathogens unrecognized by available clinical and surveillance laboratory tests, such as Zika virus, Chikungunya virus, and *Plasmodium knowlesi* (Yek et al., 2022). These discoveries have informed national surveillance strategies for malaria elimination and vector control policies. In 2020, the ICER team partnered with the Cambodia Ministry of Health to develop SARS-CoV-2 genomic surveillance capability and sequence the index case of viral infection in the country. The Cambodia Ministry of Health now has a trained workforce for both wet lab protocols and dry lab pipelines; work is ongoing to extend this capacity to national surveillance of antimicrobial resistance (AMR). As part of a pilot study to map the unknown landscape of AMR genes across Cambodia, local hospitals have contributed over 1,000 bacterial blood culture isolates for whole genome sequencing. The ICER team has sequenced >700 isolates to date, with several notable preliminary findings: i) approximately 20% of isolates are misidentified by local microbiology labs, complicating accurate reporting, ii) rates of *in silico* resistance to first-line antibiotics are high and may be underestimated by traditional antibiotic susceptibility testing due to lacking reagents and inconsistent reporting. There continue to be obstacles to scaling metagenomics in low- and middle-income countries like Cambodia including poor sample collection, supply chain issues, and instrument maintenance, among others. Nonetheless, work at ICER Cambodia highlights the feasibility of targeted, centralized metagenomics to detect, characterize, and monitor infectious disease threats at the frontlines of their emergence.

“Unbiased RNA metagenomic sequencing to shed light on the dark side of meningitis in Bangladesh” by Senjuti Saha, PhD; Child Health Research Foundation (CHRF)

Dr. Senjuti Saha and her team led a meningitis surveillance network in Bangladesh for the pediatric population, serving over 23,000 hospitalized children at a time. Over the past two decades, CHRF added several diagnostic and surveillance methods to detect the etiology of meningitis, but despite their efforts, 80% of all cases of suspected infectious meningitis remained of unknown etiology. Beginning in 2018, Dr. Saha and her team began utilizing metagenomics to perform a retrospective study of samples from a previous meningitis outbreak. During this study, they discovered that CHIKV can cause meningitis, a previously unknown etiology for meningitis as it was not known that CHIKV can infect the brain (Saha, et al., 2019). With this knowledge, the team designed a qPCR assay to detect CHIKV in hundreds of cerebrospinal fluid (CSF) samples demonstrating that neuroinvasive CHIKV was responsible for this outbreak the year before and equipping local hospitals with this assay to inform treatment and care for future cases of meningitis. Through sequencing of other idiopathic meningitis samples, the team also encountered PARV4 in a significant number of these samples though only a little was known about this virus. After identifying PARV4 as a possible pathogen of interest, Dr. Saha and her team developed qPCR assay to understand the prevalence of PARV4 in meningitis cases in Bangladesh. After analysis of more than 2,000 CSF samples, the team found that 21% of

the samples were PARV4-positive. After ruling out that PARV4 was not a contaminant, the team conducted a genomic epidemiology study of PARV4 circulating in Bangladesh and found that it is most common in very young (<5 weeks old) children. They also found that 20% of cases have co-infections with other pathogens. A lot more work remains to be done. Still, the team believes that unbiased mNGS is vital for building a pathogen atlas and designing new diagnostics with significant public policy and public health benefits. However, they also noted that it is not a routine clinical test in the foreseeable future in low-to-middle-income countries.

"Clinical metagenomics for enhanced surveillance and diagnosis of infections in New South Wales, Australia" by Ki Wook Kim, PhD; University of New South Wales

