SPRING 2021

microcosm

PUBLICATION OF THE AMERICAN SOCIETY FOR MICROBIOLOGY

DIAGNOSING THE SOLUTION

Contents

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microcosm SPRING 2021

From the Editor

BY STANLEY MALOY, PH.D.
EDITOR-IN-CHIEF

Infection, Identification and Intervention

The COVID-19 pandemic has taught us that human health depends on proactive planning rather than trying to rapidly develop and disseminate public health interventions in response to a new infectious disease. This preparation relies on basic and applied microbiology research, well-equipped and staffed clinical laboratories, and innovative life sciences companies.

Effectively responding to an infection requires a quick and accurate way to identify whether a person is infected with a particular pathogen. Before we understood the microbial nature of infectious disease, diagnoses were based solely on a person's symptoms. Because so many different infections share common symptoms, this approach often led to lumping many disparate diseases together. For example, typhus was often mistakenly identified as typhoid fever. Once it became possible to culture bacteria, the culprit could sometimes be identified in a clinical laboratory. Although much better than simply monitoring symptoms, the difficulty of culturing many bacteria, viruses and eukaryotic microbes often makes this approach challenging. Similar arguments can be made for basic microscopy and biochemical tests.

The advent of nucleic acid-based diagnostics, immunological assays and microfluidics dramatically changed our ability to rapidly identify potential pathogens with high levels of sensitivity. And, as described in the article "Tracking Pathogens via Next Generation Sequencing," new genomic and metagenomic approaches have provided an exquisite ability to distinguish different strains of closely related pathogens, facilitating our ability to track where outbreaks are coming from and possibly suggesting an upstream intervention to limit further infections. The applications of these approaches are wide-ranging, including prediction of antimicrobial resistance and vaccine-resistant variants.

Rapid and precise identification of pathogens often allows quick medical interventions that reduce the morbidity and mortality due to infectious diseases. However, there are still many clinical laboratories around the globe that lack the resources to run these technologically sophisticated diagnostic tests. This problem may be reduced by the development of point-of-care diagnostics that do not require an advanced laboratory.

These approaches have also allowed us to shift from focusing on the person who is infected to the transmission of pathogens in the environment. For example, testing wastewater for the SARS CoV-2 virus has provided valuable insights into the location of infections during the COVID-19 pandemic. This focus on transmission also allows implementation of the One Health lesson that human diseases are frequently acquired from animals or the environment. The focus on monitoring potential pathogens in the environment has catalyzed the development of a variety of new approaches for pathogen detection, from the use of smartphones and nanomechanical sensors to smart dust. Several of these innovative new approaches are described in the article "One Health: The Benefits (and Risks) of a Comprehensive Diagnostic Approach." But there is clearly more to come: This is rapidly evolving field, with many entrepreneurial opportunities that will likely compete in the marketplace over the next decade.

Some major challenges we have faced with testing for SARS CoV-2 have included the limited availability of supplies needed for testing, inadequate numbers of trained microbiologists in clinical laboratories, and a distrust in some communities that the results are important, reproducible and accurate.

For any of these diagnostic approaches to have sufficient impact to thwart the COVID-19 pandemic or the next pandemic, the public has to trust that the approach is effective and reliable, and that their privacy is protected. For example, the article on "One Health" emphasizes potential privacy issues that may arise from environment-based diagnostic approaches that may pinpoint a particular community. We need to solve these issues before they become a problem that negatively impacts people's lives, so scientists cannot shy away from ethical issues when designing diagnostic solutions.

Sadly, another concern is fraudulent claims about the effectiveness, sensitivity and reliability of diagnostic tests. The article "Consumer Beware: The Cost of the Rare Fraud in the Biomedical and Diagnostics Industry" describes examples of ineffective products that were promoted by unethical charlatans enticed by financial rewards. These cases are rare in the life sciences industry because of the scrutiny of the FDA, as well as the role of peer reviewers and the scientific community in carefully analyzing the published data that is the cornerstone of most new products. These rare infractions emphasize why it is so important for scientists to have a robust knowledge of scientific ethics, and why this is such a critical component in the training of new researchers.

Although microbiologists have made tremendous strides in responding to infectious diseases, a core concept of microbiology is that there is a tremendous number of microbes, and these numbers allow them to evolve new properties very rapidly. Insights into these mutated microbes often depend on knowledge that we don't yet have and can't predict a priori. As argued in Vannevar Bush's 1945 report "Science: The Endless Frontier," critical new insights frequently come from fundamental research. So, if we are going to be prepared for the next new pathogen or the next pandemic, we need to pay close attention to basic research and research on pathogenesis per se. The article 'What's Hot in the Microbial Sciences' includes some published articles that address directed research on pathogens, but also some articles that we thought were interesting, clever and – who knows – may someday lead to an application that makes the world a healthier place.

Stanley Maloy, Ph.D.
Microcosm Editor-in-Chief

Tracking Pathogens via Next Generation Sequencing (NGS)

BY ASHLEY HAGEN, M.S.

Since the discovery and isolation of DNA, investigative processes aiming to decipher life's complex and variable genetic codes have become indispensable to public health infrastructure. Advances in nucleic acid sequencing have revolutionized the way we identify, characterize and track causative agents of disease. Where symptomology was once considered the gold standard of diagnostics, next generation — or high-throughput — sequencing has now become the enabling instrument of "precision public health," with applications in emerging infectious diseases, foodborne illness, antimicrobial resistance, biosurveillance, bioforensics and epidemiology, allowing for earlier detection and management of outbreaks and disease. Here, we discuss some of the broad applications of pathogen genomics, as well as past, present and ongoing developments in this powerful area of research.

NEXT GENERATION SEQUENCING IN THE MICROBIOLOGY LAB

Next generation sequencing (NGS) encompasses any high-throughput sequencing method that can process millions of individual DNA fragments (or cDNA fragments from RNA) at one time, and is predated by first-generation sequencing methods such as Sanger sequencing (also known as dideoxynucleotide chain termination sequencing) that process only one nucleotide per reaction. There is considerable debate about what defines the generations of DNA sequencing technology. While some purport that NGS began when massively parallel pyrosequencing was commercially released in 2005, others link the beginning of the NGS era to the evolution of single molecule sequencing (SMS) technology.

Either way, genomic sequencing facilitates genome assembly and metagenomic analysis. And according to Dr. Trish Simner, director of the Medical Bacteriology and Infectious Disease Sequencing Laboratories at the Johns Hopkins Hospital and Early Career At-Large representative for the American Society for Microbiology (ASM) Council on Microbial Science, among the many sequencing platforms in use today, "NGS has three main applications in the clinical microbiology lab."

1. Whole genome sequencing (WGS) allows for comprehensive analysis of entire microbial genomes. This approach is often applied to pure, isolated colonies of organisms in the lab and provides large amounts of data in a short amount of time.

2. Targeted next generation sequencing allows for sequence analysis of specific areas of the genome. The approach increases sensitivity directly from the specimen. However, selecting for specific targets in the sequence requires the use of an amplification approach by PCR or a hybridization approach after the library is prepared.

3. Metagenomic sequencing (mNGS) allows for agnostic analysis of all nucleic acid in a given sample. Nothing specific is targeted, resulting in both host and microbial nucleic acid being sequenced. mNGS data can then be further mined to detect microbial nucleic acid and determine whether a pathogen of interest is present in the sample.

PATHOGEN IDENTIFICATION

A battery of tests are typically ordered in an attempt to establish a diagnosis that is guided by physician ordering practices, but mNGS provides a hypothesis-free approach to detecting all microbial groups in a given sample. Detecting all nucleic acid in a sample gives researchers the ability to look at any portion of the genome sequenced, uncover coinfections, and identify new or unexpected organisms.

RNA-based mNGS of a respiratory sample from a patient in Wuhan is what allowed researchers to identify the cause of an outbreak of pneumonia spreading through China in late 2019. The causative agent turned out to be the novel coronavirus, later named SARS-CoV-2, which is responsible for the ongoing COVID-19 pandemic.

"It shows you the power of the method," said Dr. Simner. "Because it's so broad, it allows you to capture the rare, atypical or unknown pathogens."

How do researchers know when they have a novel pathogen on their hands? Sequencing is still considered a complex, expensive and relatively new diagnostic technique in the clinical lab. But when a cluster of patients presents with similar symptoms (like in Wuhan), for which standard diagnostic methods fail to identify a likely cause, it's a good sign that something unique is taking place, and a deeper analysis is warranted. At that point, sequencing approaches such as metagenomics can be especially helpful.

"You can sequence any nucleic acid in your sample, and the fun thing with metagenomics is that you can extract just the reads assigned to a single pathogen. And if you got sufficient coverage, you can actually assemble the genome with those reads and taxonomically classify the organism to its closest relative," Dr. Simner explained. In the case of SARS-CoV-2, that was closest to a bat coronavirus species called RaTG13 virus.

