Journal of the Pediatric Infectious Diseases Society

SUPPLEMENT ARTICLE

Metagenomic Next-Generation Sequencing (mNGS): SARS-CoV-2 as an Example of the Technology's Potential Pediatric Infectious Disease Applications

Andrew S. Handel,[1] William J. Muller,[2,3] and Paul J. Planet[4,5,6]

[1]Department of Pediatrics, Division of Infectious Diseases, Stony Brook Children's Hospital, Stony Brook, New York, USA, [2]Department of Pediatrics, Northwestern University, Chicago, Illinois, USA, [3]Division of Pediatric Infectious Diseases, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, USA, [4]Division of Pediatric Infectious Diseases, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA, [5]Department of Pediatrics, Perelman College of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA, and [6]Sackler Institute for Comparative Genomics, American Museum of Natural History, New York, New York, USA

Metagenomic next-generation sequencing (mNGS) has emerged as a potentially powerful tool in clinical diagnosis, hospital epidemiology, microbial evolutionary biology, and studies of host-pathogen interaction. The SARS-CoV-2 pandemic provides a framework for demonstrating the applications of this technology in each of these areas. In this Supplement, we review applications of mNGS within the discipline of pediatric infectious diseases.

Keywords: diagnostics; infectious diseases; metagenomic next-generation sequencing; pediatrics; SARS-CoV-2.

In January 2020, the world first learned of a mysterious cluster of patients with severe pneumonia in Wuhan, China [1]. Although the causative agent was initially unclear, SARS-CoV-2 was soon isolated and identified [2–4], followed quickly by a global explosion of COVID-19 cases and deaths. In response to the emerging pandemic, the biomedical research world placed unparalleled focus on this novel pathogen. From diagnostic and surveillance assays, to preventive vaccines, to therapeutics, rapid advances were made within months. Central to this progress was next-generation sequencing (NGS), described as an approach for rapidly sequencing genetic material, and metagenomic NGS (mNGS) to identify a microbial source.

As we track the scientific strides made throughout the pandemic, studies utilizing NGS have repeatedly provided crucial information for understanding SARS-CoV-2. In this *JPIDS* supplement, we provide the pediatric infectious disease community with an introduction to next-generation sequencing (NGS) and its potential clinical applications. The focus will be on metagenomic NGS (mNGS). The included articles explore the roles of mNGS in clinical care and infectious disease research, considering both its current uses and potential future applications.

mNGS is a novel diagnostic that facilitates the sequencing and identification of microbial DNA or RNA from a clinical sample. The mNGS process is complex and requires expertise at several steps to efficiently yield accurate results. Both "wet labs" and "dry labs" are involved. Prior reviews have provided in-depth descriptions of the mNGS process [5–9]. Briefly, a clinical specimen is obtained, such as blood, cerebrospinal fluid, or bronchoalveolar lavage (BAL) fluid. DNA and/or RNA is extracted and purified from the sample. Through various methods, the genetic material is separated into small fragmented strands, facilitating massive parallel or "shotgun" sequencing. Within the sequencer, universal adapters bind to millions of fragments, which are then amplified through polymerase chain reaction (PCR). The amplified fragments are then sequenced simultaneously, allowing for identification of billions of nucleotide base pairs in a short time. The nucleotide sequences are then analyzed through a bioinformatics processor that aligns the individual reads with known genomes. In human samples, >99% of the reads are typically human in origin. In some applications, the remaining reads are aligned with a database of known microbe genomes. If the DNA/RNA of an organism is present in sufficient quantities above the threshold determined by a healthy control population, that organism is "called" and said to be present in the sample, potentially identifying an organism with clinical correlation that might be determined to be a pathogen. In many cases, the process from sample collection to pathogen call may require only a couple of days.

Because of its unbiased nature, mNGS has been met with considerable enthusiasm for its potential as a diagnostic tool for new or unanticipated diseases. As mentioned, within weeks of the observation of a cluster of cases of severe respiratory disease, SARS-CoV-2 was identified through next-generation sequencing of BAL fluid from infected patients [2–4]. Our supplement addresses the potential utility of mNGS for clinical infectious disease diagnosis through 2 complementary review articles. The first, by Edward and Handel [10], provides a

Corresponding Author: Andrew S. Handel, MD, Department of Pediatrics, Division of Infectious Diseases, Stony Brook Children's Hospital, 101 Nicolls Road, HSC-T11, Stony Brook, NY 11794, USA. E-mail: andrew.handel@stonybrookmedicine.edu.

Journal of the Pediatric Infectious Diseases Society 2021;10(S4):S69–70

https://doi.org/10.1093/jpids/piab108

broad overview of mNGS performance in a variety of conditions including bacteremia, febrile neutropenia, osteomyelitis, and others. The article also compares and contrasts the real-world clinical effect of mNGS testing at children's hospitals as described by 4 retrospective studies [11–14]. Next, Graff et al [15] focus on the utility of mNGS for suggesting the microbial etiologies of pediatric meningitis and encephalitis, a topic that has received abundant attention. These articles provide an in-depth review of the advantages and many limitations of mNGS as a clinical diagnostic tool in a rapidly evolving field.

Health care-associated transmission of SARS-CoV-2 has been a major concern with limited experiences to guide health care epidemiology practices [16]. Whole-genome sequencing (WGS) through NGS has the potential to revolutionize how hospital-acquired infections are detected and prevented. Greninger and Zerr [17] provide a review of WGS for identification of hospital-based outbreaks of bacterial, viral, and fungal infections. They then delve into the future of NGS as a tool in health care epidemiology, providing a comparison of specific sequencing technologies of the commonly used commercial platforms Illumina (Illumina, Inc., San Diego, CA, USA) and nanopore (Oxford Nanopore Technologies, PLC, Oxford, UK), an analysis of logistic hurdles that limit the use of NGS in health care epidemiology, and an overview of reimbursement for processing such samples.