Dr. Ki Wook Kim, a Juvenile Diabetes Research Foundation (JDRF) Career Development Fellow at the University of New South Wales, based within the Virology Research Laboratory at the Prince of Wales Hospital, leads groups that leverage the power of metagenomic sequencing for human virome research and clinical diagnostics. Dr. Kim discussed a wide array of metagenomics use cases, including characterization of the longitudinal virome from pregnancy to childhood in a nationwide Australian prospective cohort study (1,500 mother-infant pairs) investigating the contribution of viral infections on the development of type 1 diabetes, surveillance of co-infections among SARS-CoV-2 cases and investigating disease etiology at clinician request. Dr. Kim and team use a variety of tools and techniques such as hybridization capture, metatranscriptomics, amplicon-whole genome sequencing, and phage immunoprecipitation sequencing. To illustrate the power of metagenomic sequencing for clinical diagnostics in Australia, Dr. Kim discussed a suspected case of Japanese encephalitis which was unexpectedly caused by a novel virus most closely related to pigeon paramyxovirus 1, an avian virus that causes Newcastle Disease (Stelzer-Braid, et al., 2022). Dr. Kim also discussed an unexpected case of dengue virus encephalitis with suspected neurotropism in a patient whose last known exposure was many years prior to the diagnosis. Sequencing plays an important role in national biosurveillance in Australia, sampling sources such as wastewater from municipal and aircraft for SARS-CoV-2 and agriculture/animal reservoirs. In addition, Dr. Kim discussed the state of clinical metagenomics in Australian healthcare, highlighting the potential for broad clinical impact and the barriers to implementation such as a lack of framework for clinical metagenomics, status as a research-use only test, lack of standardization in reporting, and lack of public funding through national healthcare. Overall, Dr. Kim's research underscores the importance of studying the virome and developing better diagnostic tools for infectious diseases.

"Integrating host response and unbiased microbe detection for enhanced diagnosis of pneumonia and sepsis" by Charles Langelier, MD, PhD; UCSF

Dr. Charles Langelier from UCSF studies the dynamic relationship between host and microbes in various diseases, particularly respiratory infections and sepsis. Sepsis, an increasing problem worsened by COVID-19, is challenging to diagnose because over 30% of sepsis cases have no identified pathogen, and non-infectious systemic inflammatory conditions mimic sepsis. This leads to misdiagnosis, inappropriate antibiotic use, and other adverse events. To address this problem, Langelier and his team developed an integrated host-microbe sepsis diagnostic model that combines transcriptional profiling and metagenomic pathogen detection from plasma (Kalantar, et al., 2022). They found that detecting a pathogen alone was often insufficient for sepsis diagnosis, but when combined with a host transcriptional profile, it achieved high accuracy for both sepsis

diagnosis and identifying the probable etiologic pathogen. Langelier noted that the key next steps before deployment in clinical settings are independent validation of findings and a clinical trial.

"The Free, Cloud-Based Metagenomics Platform for Researchers" by Katrina Kalantar, PhD; Chan Zuckerberg Biohub

Dr. Katrina Kalantar discussed CZ ID, a cloud-based bioinformatics pipeline powered by AWS that provides an easy-to-use platform for the analysis of mNGS data. With CZ ID, user-uploaded fastq files are securely stored on S3 and analyses are run automatically in the cloud. Results are accessible through the web application as well as flexible download options. CZ ID enables the characterization of all aspects of infection, including the host, pathogen, microbiome, and AMR profiles through unbiased mNGS analysis. Three case studies demonstrated the application of CZ ID for the identification of unexpected viruses in neonates (using CSF, lung, and blood samples), diagnosis of sepsis in critically ill patients from blood (leveraging pathogen-detection and host transcriptome), and identification of AMR genes in clinical samples. Data analysis remains a major challenge associated with mNGS, and CZ ID provides a solution to this challenge.

Session 2 | Advancing Clinical mNGS for Public Health Preparedness

Advancing clinical mNGS for public health preparedness is critical to enable rapid and accurate identification of novel infectious pathogens for effective outbreak control and clinical management of infected patients. In recent years, mNGS has emerged as a powerful tool for pathogen detection and identification, offering an unbiased and comprehensive analysis of microbial communities within clinical specimens. As the field of mNGS continues to evolve, there is a growing need to translate this technology into clinical practice, where it can be leveraged for rapid and accurate diagnosis of infectious diseases, identification of antimicrobial resistance, and outbreak surveillance. The following section discusses recent advances in clinical mNGS, its potential impact on public health preparedness, and the challenges that must be overcome to ensure widespread adoption and effective use in clinical settings.

"An NGS-based diagnostic test to identify any and every pathogen from a single sample" by Dev Mittar, PhD; BARDA DRIVe

Dr. Dev Mittar presented BARDA's vision about how mNGS can be a valuable tool for future pandemic preparation by emphasizing how an approved pathogen agnostic test can be immensely useful in case of a future outbreak with a novel pathogen. When regulatory guidance is issued and mNGS assays can seek FDA approval or clearance, mNGS can not only be implemented immediately, but can also generate valuable data early in a potential pandemic, which can inform the development and validation of medical countermeasures, enable monitoring the spread of the novel pathogen, and support the public health response. DRIVe catalyzed BARDA's investment in mNGS-based agnostic diagnostics for pandemic preparedness in 2022 by funding five partners through an EZ-BAA program. Additionally, BARDA’s Detection, Diagnostics, and Device Infrastructure Division is continuing its leadership in the field with a BAA program designed to take such technologies through regulatory approval or clearance.