SURVEILLANCE AND TRACKING OF VARIANTS

After SARS-CoV-2 was identified, preexisting libraries of microbial knowledge, medical tools and scientific practices were rapidly deployed or repurposed to fight the evolving pandemic. Among these, genomic sequencing has remained (rather quietly) indispensable, not only for the early detection and investigation of SARS-CoV-2 outbreaks but also for the tracking of new variants.

> ***In fact, by the time reports of a "new" SARS-CoV-2 variant, B.1.1.7, reached the general public, many research labs and medical and academic institutions around the world had already been tracking the evolution of the novel coronavirus for quite some time.***

Dr. Heba Mostafa, assistant professor of pathology at the Johns Hopkins University School of Medicine, recalls the experience vividly. "When the pandemic started and we began diagnosing [SARS-CoV-2] in the lab in March 2020, it was very logical to me that we needed to start sequencing right away to understand what kind of virus diversity we have and if there are any correlations between changes in the viral genome and the severity of the disease," she shared, adding that they were already detecting diversity in the SARS-CoV-2 genome at the time.

Dr. Jacques Ravel, associate director of the Institute for Genome Sciences at the University of Maryland School of Medicine and an American Academy of Microbiology Fellow, described a similar experience. "We established genome sequencing of variants a long time ago, because that's what we do. We are a genomic center. We sequence things, and we are really good at that," he explained. "But you know, way back in the summer (2020), nobody cared about it." Genomic surveillance is expensive, with WGS typically costing approximately $150-$200 per sample, and despite having the required expertise and equipment, in the absence of regular funding many labs were unable to support the necessary ongoing sequencing efforts.

Meanwhile, the U.K. invested in epidemiological surveillance early in the pandemic. The COVID-19 Genomics Consortium UK (COG-UK) was created in late March 2020 with £20 million in funding from UK Research and Innovation, and was granted an additional £12.2 million from the Department of Health and Social Care's Testing Innovation Fund in November 2020 to build a real-time surveillance system of emerging outbreaks. Early action and adequate funding enabled the U.K. to discover the B.1.1.7 variant and alert much of the world that SARS-CoV-2 was evolving.

As news of circulating SARS-CoV-2 variants, including B.1.351 and P.1, increased, so did local, national and international efforts to increase sequencing capacity. Both the University of Maryland and Johns Hopkins University are now working closely with the Maryland Department of Health to ramp up sequencing, with a goal of processing 10% of the total positive cases in Maryland, a number that, according to Dr. Ravel, epidemiologists say is a good number.

> *"First you need to catch where the variants are located, and then you can do your epidemiological study to capture contacts and so on," Dr. Ravel said. "Together, this gives a good picture of the penetration in a given area." Many other academic institutions and research and medical centers across the country are collaborating in a similar manner.*

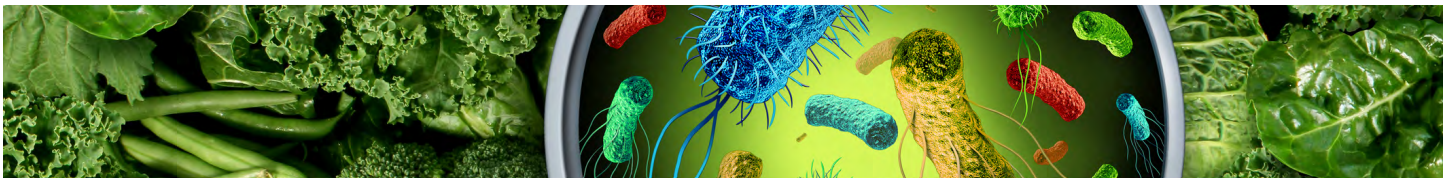
In an NPR interview, Dr. Vaughn Cooper, professor of Microbiology and Molecular Genetics and Computational and Systems Biology at the University of Pittsburgh School of Medicine, and an ASM COMS-elected Board director and co-founder of the Microbial Genome Sequencing Center (MiGS), which has also sequenced 1000s of isolates of SARS-CoV-2 for many customers and collaborators, stated, "There are a few big companies being contracted to do sequencing by the CDC, but they've also contracted with a relatively small number of academic medical centers. And I understand that number of contractees is growing. We really need to engage researchers at academic medical centers who have this ability to join the effort."

According to Dr. Cooper, a major unmet need in legislation is training people in county and state public health labs and academic partners to turn WGS data into knowledge in a timely fashion. "Sequencing is cheap; analysis is expensive," he said. "Our training and funding should reflect these facts."

The Centers for Disease Control and Prevention's (CDC) Advanced Molecular Detection program (AMD) has been working to increase the availability of next generation sequencing in state and local public health systems since its inception in 2014, with the goals of faster disease and outbreak detection and protection from emerging and evolving disease threats. ASM is a leading advocate for greater investment in pathogen genomics and strongly supports the AMD goals.

> *"The work of CDC's AMD program is fundamental to U.S. leadership in sequencing SARS-CoV-2 samples, and strengthening this effort will allow us to get ahead and stay ahead of the COVID-19 variants of concern as they emerge and circulate," said ASM CEO Dr. Stefano Bertuzzi.*

On March 11, 2021, $1.75 billion was allocated to the CDC's AMD program as part of the Energy and Commerce Committee's COVID-19 relief budget reconciliation package. Thanks to the dedicated efforts of many microbiologists who have tirelessly built a foundation upon which SARS-CoV-2 surveillance can now expand, the increased funding will help efficiently ramp up sequencing capacity to track SARS-CoV-2 evolution; elucidate the source, timing, transmission and spread of circulating variants; inform public health practices; and guide vaccine rollouts.



SCREENING FOR FOODBORNE PATHOGENS

Before, during and after the COVID-19 pandemic, a number of other vital applications for NGS have also expanded. For example, NGS is revolutionizing food microbiology. Where pulsed field gel electrophoresis (PFGE) was once the gold standard for characterizing outbreaks, WGS is now being used to screen for pathogens that are contaminating the food chain. One advantage of WGS is that it provides data about evolutionary relationships between bacterial isolates, and comparison of lineages can help provide links between cases and transmission. Routine surveillance may therefore prevent future outbreaks of foodborne illness.

In 2012, the U.S. Food and Drug Administration (FDA) created the GenomeTrackr Network as a means to aggregate and share WGS data of foodborne pathogens collected by public health and university laboratories across the country. The data is housed and analyzed by the National Center for Biotechnology Information (NCBI) but can also be publicly accessed for real-time comparison and analysis.

Thanks to information collected by this network, 709 public health actions have been taken to prevent foodborne illness since 2013, including, but not limited to, investigation of *E. coli* infections linked to flour (2017), *Salmonella* linked to dried coconut (2018) and numerous pet food recalls. Furthermore, the FDA has partnered with the CDC to sequence all *Listeria monocytogenes* isolates in the U.S., with state labs in Washington, Minnesota and New York to conduct real-time samplings of food. Environmental and clinical samples of *Salmonella, E. coli, Campylobacter, Vibrio, Cronobacter,* parasites and viruses are also beginning to be sequenced by labs in the GenomeTrackr network, and it's exciting to consider where analysis of that data might lead.

ASSESSMENT OF ANTIMICROBIAL RESISTANCE AND PREDICTING ANTIMICROBIAL SUSCEPTIBILITIES

Antimicrobial resistance remains one of the greatest public health threats of our time and is responsible for more than 700,000 lives lost annually, according to World Health Organization (WHO) estimates. On a global scale, most of these deaths are caused by resistance in malaria, tuberculosis and HIV. In highly developed countries, hospital-acquired infections, such as methicillin-resistant *Staphylococcus aureus* (MRSA), enterobacterales with extended-spectrum beta-lactamase (ESBL) resistance, and other emerging pan-resistant Gram-negative bacteria are often the culprits.

NGS offers the technology to identify genetic determinants of antimicrobial resistance and the power to monitor events related to the emergence and spread of AMR. Sequence-based approaches are being employed to analyze the microbial resistome (collection of antibiotic resistance genes) in a variety of ways. For example, WGS has been used to predict the species and drug susceptibility profile of mycobacteria with 93% accuracy, analyze the origins of MRSA to reveal that resistant strains of the bacteria emerged long before methicillin was introduced into clinical practice, and provide useful information about detecting known and new mechanisms of drug resistance in *Leishmania*, to name a few.