In light of the frequent emergence of novel SARS-CoV-2 variants, viral evolutionary biology has become a topic of discussion. In this supplement, Moustafa and Planet [18] provide a detailed review of the concepts needed to fully appreciate SARS-CoV-2 mutations and their clinical significance. Central to this work is the massive growth in next-generation sequencing of the virus throughout the pandemic. As their title, "Jumping a Moving Train: SARS-CoV-2 Evolution in Real Time" suggests, mutations inherently leave evolutionary biologists a step behind. Their article provides clinicians with the tools needed to understand novel strains as they emerge.

The crucial role of the immune response to SARS-CoV-2 has been made clear as the pandemic progressed. Silverman and Green [19] introduce readers to an exciting new application of NGS, microbial flow cytometry coupled to NGS (mFLOW-seq) that is used to better understand the interaction of microbes and antibodies. This technique uses flow cytometry to sort antibody-coated microbes from those that are not coated, followed by NGS to identify the microbes in each group. As described in their article, mFLOW-seq has provided information crucial for understanding the pathogenesis of neonatal necrotizing enterocolitis, the mechanism of action of fecal microbiota transplants, and the potential utility to treat patients with sepsis with bacterial species-specific antibodies.

As demonstrated by the global response to the COVID-19 pandemic, awareness of future pandemics is necessary for clinicians and scientists. Haslam [20] explores this topic with his article, "Future Applications of Metagenomic Next Generation Sequencing for Infectious Diseases Diagnostics." The work first describes how mNGS is performed, then considers its challenges and how these may be overcome. From this framework, the article then proposes clinical applications for mNGS.

The COVID-19 pandemic has demonstrated the importance of applying cutting-edge approaches, such as mNGS, to novel challenges. We hope that this supplement provides readers with a deeper understanding of mNGS science and use, as well as with inspiration to identify new applications of this emerging clinical tool.

Notes

Potential conflicts of interest. Drs. A.S.H. and W.J.M. have received research support from Karius, Inc. unrelated to the current manuscript.

All authors have submitted the ICMJE Form for Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Supplement sponsorship. This supplement was sponsored by Illumina and IDbyDNA.

References

1. COVID-19 – China. **2020**. Accessed September 13, 2021. https://www.who.int/emergencies/disease-outbreak-news/item/2020-DON229
2. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature **2020**; 579:265–9.
3. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature **2020**; 579:270–3.
4. Zhu N, Zhang D, Wang W, et al.; China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med **2020**; 382:727–33.
5. Chiu CY, Miller SA. Clinical metagenomics. Nat Rev Genet **2019**; 20:341–55.
6. Gu W, Miller S, Chiu CY. Clinical metagenomic next-generation sequencing for pathogen detection. Annu Rev Pathol **2019**; 14:319–38.
7. Gu W, Deng X, Lee M, et al. Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids. Nat Med **2021**; 27:115–24.
8. Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet **2016**; 17:333–51.
9. Bender JM, Dien Bard J. Metagenomics in pediatrics: using a shotgun approach to diagnose infections. Curr Opin Pediatr **2018**; 30:125–30.
10. Edward P, Handel A. Metagenomic next-generation sequencing for infectious disease diagnosis: a review of the literature with a focus on pediatrics. J Pediatric Infect Dis Soc **2021**; 10(S4):S71–7.
11. Lee RA, Al Dhaheri F, Pollock NR, Sharma TS. Assessment of the clinical utility of plasma metagenomic next-generation sequencing in a pediatric hospital population. J Clin Microbiol **2020**;58(7):e00419–20.
12. Rossoff J, Chaudhury S, Soneji M, et al. Noninvasive diagnosis of infection using plasma next-generation sequencing: a single-center experience. Open Forum Infect Dis **2019**;6(8):ofz327.
13. Niles DT, Wijetunge DSS, Palazzi DL, et al. Plasma metagenomic next-generation sequencing assay for identifying pathogens: a retrospective review of test utilization in a large children's hospital. J Clin Microbiol **2020**;58(11):e00794–20.
14. Hogan CA, Yang S, Garner OB, et al. Clinical impact of metagenomic next-generation sequencing of plasma cell-free DNA for the diagnosis of infectious diseases: a multicenter retrospective cohort study. Clin Infect Dis **2021**; 72:239–45.
15. Graff K, Dominguez S, Messacar K. Metagenomic next generation sequencing for diagnosis of pediatric meningitis and encephalitis: a review. J Pediatric Infect Dis Soc **2021**; 10(S4):S78–87.
16. Richterman A, Meyerowitz EA, Cevik M. Hospital-acquired SARS-CoV-2 infection: lessons for public health. JAMA **2020**; 324:2155–6.
17. Greninger AL, Zerr DM. NGSocomial infections: high resolution views of hospital-acquired infections through genomic epidemiology. J Pediatric Infect Dis Soc **2021**; 10(S4):S88–95.
18. Moustafa AM, Planet PJ. Jumping a moving train: SARS-CoV-2 evolution in real time. J Pediatric Infect Dis Soc **2021**; 10(S4):S96–105.
19. Silverman MA, Green JL. Insight into host-microbe interactions using microbial flow cytometry coupled to next generation sequencing. J Pediatric Infect Dis Soc **2021**; 10(S4):S106–11.
20. Haslam DB. Future applications of metagenomic next generation sequencing for infectious diseases diagnostics. J Pediatric Infect Dis Soc **2021**; 10(S4):S112–7.