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"CRISPR-powered NGS-based agnostic diagnostics" by Keith Brown; Jumpcode Genomics

Keith Brown discussed the importance of removing uninformative molecules from sequencing libraries prior to sequencing to increase the prevalence of the target. Low sample input with high human nucleic acid background is like finding a needle in the haystack, which is the reason for the low sensitivity and higher cost of mNGS assays. He introduced CRISPRClean, which harnesses the specificity of CRISPR-Cas9 to deplete abundant sequences, cut through the noise of uninformative transcripts, and boost discovery. This CRISPR-Cas9 system was able to remove non-target abundant sequences effectively. It demonstrated the sensitivity of mNGS assay equivalent to RT-qPCR for detecting SARS-CoV-2, notably at high Ct values (Chan et al., 2023). The CRISPRClean NGS workflow is pathogen agnostic and can enhance the sensitivity of the assays and reduce the cost of infectious disease sequencing assays.

"Metagenomic sequencing for diagnosis and surveillance of emerging infections" by Charles Chiu, MD, PhD; UCSF

Dr. Charles Chiu, Professor of Laboratory Medicine at UCSF and Director of the UCSF Clinical Microbiology Laboratory, has identified a triad of diseases in hospitalized patients that are challenging to diagnose: pneumonia, fever/sepsis, and neurological syndromes such as meningitis, encephalitis, and myelitis. He developed an mNGS assay that can detect a wide spectrum of pathogens, including viruses, bacteria, fungi, and parasites, and is thus more efficient than traditional microbiologic testing methods, which rely on a targeted "fishing pole" approach to find pathogens - where pathogens are detected in a single-plex manner. Enhancements have been made to the SURPI ("sequence-based ultra-rapid pathogen identification") pathogen detection and discovery pipeline (Miller et al., 2019), including identification of novel, sequence-divergent viruses using automated *de novo* assembly and amino acid-based alignment algorithms and quantification of viral loads using quantified reference controls that are added to extracted nucleic acid samples. In a current *in silico* study, Chiu removed all reference sequences from human outbreak-associated pathogens from the database and tested the ability of the enhanced SURPI pipeline to identify these now "novel" viral pathogens. The pipeline was successful in detecting all viral pathogens. The remaining challenges to overcome with mNGS include turnaround time, accuracy, and clinical interpretation. Chiu's recent work has aimed at directly addressing some of these challenges, including (1) the identification of rabies virus infection in just 15 minutes of sequencing using a pocket-sized MinION nanopore sequencer, (2) detection of respiratory viruses using a clinically validated mNGS test with comparable sensitivity to virus-specific PCR (REF), and (3) development of machine learning based RNA gene classifier models from mNGS data that can discriminate between viral, bacterial, fungal, and parasitic infection as well as non-infectious etiologies of acute illness such as autoimmune disease based on the patient's host response.

"Enabling clinical NGS with tailored analysis and rapid sequencers" by Sam Chorlton, MD; BugSeq Bioinformatics

BugSeq's Dr. Sam Chorlton emphasized the importance of a tailored approach to building analysis pipelines for the clinical setting, as in-house pipelines cannot meet the needs of this setting and may not be maintainable in the long run. Simple, automated, and tailored bioinformatics are needed to adopt NGS in clinical microbiology rapidly. Additionally, he highlighted the need for dedicated resources to curate complex databases and provide high-quality analysis. To offer usable results to clinicians, he emphasized the importance of service-level support for clients and intuitive reporting. Once lab sequenced samples are received, BugSeq detects all

pathogens in the sample, eliminating the need for single-pathogen tests. BuqSeq predicts the antimicrobial resistance of each pathogen in the sample, even in the presence of thousands of background microbes. Then BugSeq characterizes organisms to track their spread in hospitals and communities, all while in a secure cloud network. BugSeq is leading the way to broadly adopted NGS by building a platform dedicated to clinical and public health microbiology.