> *Today, broad-based panels that use targeted NGS to identify pathogens or profile bacteria based off 16S rRNA gene sequencing are beginning to include wide varieties of AMR genes on their panels.*

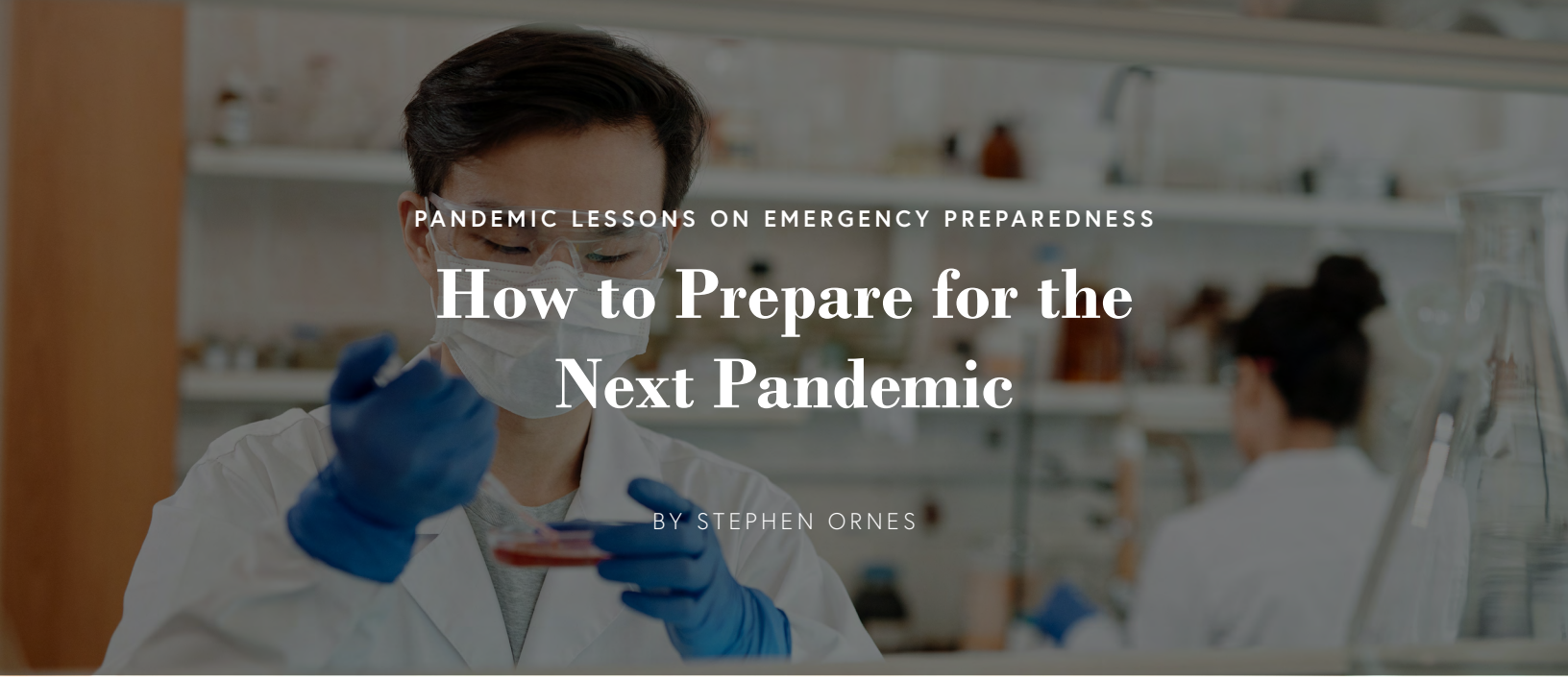
"This antimicrobial resistance detection by next gen sequencing is one of my interests," explained Dr. Simner. *Carbapenem*-resistant organisms are one of her primary research foci. "If we think about direct-from-specimen detection of antimicrobial resistance, the targeted approach is going to be the best approach because oftentimes for antimicrobial resistance, we're only looking for a specific gene or a single nucleotide variant, as opposed to trying to sequence any part of the genome, which is used by a metagenomic approach." She expects that we will one day see broad-based targeted NGS platforms expanding past pathogen identification and AMR detection to include other characteristics like virulence factors. And as NGS becomes more affordable and bioinformatic analysis of NGS data improves, analysis of the entire genome may allow us to see things we might not have otherwise predicted, including suppressor mutations.

PRECISION MEDICINE: THE FUTURE OF PATHOGEN GENOMICS

Genomic sequencing has certainly come a long way since the first bacterial genome (*Haemophilus influenza*) was sequenced in 1995 at the Institute for Genomic Research (TIGR) in Rockville, Md., where Dr. Ravel was an assistant investigator before accepting his current position with the University of Maryland School of Medicine. In the near future, high-throughput sequencing of metagenomic samples could revolutionize the speed and accuracy with which we diagnose pathogens and treat infection. Metagenomic analysis of patient samples will revolutionize the way we conduct medicine on the whole. Metagenomic analysis picks up not only microbial DNA/RNA, but also the host transcriptome (RNA-sequencing approach), something that researchers currently ignore during analysis, but Dr. Simner hopes it will one day be used to our advantage.

> *"There's so much host information there," Dr. Simner pointed out. "Novel diagnostic tools that rely on biomarkers are being developed, but we already sequence the host biomarkers in the metagenomic approach."*

Next steps include looking at the combination of biomarkers to try to identify whether there is a host response to the pathogen, and searching for shifts in the microbiome that might make the host more prone to infection, rather than simply acquiring or being colonized by a particular pathogen. Dr. Simner adds, "It truly is a precision medicine test!"



PANDEMIC LESSONS ON EMERGENCY PREPAREDNESS

How to Prepare for the Next Pandemic

BY STEPHEN ORNES

The pandemic has exposed vulnerabilities in the public health system. Can we learn those lessons in time to prepare for the next one?

Last March, as the COVID-19 pandemic began to spread like wildfire across the country, clinical laboratory scientist Brandy Gunsolus, D.C.L.S., M.L.S. (A.S.C.P.)^{CM}, saw an unexpected problem fast approaching. Her diagnostic testing lab at Augusta University Health in Georgia was quickly running out of nasal swabs. Ordinarily, she'd just order more, but her supplier had also run out. This was a huge problem: Without swabs, Gunsolus and her staff couldn't collect patient samples to test, and without tests, new cases of the disease couldn't be confirmed.

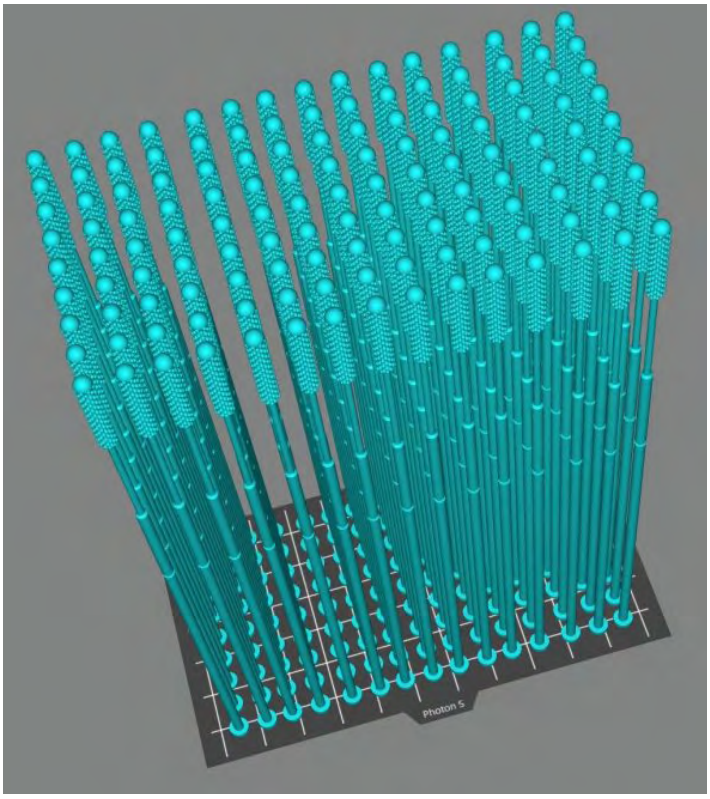


Photo credit Augusta University

So she turned to an unlikely source: The school's dental college. Her colleagues there were already using 3-D printers to create customized, transparent teeth aligners for patients. She asked whether they could use the same material to print swabs. Within days, she had prototypes to test, and once they had settled on a design, the printers started printing.

"We validated that printed swab against the few other swabs that we had left, and we went for it," Gunsolus said. The printed swabs proved to be a lifeline. "We lived on 3D swabs for several months. Not until October were we able get supplies of our regular swabs again."

The swabs were the first of many shortages that emerged as the pandemic — and the need for testing — ballooned. After swabs, Gunsolus had to scramble to find a viral transport medium. Then she had to look for the plastic tips used in the lab's automated liquid-handling system. That manufacturer had run low on raw material, Gunsolus said, and couldn't keep up with the surge in demand. In desperation, she began searching the internet — and found a solution.

"We found them on eBay," she said, "and that's how we were able to keep COVID-testing."

Gunsolus' situation wasn't unique. The pandemic plunged diagnostic labs around the country — and the world — into a tailspin, with lab workers forging creative solutions on the fly. "If I had to sum it up in two words, they would be 'roller coaster,'" said medical laboratory scientist Ian Wallace, M.L.S. (A.S.C.P.)[CM], of Saint Joseph Hospital in Denver.