"SwabSeq agnostic test: Adapting a targeted diagnostic to an agnostic test" by Eleazar Eskin, PhD; University of California Los Angeles (UCLA)

Dr. Eleazar Eskin described how UCLA was adapting the Swab-Seq targeted COVID-19 diagnostic to an agnostic test. SwabSeq is a new diagnostic technology invented in April 2020 that attaches molecular barcodes during PCR amplification which is followed by next generation sequencing (Bloom et al., 2021). In SwabSeq, samples are subjected to reverse transcription (RT) and PCR in 384-well plates with primers that contain unique molecular barcodes, allowing samples to be identified by indexed genomic sequence. Barcoded reaction products are pooled for sequencing, and diagnosis is made by comparing the number of reads matching the virus to the number matching the spike-in control. A key innovation in the technology is using a synthetic spike sequence similar to the amplification target but can be distinguished by the sequencer. A known quantity of the spike sequence is added to each sample, enabling a final readout of the test to be a ratio of SARS-CoV-2 and spike reads. The ratio makes the test quantitative, robust, with high precision and accuracy. Since deployment in November 2020, SwabSeq has performed almost 2,000,000 diagnostic tests for SARS-CoV-2. SwabSeq obtained NIH Rapid Acceleration of Diagnostics (RADx) funding to expand the facility to have a capacity of over 25,000 tests per day. SwabSeq provided testing for multiple organizations including multiple University of California campuses (UCLA, UCSB, UCSC, UCI), the UCLA Health Care workers for their asymptomatic testing program, other universities, the Los Angeles Unified School District and is now partnering with the California Department of Public Health. The SwabSeq targeted workflow is being adapted for the SwabSeq agnostic diagnostic. In the agnostic test, after barcodes are added to the samples, the samples are pooled together and Jumpcode depletion is utilized to reduce the amount of host RNA. The remaining RNA is then sequenced to identify pathogens.

“Impact of Genomics in Low-Middle Income Countries” by Sergio Carmona, MD, PhD; FIND

Dr. Sergio Carmona from FIND, the foundation for innovative diagnostics, broadened the focus of genomics to communicable diseases in low-to-middle-income countries with a primary focus on neglected diseases. The goal was to make genomics accessible and affordable and to promote, implement, collaborate for equitable use and responsible sharing of information obtained with genomic methods through effective oversight and national and international rules and standards in genomics.

“Clinical Implementation of mNGS: Challenges and Opportunities” moderated by Charles Chiu, MD, PhD; UCSF with Panelists: Tim Blauwkamp, PhD, Karius; Matt Binnicker, PhD, Mayo Clinic; and Robert Schlaberg, MD, PhD, Illumina

Dr. Chiu opened the discussion by urging the panel and participants to promote the integration of mNGS into clinical practice. The moderated forum focused on four main themes: the clinical usefulness of mNGS testing,

implementation in clinical laboratories, assay performance and validation, and the availability and dissemination of mNGS testing.

The lack of clinical guidelines for using and interpreting mNGS results was highlighted, despite the presence of review articles and efforts by professional societies and public health agencies. Guidelines would clarify when to use mNGS and help establish reimbursement policies. Standardizing methods and reporting would enhance the acceptance of mNGS studies beyond the mNGS community.

The panel emphasized the importance of diagnostic stewardship and reducing the reliance on mNGS as a last-resort test. Collaborations between clinicians and laboratorians were crucial for improving the diagnostic value of mNGS. Clear and actionable reporting of results within the clinical context could offset the cost of sequencing by improving patient outcomes. Although barriers like cost, assay complexity, and bioinformatic challenges exist, the panel discussed the potential for mNGS to move from centralized laboratories to point-of-care settings.

The context and resources of laboratories performing mNGS assays play a significant role in determining the best implementation approach. Some labs may develop in-house solutions to retain control, while others may prefer commercial off-the-shelf options for streamlined protocols and comparability with external references.

The panel addressed the challenge of assessing mNGS assay performance without a gold standard for comparison. *In silico* challenge datasets, alongside biological reference materials, were suggested as a possible approach. Discordant results could be investigated through precision demonstration, alternative sequencing platforms, or targeted molecular assays.

Regarding the availability and dissemination of mNGS testing, a collaborative effort among public health agencies, industry, and academia was emphasized. Breaking down silos and fostering coordination between stakeholders were crucial for progress and stakeholder buy-in. The panel also discussed making mNGS more accessible and equitable for underserved populations and the importance of technological advancements to reduce logistical burdens and dependency on cold chain and computing power. The developer community was encouraged to share data, experiences, and results to propel the field forward.

“Regulatory Considerations for Clinical mNGS Technologies” by Elliot Cowan, PhD; Partners in Diagnostics

Dr. Elliot Cowan presented an overview of how the FDA views the regulation of mNGS for infectious disease, what FDA looks for in a submission, and how to initiate a discussion with the FDA on the scientifically based rationale to get relevant feedback. Since most mNGS workflows are currently run as laboratory-developed tests (LDTs), Dr. Cowan clarified the difference between in vitro diagnostic (IVD) and laboratory-developed test (LDT) pathways and highlighted that LDTs remain controversial. Recently, in 2023, the VALID Act was presented which proposed, among other elements, regulation of LDTs by the FDA (Bonislawski, 2023). After consideration by Congress, LDT regulation by FDA was removed from the VALID Act. The FDA has considered regulating mNGS through its 2016 draft guidance, through which the FDA sought comments from the public and other parties. FDA received a number of comments. However, between 2016 and 2022, FDA made no additions or modifications to the draft guidance. FDA appears to be taking a less prescriptive and more flexible approach, as it is very challenging to fit all intended uses into one guidance; therefore, the 2016 draft guidance

is largely irrelevant, and the FDA seeks additional input to work constructively with stakeholders as they draft the next version.

Additionally, the FDA reviewers are scientists who value technological innovation and seek stakeholder feedback. The FDA is open to discussing specific intended use cases, risks associated with these devices, and their limitations. They are also open to new approaches such as *in silico* approaches, what would be acceptable error rates, different sequencing platforms, and least burdensome and most efficient approaches to expand claims.

The likely regulatory pathway for an mNGS test would be de-novo, which will involve generating special controls since there is no predicate device. Special controls are going to be a listing of information regarding what information needs to see and what the performance needs to be. For example, how the quality of databases for bioinformatics analysis will be maintained with curation methods are critical. Although the FDA started the ARGOS database and published a paper at that time (Sichtig et al., 2019), their thinking has evolved over the years, and they want stakeholders to propose ideas on how they will determine the performance of the test. To get FDA feedback, the mNGS community is encouraged to submit a Pre-Submission to FDA and ask pertinent and specific questions in their submissions. There are no fees for submitting a Pre-Submission or seeking guidance from the FDA. Overall, FDA's ground rules are that the device is safe and effective for its intended use, and the benefits such as standard of care must outweigh the risks which must be mitigated to acceptable levels using a scientific, data-driven, and least burdensome approach.

"Bioinformatics Challenges in implementation of mNGS in clinical labs" moderated by Dev Mittar, PhD; BARDA DRIVe with Panelists: J. Rodney Brister, PhD, National Center for Biotechnology Information; Sam Chorlton, MD, BugSeq Bioinformatics; and Katrina Kalantar, PhD, Chan Zuckerberg Biohub

Dr. Mittar led a discussion on the bioinformatic challenges of implementing mNGS in clinical laboratories for routine care. The panel focused on validating bioinformatic tools, user-friendly pipelines, regulatory-grade databases, harmonizing sequencing data, and reporting clinically relevant results.

The panel addressed the need to verify and validate the numerous bioinformatic tools and pipelines available for mNGS analysis. They emphasized the importance of fitness for the intended purpose and the need for common reference standards to benchmark accuracy and standardization. While efforts like the Critical Assessment of Metagenomics Interpretation (CAMI) challenge (Meyer et al., 2022) using *in silico* datasets have been made, evaluating pipelines for clinical practice requires real-world samples. Synthetic sets are useful but challenging for testing the detection of unknown pathogens. Lessons learned from the SARS-CoV-2 response, including analyzing the same dataset across different pipelines, provided insights into discrepancies. The panel acknowledged that this problem remains challenging but crucial.

Simplifying complex bioinformatic pipelines into user-friendly interfaces that clinical lab technicians can routinely use was discussed. Efforts by organizations like CZ Biohub and Bugseq to create accessible web-based portals were highlighted. Targeting the end-user and ensuring easy access to quality control information and interpretable results were key considerations. The community continues to focus on simplifying report interpretation and making pipelines easier to use and yield clinically actionable results.

The value and necessity of regulatory-grade databases were examined. The panel agreed on the importance of aligning tools, databases, and analysis pipelines with specific use cases. Different use cases require different databases, structures, and maintenance to support decision-making. Curated databases can enhance pathogen representation, and automation methods can expedite curation. Defining the clinical need or databases to determine appropriate clinical use cases posed a chicken-and-egg problem.