Since the start of the pandemic, labs in the U.S. have conducted more than 360 million COVID-19 tests. Just over 31 million have tested positive, and more than 600,000 people have died of the disease. Virtually no aspect of 21st century life has been unaffected. But many people saw it coming: Virologists and epidemiologists have been warning at least since the late 1980s that a pathogen as contagious and dangerous as SArS-CoV-2 could wreak havoc on the world. Researchers also warned that the U.S. would not be prepared because of a paucity of public health funding and a workplace shortage. According to a 2020 report from the National Association of County and City Health Officials, government spending on local health departments fell by 18% between 2010 and 2019. In 2008, the Association of Schools of Public Health predicted a shortage of 250,000 public health workers by 2020. Tens of thousands of public health jobs that disappeared between 2007 and 2009, during the Great Recession, were never replaced.

> *"One can't replace those professionals overnight. We must have a sustained pipeline of these highly trained professionals."*
>
> - Rodney E. Rohde, Ph.D., S.M. (A.S.C.P.)[CM], S.V.[CM], M.B.[CM], who has been writing and speaking about the issue for more than two decades.

Nevertheless, those warnings and signs went largely unheeded. As a result, underfunded and understaffed public health systems were underprepared to respond to an emergency of COVID-19's magnitude

Clinical microbiologists, public health personnel and medical laboratory professionals in labs at hospitals, clinics and other facilities were caught in the middle. Almost overnight, starting in February 2020, scientists like Gunsolus found themselves scrambling to test, as quickly as possible, an unprecedented number of incoming samples, using a variety of platforms, for a potentially lethal pathogen that had only recently been sequenced and about which, in the early weeks of the pandemic, experts knew very little.

Swabs and tips weren't the only materials in short supply. Personal protective equipment, or PPE, became a scarce commodity. Labs ran out of pipettes, pipette tips and tests. "Almost immediately there were not enough tests available," says Gunsolus. "We ended up using five different platforms to keep up with the testing demand and the reagents that were available."

Microbiologist J. Michael Miller, Ph.D., who runs a company in Dunwoody, Ga., that consults with government and private labs across the country, said that the pandemic disrupted other tests as well. "The supply shortages started with COVID supplies but then extended into all other areas of the lab," he said. Many labs that he worked with managed to keep functioning through local cooperation — often connecting through the CLIN MICRONET and DIV C listserves hosted by the ASM.

"We reached out to sister laboratories nearby and shared what we could," he said. "We all shared the same problems and same roadblocks."

Material shortages were matched — and worsened — by the lack of trained personnel. Many labs run on a skeletal staff. The pandemic highlighted the severe shortfall of workers needed to run the tests and keep the system functioning, said clinical microbiologist Amy Leber, Ph.D., at Nationwide Children's Hospital in Columbus, Ohio.

"We already had holes in our staffing," Dr. Leber said. "When we pile on top of this a pandemic, it exaggerates and exacerbates our need for extra personnel. And it's not like we can hire more people because we can't even fill the positions we had open to begin with."

Dr. Leber called the shortage of scientists in medical laboratories a "hidden crisis." Part of the problem, she said, is a lack of training programs. Between 1983 and 1999, enrollment in medical laboratory scientist (MLS) certification programs dropped from over 8,000 to nearly 5,000, and the number of programs fell from 638 to 273, according to the National Agency for Clinical Laboratory Sciences. As of 2017, the U.S. had 234 MLS programs and 244 MLT (medical laboratory technician) programs.

"Our programs have fallen way off," said Dr. Rohde, who used to work for the Texas Department of Health and now runs the Clinical Laboratory Science Program at Texas State University in San Marcos. Many labs are staffed by scientists who are nearing retirement, he said, and he worries that their expertise will be lost without a younger generation to train.

Dr. Leber responded to her lab's shortage by appealing to the research institute affiliated with the hospital. "We had to find people who might be able to come in and work in the lab, and had to reposition people from other areas," she said. "People brought in nontraditional workers during this period of all flavors and types, including students and doctoral candidates."

Before COVID-19, Gunsolus said, her lab had been trying to hire a new recruit for one open position. "We were already short-staffed, and we have a large number who are at or past retirement age across all our labs, including microbiology," she said. But when case numbers rose, the lab faced a mass exodus of older scientists who interpreted the pandemic as a sign that it was time to retire. Now, she says, the lab has six open positions and remains understaffed.

As vaccinations have become available and case numbers have steadied or declined in many parts of the country, clinical microbiologists and other experts are considering how labs and other public health services can prepare for the next catastrophe.

To assess the state of workplace shortages in clinical microbiology, for example, the ASM invites its members to take part in the 2021 Clinical Microbiology Workforce Survey that launched this month. Dr. Rohde, in Texas, believes that public health and medical laboratory training programs should be considered for line-item (eternal) funding, similar to how the Department of Defense is treated. "Shouldn't we treat pathogens, which are stealthy and always mutating, [as being] at least as dangerous as our worst terrorist event?," he asked, pointing out that pathogens have killed more people than many wars combined.

> *"We must fix the shortage issue with long-term committed funds for not only more medical laboratory programs, but also medical laboratory scholarships, awareness campaigns and the critical need to raise clinical internships and rotation slots for our students."*

In a New York Times editorial published in April 2020, ASM chief executive Stefano Bertuzzi, Ph.D., and ASM president Robin Patel, Ph.D., called for a "microbiologist National Guard" to address the workforce shortage. During a pandemic, trained graduate students, postdoctoral scientists and research scientists whose labs have closed would be able to fill in the gaps in non-critical roles in clinical microbiology labs and help with testing. (Unless they'd completed an accredited program, Dr. Rohde notes, they wouldn't be qualified to swap in for every job.)

Dr. Leber says an increased investment in MLT and MLS training programs — as well as outreach programs that raise awareness among high school students — could bolster the workforce; she encourages ASM members who run labs to consider becoming a training site.

Dr. Miller, in Georgia, said he thinks labs should address pandemics in their in-house emergency-preparedness training. "We used to think of emergency preparedness for bioterrorism," he said. "This wasn't a bioterrorism event, but the pandemic did give us a good, sobering view of how fast something like this can occur."

Preparation should also include addressing the supply shortage, said Gunsolus. "As a health system, we've identified that we do need a reserve stock of things like gloves, lab coats, and basic PPE," she said, "but we don't want to horde. We don't want it to be like toilet paper."

"Also, we need stockpiles of swabs," Dr. Leber said. For other materials, knowing how much to keep on hand will be trickier. Lab reagents, for example, often expire after a year, and "it's hard to keep stockpiles of stuff that expires," said Dr. Leber.

By bolstering the workforce, funding and supply shortages, the public health system may be better prepared for the emergence of the next virus that wreaks havoc on the world.

"I'm hoping that we never have to experience this thing again," said Gunsolus, "but as Rodney Rohde said at the beginning of this pandemic, 'viruses are going to virus.' There will be another respiratory pathogen, and we've just got to be ready for it."

ONE HEALTH

The Benefits (and Risks) of a Comprehensive Diagnostic Approach

BY GEOFFREY HUNT, PH.D.

Even as society continues to reckon with COVID-19, scientists and public health officials are already taking steps to prevent the next outbreak. Such measures will utilize diagnostic techniques that rely upon the robust, coordinated surveillance of individuals, communities, populations and environments, a concept known as "One Health." Yet when considering the development and implementation of novel diagnostic technology with such large societal impact, the research community must take into account the potential implications and inherent trade-offs that accompany innovation in order to ensure that scientific solutions are moral and equitable and minimize risk.

One Health "recognizes that the health of people is closely connected to the health of animals and our shared environment." Or as Dr. Sanjana Mukherjee, an ORISE Public Health Policy and Regulatory Research Fellow at the Food and Drug Administration (FDA), puts it, "People are really figuring out that you won't be able to solve a [health] crisis by just focusing on humans." Instead, the approach promoted by One Health involves close monitoring of animal populations, intense research into the behavior of plants, fungi and other microorganisms, and studying (and minimizing) the impact of environmental changes, all while improving current diagnostic tools used to prevent disease in humans.

"There is a clear need for simple, rapid, point-of-care diagnostics in the field or at home," says Dr. Changchun Liu, associate professor of Biomedical Engineering at the University of Connecticut. That's why, according to Dr. Liu, "the smartphone is the ideal tool for pathogen detection." His team is utilizing the ubiquity, computing power and high-resolution imaging capacity of mobile devices to develop straightforward diagnostic applications that can aid both patients and medical professionals.

Other novel diagnostic approaches seemingly come straight out of science fiction. A glowing liquid that indicates the presence of pathogens? Researchers in Germany have developed a technique in which carbon nanotubes fluoresce upon coming in contact with molecules emitted by certain bacteria, eliminating the need for costly analysis of tissue samples. Particle-sized mechanical sensors released into the atmosphere capable of monitoring minute changes in environmental conditions over large geographic areas? That's the concept behind smart dust (also known as "motes"), which researchers propose deploying in a variety of facets, including crop monitoring and the observation of animal habitats.

More traditional tools and techniques are being reimagined and adapted for new purposes as well. Wastewater analysis has been used for decades to monitor the presence and environmental impact of pharmaceutical products, as well as to detect illicit drug use. Thanks to COVID-19, the technique has received a renewed focus for its use in disease detection, according to Dr. Wayne Hall, emeritus professor at the National Centre for Youth Substance Use Research in Queensland, Australia. "The idea has been around for a while, it just hasn't really been applied on a wide scale," he says. "I guess COVID has kind of given us a huge kick along." So much so, in fact, that since the beginning of 2020, there have been nearly 300 papers published on the detection of SARS-CoV-2 in wastewater.

Elsewhere, researchers are building off of existing laboratory-based methods, including PCR, CRISPR, DNA microarrays and detection assays such as ELISAs and immunoblots, to develop improved diagnostic tests that have higher sensitivity, increased processing capacity, and the ability to obtain results more quickly. Such advances will allow researchers to better survey, detect and diagnose potential and active threats, leading to faster, more rapid therapeutic interventions.

While promising from a scientific perspective, the comprehensive, all-encompassing nature of the One Health approach does raise uncomfortable questions related to risk, ethics, privacy and legality.

How can community members be informed so that they understand the issues at stake with respect to widespread surveillance and diagnostics? Do citizens get any input into which measures are enacted by their elected officials? And what can individuals do that would help with these technological solutions while still retaining their rights and voices?

The way Dr. Hall sees things, it is incumbent on scientists to consider these issues before, not after, conducting their research. "It's thinking how might this technology be used, not just for the benefit of the greater good, but how ... uninformed and naive use of this technology [could] harm people," he says. "You can't know that in advance, but it's certainly well worth thinking about as you're developing the methods."

For his part, UConn's Dr. Liu is quick to recognize the potential risks of the smartphone technology his group is developing. The "first thing[s] we need to address," he says, are "the privacy issue and the security issue." Similar concerns have been raised about other technologies: Scholars have argued that the "privacy and environmental risks which follow smart dust far outweigh its benefit."

It all comes down to balance, says Dr. Jeremy Prichard, associate professor in the Faculty of Law at the University of Tasmania. "Appropriate research ethics," he points out, "is about balancing sometimes competing or different considerations. It would be wrong to think that the best way to approach ethics is to avoid risk at all costs — even if that means preventing beneficial research."

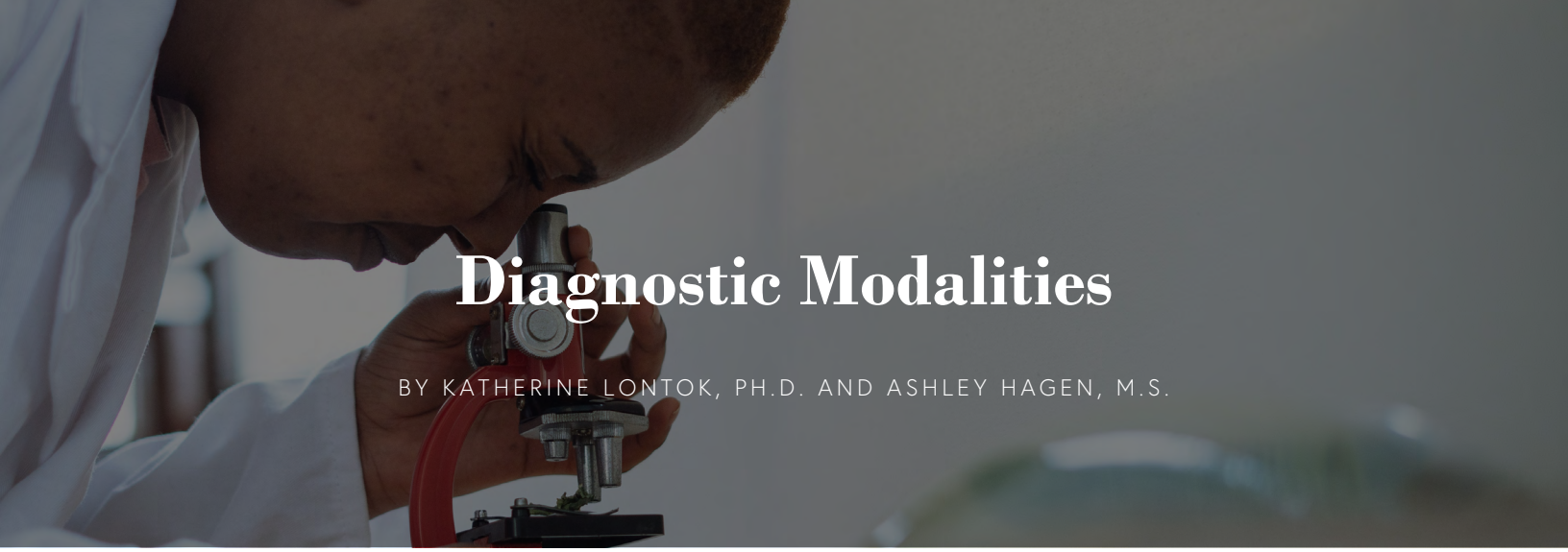
Contemplating how to advise researchers to achieve this balance is what led Drs. Hall and Prichard and their colleagues to develop a set of voluntary ethical guidelines around issues related to wastewater analysis. By putting the onus on scientists to understand the impact of their work and communicate with affected populations, the guidelines represent an intriguing model for other fields to consider using.

A proactive approach on the part of scientists to address these issues would have the added benefit of obviating the need for a long, drawn-out push for legislative action. As Dr. Mukherjee points out, the "FDA's regulatory authority is enforced by statutes and laws passed by Congress. And having Congress involved is going to be a super-long process."

Looking to the future, preventing the global havoc wrought by disease outbreaks like COVID-19 and Zika is paramount. While the scientific research and public health communities will keep moving forward with technological advancements such as One Health, bringing the greater public along presents an equally significant challenge, especially when considering humanity's stupefying ability to ignore intangible threats. "I think, from what we've seen with COVID, people don't really prepare," says Dr. Mukherjee. "I think they're very reactionary."

Lowering the activation energy needed to make society better prepared requires that scientists promote education and awareness, and engage in dialogue that invites input and feedback from all communities. Doing so will demonstrate that One Health truly is for the benefit of everyone.

** Dr. Sanjana Mukherjee's views are her own and do not represent those of the FDA.*



Diagnostic Modalities

BY KATHERINE LONTOK, PH.D. AND ASHLEY HAGEN, M.S.

The diagnostic modalities depicted here represent simplified versions of key laboratory and point-of-care techniques used to identify and characterize microorganisms from patient samples and/or culture isolates. In general, these tests rely on the detection of specific nucleic acid sequences or proteins for diagnosis. Platform variations and examples have been included.

INPUT: PATIENT SAMPLE

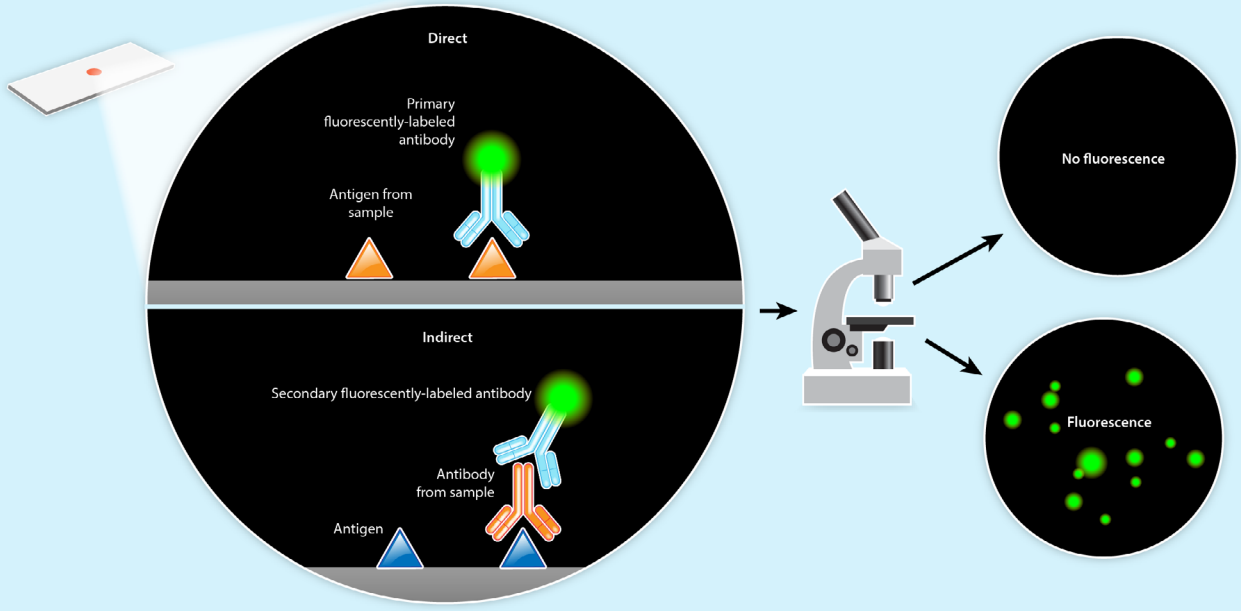
IMMUNOFLUORESCENCE ASSAYS (IFA)

WHAT IT DETECTS:
An antigen from a pathogen or an antibody from the patient.