The panel also discussed the need to harmonize sequencing data from different platforms. While data characteristics have converged among manufacturers, middleware can be used to achieve consistent bioinformatic results.

Defining signal and background in an mNGS report was another topic. The panel highlighted the significance of technological/process controls and clinical context in defining thresholds, cutoff values, and distinguishing real results. Best practices included using controls, spike-ins, blanks, generating "kit-omes" to define background, considering clinical context, and conducting contamination studies. Parameters such as read counts, coverage, quality, and result uniqueness confirmed the signal. Understanding the clinical context and disease progression was crucial for providing meaningful diagnostic information.

Overall, the panel addressed challenges related to validating bioinformatic tools, creating user-friendly pipelines, developing regulatory-grade databases, harmonizing sequencing data, and defining signal and background in mNGS reports, aiming to enhance the implementation of mNGS in clinical care.

Perspectives

A pathogen-agnostic test like mNGS can detect any and every pathogen including new, emerging, and existing; mNGS could also reshape the public health response and aid in tailoring interventions to improve patient outcomes. Despite this, the translation of this technology to be broadly used clinically has not happened due to many hurdles including:

1) **Regulatory Guidance:**
   The FDA released a guidance document in 2016 related to the use of mNGS in determining antimicrobial resistance. To broaden clinical use of mNGS technology, more public guidance is needed in terms of analytical and clinical validation needs for a pathogen-agnostic diagnostic test. Several academic, industry, and public health labs are currently performing analytical and clinical validation on the use of mNGS in diagnostics, including DRIVe's partners in the agnostic diagnostic program. This scientific rigor and innovation, combined with investment, indicates that this technology is ready to enter clinical laboratories. Given that the field is at an inflection point, the FDA guidance on analytical and clinical validation of this technology would be immensely helpful. The group was strongly encouraged to interact with the FDA for bidirectional understanding of technologies and regulatory needs.
2) **Foster collaboration with industry and academia:**
   Given that unbiased diagnostic approach is novel, and there are no predicate tests available, there is a need to foster collaboration between industry and academia in order to develop consensus on the

requirement of validation methods. This collaboration will be a great learning opportunity to advance the entire field of mNGS.

3) **Bioinformatic analysis and the regulatory grade database:**
Approaches are needed for meaningful interpretation of the tranche of data resulting from a clinical mNGS test. When should a "hit" translate to a diagnosis? Agreement is needed on how much resulting data should be shared with clinicians and how best to communicate it. The FDA recognized the need for a standardized bioinformatic database in its 2016 draft guidance; the FDA introduced the ARGOS database as a reference for industry and developers to source (Sichtig et al., 2019); however, this database is not comprehensive. There is a strong need to document the requirements for a clinical and regulatory grade database. A public-private partnership can address this problem of database curation, maintenance, validation, and quality control.
4) **Clinical utility and interpretation of results:**
Despite the numerous published cases where mNGS has been used clinically, there remains a need for prospective, longitudinal, randomized clinical trials to demonstrate the clinical utility of mNGS in different settings. Amongst many other endpoints, these studies would demonstrate the clinical impact, diagnostic yield, and economic impact of mNGS compared to the current standard of care. Well-designed studies examining the use of mNGS earlier in the diagnostic algorithm could assist in adopting mNGS; however, these studies remain costly and lengthy and, given the lack of regulatory guidance, are challenging to design. Additionally, unlike traditional targeted tests, there are no well-defined references or gold standards for comparison of clinical performance.

Final thoughts

There is an urgent need to be prepared for the next pandemic. Combining the clinical use of mNGS with its current use for surveillance would be immensely helpful in rapidly detecting, managing, and monitoring emerging pathogens. Beyond pandemic preparedness, mNGS offers a paradigm shift in infectious disease testing by enabling the detection of any pathogen in a sample at once - this ability to characterize the sample in one shot can potentially shorten long and laborious diagnostic pathways. In conclusion, mNGS has the potential to revolutionize the clinical diagnosis of infectious diseases by providing a comprehensive and unbiased approach to pathogen detection. As technology advances, mNGS is expected to play an increasingly important role in infectious disease diagnostics, leading to improved patient care, better management of future outbreaks, and complicated hospital infections.

FROM BENCH TO PATIENT
TACKLING HURDLES TO IMPLEMENTING NGS-BASED AGNOSTIC PATHOGEN DETECTION

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