VARIATIONS:
- Direct (DFA)
- Indirect

EXAMPLES:
- *Rabies virus*
- *Rickettsia rickettsii*

HOW IT WORKS:



Sample is mixed with fluorescently labeled primary antibody that binds a particular antigen (direct) OR with unlabeled antigen and then fluorescently labeled secondary antibody that binds the tail (Fc) region of patient antibodies (indirect). Fluorescence is then detected by microscopy.

INPUT: PATIENT SAMPLE

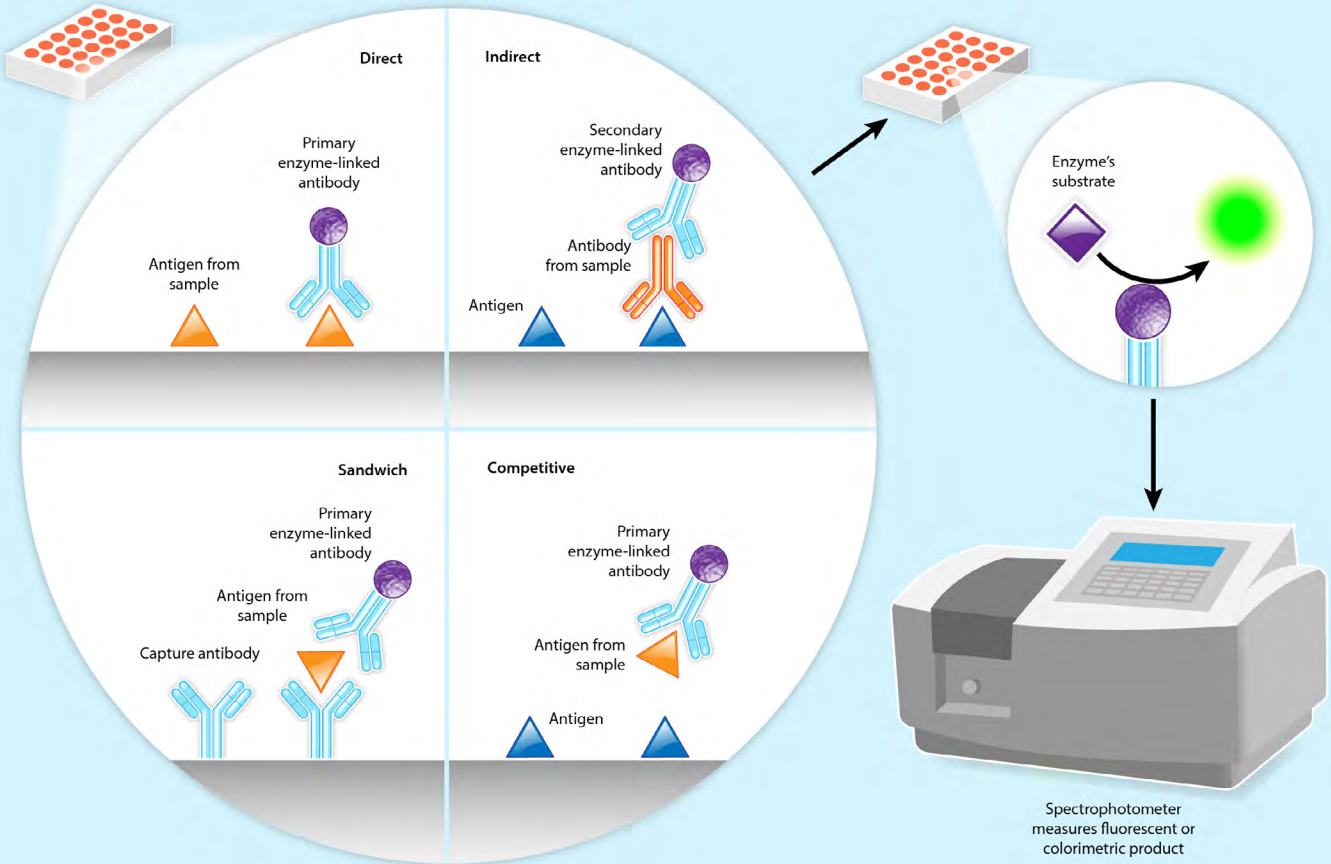
ENZYME IMMUNOASSAYS (EIA)

WHAT IT DETECTS:
An antigen from a pathogen or an antibody from the patient.

VARIATIONS:
- Direct
- Indirect
- Sandwich
- Competitive

EXAMPLES:
- Hepatitis C virus
- Legionella pneumophila serogroup 1

HOW IT WORKS:



Direct and indirect EIAs are analogous to direct and indirect IFAs: The primary or secondary antibody is enzyme-linked instead of fluorescently labeled. Substrate for the enzyme is added, and the amount of chromogenic or fluorescent product is measured by spectrophotometer.

Sandwich EIAs use immobilized, unlabeled antibody to bind antigen from a patient sample, then an enzyme-linked form of the same antibody for detection. In competitive EIAs, a patient sample is mixed with enzyme-linked antibody, then added to immobilized antigen. Conversion of the enzyme's substrate detects the amount of primary antibody captured by the immobilized antigen, which inversely correlates with the amount of antigen in the patient sample.

INPUT: PATIENT SAMPLE

LATERAL FLOW IMMUNOASSAYS

WHAT IT DETECTS:

An antigen from a pathogen or an antibody from the patient.

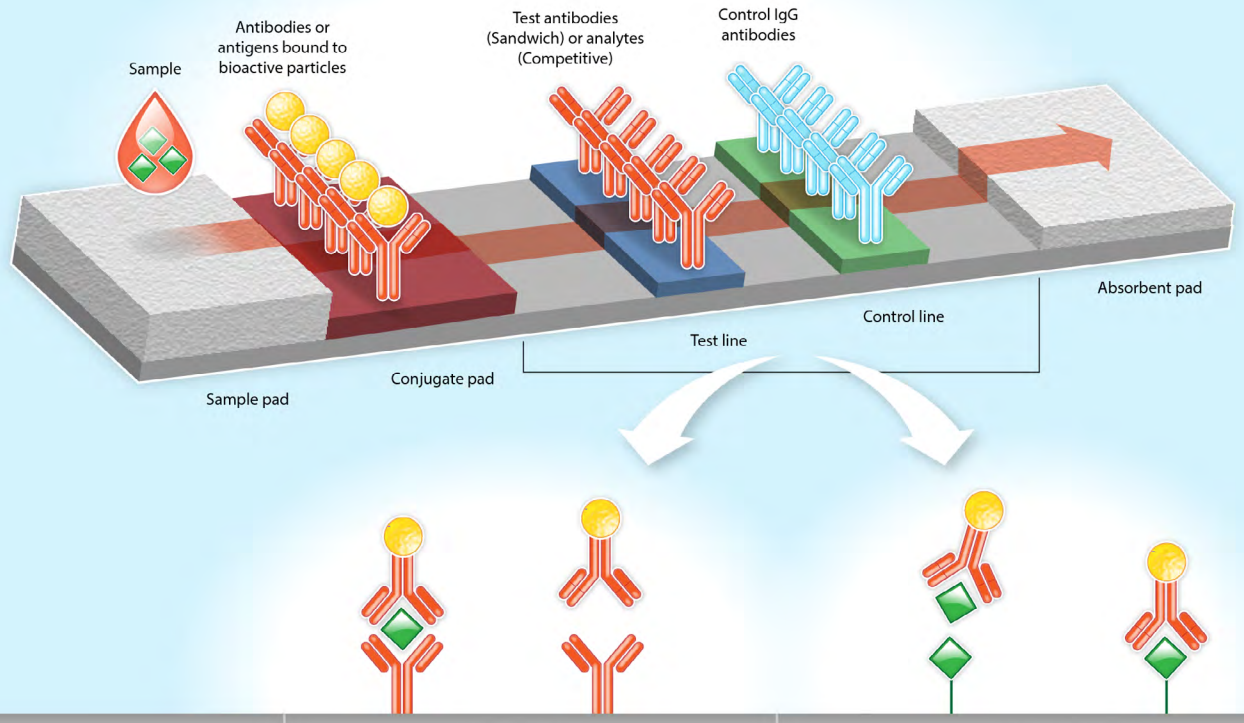
VARIATIONS:

- Competitive
- Sandwich

EXAMPLES:

- Rhinovirus, PIV and Influenza virus rapid antigen detection
- Epstein-Barr serology

HOW IT WORKS:



	Sandwich Assay		Competition Assay	
Analyte present?	✓	✗	✓	✗
Signal readout?	✓	✗	✗	✓

Sample is drawn by capillary action through a series of overlapping components, including conjugate pad, test and control lines. As it flows laterally through the assay, the analyte mixes with freeze-dried bioactive particles, called conjugates, that have immobilized antibodies (or antigens) displayed on their surfaces.

If the target molecule is present in the sample, it will bind to its chemical partner in the conjugate pad.
In sandwich lateral-flow assays, the test line also contains antibodies that are specific to the target analyte. If present, that analyte will also become associated with or bind to the molecules in the test line and display a visible signal (often indicated by color change or fluorescence).

Competitive lateral-flow assays are generally used to detect smaller analytes that have fewer binding sites. Copies of the target analyte are immobilized in the test line. Only when the analyte is absent from the sample will the conjugate antibodies bind and display a visual signal in the test line.

In both types of assays, the control line shows whether the sample has flowed through the assay and the biomolecules are active.

INPUT: NUCLEIC ACID

NUCLEIC ACID AMPLIFICATION TESTS (NAAT)

WHAT IT DETECTS:

Specific genomic sequence(s) of a target pathogen in a sample.

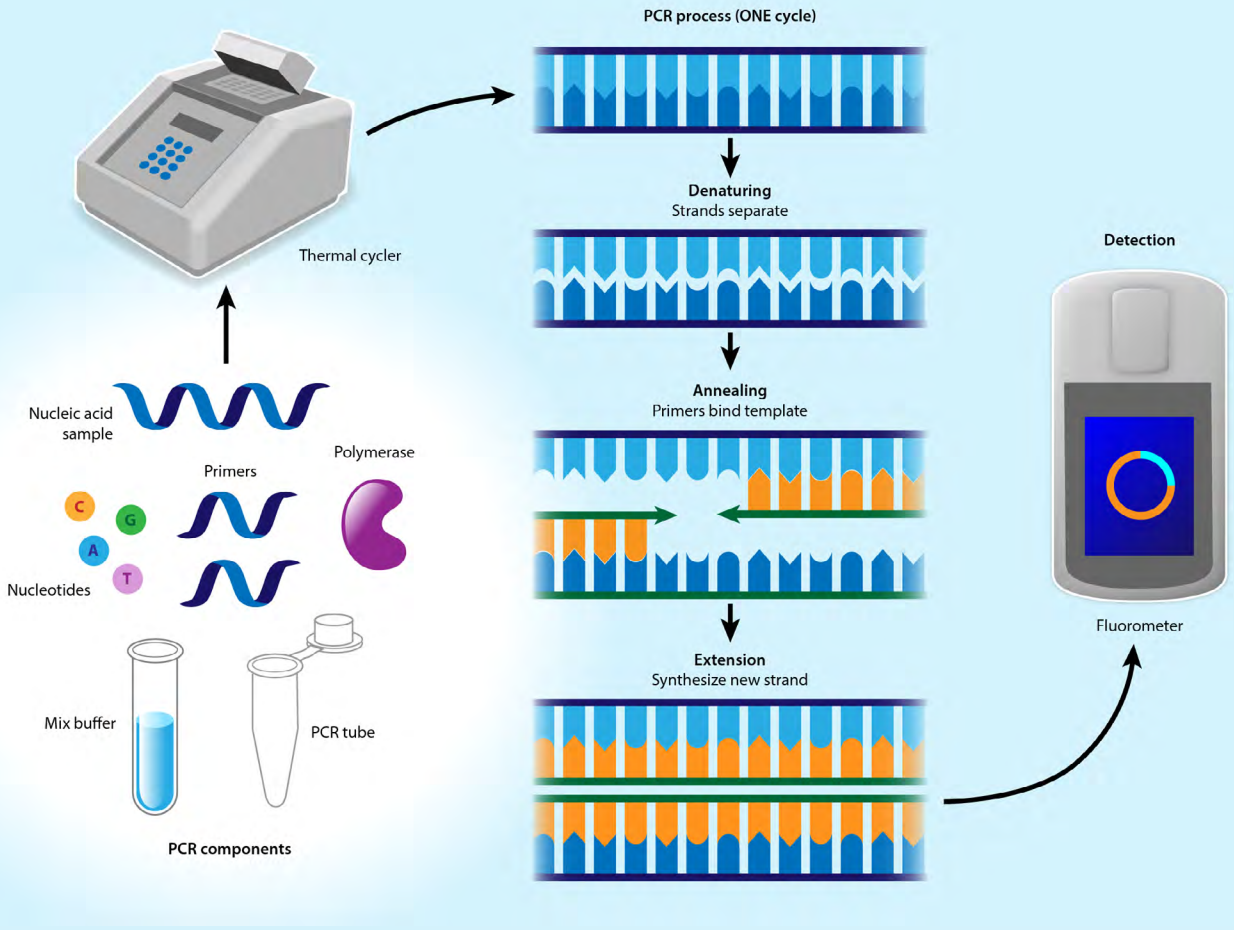
VARIATIONS:

- RT-PCR
- Multiplex-PCR (multiple primer pairs designed to amplify several regions of the same pathogen's genome or regions across several pathogens' genomes),
- Loop-mediated isothermal amplification (LAMP)
- Strand displacement

EXAMPLES:

- Respiratory virus panels
- HIV, HSV, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

HOW IT WORKS:



Primers for one or more genomic sequences are used to amplify nucleic acids from a pathogen or group of pathogens. Amplification products are then detected using fluorescent dye, sequence-specific labeled probes or electrophoresis.

INPUT: NUCLEIC ACID

NEXT GENERATION SEQUENCING (NGS)

WHAT IT DETECTS:

All or part of the nucleic acid sequence(s) of a single pathogen or mix of pathogens in a sample.

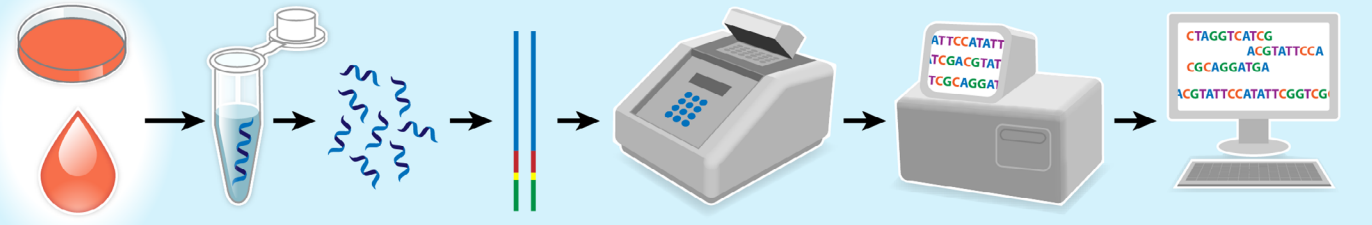
VARIATIONS:

- Whole Genome Sequencing (WGS)
- Metagenomic Next Generation Sequencing (mNGS)
- Targeted Next Generation Sequencing

EXAMPLES:

- Genomic surveillance and outbreak identification
- Detection of foodborne pathogens

HOW IT WORKS:



Sample collection
Culture isolates or patient sample

Nucleic Acid Extraction
Nucleic acid is isolated and purified

Library Prep
Nucleic acid is sheared to create fragments of known lengths
Specific adaptors and barcodes for multiplex sequencing are ligated to nucleic acid fragments

Amplification
Nucleic acid is amplified using PCR or hybridization approach

Sequencing
Nucleic acid fragments are sequenced to produce "reads"

Genome assembly and analysis
"Reads" are assembled in the correct order for analysis

Various technologies capture all nucleic acid sequences of organisms present in a sample. Data can be analyzed for full genomes of a predominant pathogen (*i.e.*, typing or outbreak-tracing) or a mix of pathogens.

INPUT: CULTURE ISOLATES

SPECTROSCOPY/SPECTROMETRY

WHAT IT DETECTS:

Protein (or other biomolecular) profile of a single pathogen.

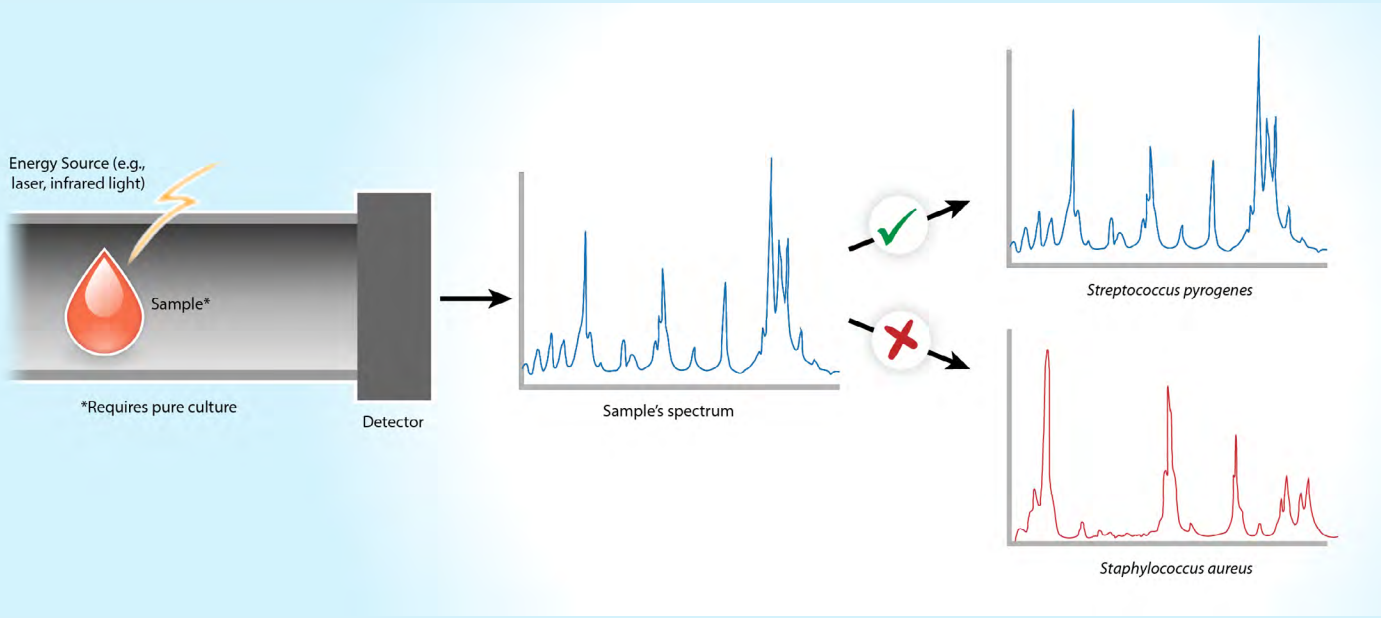
VARIATIONS:

- Fourier-transform infrared spectroscopy (FTIR)
- Matrix-assisted laser desorption/ionization-time of flight (MALDI-ToF)

EXAMPLES:

- *Candida auris*
- *Enterobacter cloacae* complex typing

HOW IT WORKS:



An isolate is either bombarded with infrared light (FTIR) or ionized and run through a detector (MALDI-ToF) to capture a fingerprint-like spectrum. Comparison to a database of known spectra enables organism or strain identification.

CONSUMER BEWARE:

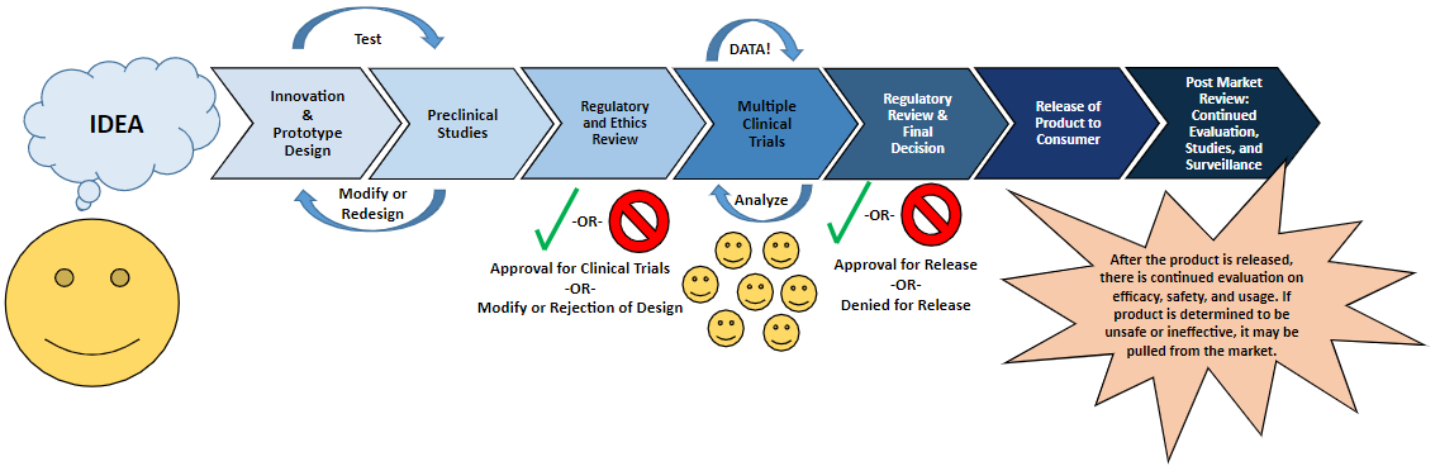
The Cost of the Rare Fraud in the Biomedical and Diagnostics Industry

BY AMY KULLAS, PH.D.

In vitro diagnostics (IVDs) are an integral part of our health care system. Medical providers regularly rely on these diagnostic tests, which analyze samples like blood, saliva or urine, to assist with the diagnosis of disease or other medical conditions. Laboratory tests are routinely included in ~1/4 of adult patient care visits to assist with improved disease management and overall patient wellness. There are approximately 13 billion laboratory and diagnostic tests performed in the U.S. each year. This equates to the IVD and clinical laboratory services industries generating an estimated $87.3 billion in revenue! The extraordinary number of tests performed and the estimated revenue present an enticing opportunity for potential abuse and fraud, however. Although the number of cases tried for abuse and fraud in the biomedical industry is relatively low, results from investigations suggest that the losses due to fraud can be in excess of "tens or as high as hundreds of millions of dollars."

> *Unfortunately, the COVID-19 pandemic allowed for a surge of companies to profit from and prey on people's fears by selling unproven products that made bogus claims of efficacy and/or prevention to offer a fake sense of security.*

The U.S. Food and Drug Administration (FDA) plays an incredibly valuable role in both the approval of IVDs and identification of fraudulent products. The FDA regulates IVDs as medical devices for which there is an established "risk-based device classification system" (Classes I [low risk]-III [significant risk of illness or injury], accounting for regulations needed to ensure reasonable safety and effectiveness assurance). The Food, Drugs, and Cosmetics Act, Section 201(h), defines a medical device as a health care product for the "use in the diagnosis of disease or other conditions … that does not achieve its principal intended purpose by chemical action or by being metabolized."



Product Idea to Market Release. Early pilot and preclinical studies aim to understand the general feasibility of the product and to collect safety and device performance data. They also provide insight into whether modifications are necessary. Clinical trials are large studies to confirm efficacy, safety and potential risks. They are guided by data, and the analysis is statistically driven. After the release of the product to the consumer, there is still a post-market review that continues to monitor long-term efficacy, safety and usage in the marketplace. (Figure is based on information from the FDA's 'Pathway to Approval.')

Biotech companies are not analogous to tech companies. It takes significantly longer to develop a medical device, new drug or vaccine. Taking a product from the idea phase to approval takes an average of three to seven years and often involves several regulatory reviews and multiple clinical trials (Figure 1). The FDA provides two principal premarket review pathways for IVDs. Generally, for IVDs that fall into Class I or II, the pathway is premarket notification, or the 510(k) pathway. This pathway is largely for products that can be considered "substantially equivalent" to one that is currently in the market or products that are low to moderate risk. Class III products (approximately 10% of IVDs) go through a more rigorous process, a premarket approval pathway, which requires a demonstration of both safety and efficacy before the product can be marketed. Even after a product comes to market, there is continued surveillance and post-market studies that occur to ensure the continued safety and evaluation of the product (Figure 1).

However, like in all other human endeavors, there are people who make false claims for their own personal profit. A few examples emphasize what can go wrong. A widely publicized example was the rise and fall of Theranos, a company that claimed it would transform the diagnostics industry. The company claimed that they had developed a technology to quickly and accurately perform 70 diagnostic tests on a drop of blood; this is roughly 1/100-1/1000 of what other tests require. Thus, the company also claimed that the cost of their test was also significantly lower — approximately 10% compared to the cost of centralized laboratories.

Theranos, founded in 2003, quickly raised more than $700 million from investors. This allowed the company to boast of an astounding $9-10 billion valuation at the company's peak in 2014. Consequently, the CEO, whose own net worth was estimated at $4.5 billion, revealed in the company's recognition and publicity, promoted the technology in a variety of venues, from a TEDMED conference to the Clinton Global Initiative, among others. In July 2015, Theranos received its first (and only) FDA approval, for a herpes test. Shortly afterward, the company began to tumble because data (or lack of it) does not lie.

A steep downward spiral soon started for Theranos following reports about the inaccuracy of the tests. A researcher wrote in the Journal of the American Medical Association that the company had not published any studies in academic or peer-reviewed journals. Others in the scientific community scrutinized the technology and determined that "most of the company's claims are exaggerated." In late 2015, the company lost a major partnership with the Safeway grocery store chain. Then in January 2016, Theranos failed a laboratory inspection. By that April, the U.S. Attorney's Office and the Securities and Exchange Commission (SEC) were involved and investigating the company. The company lost its Clinical Laboratory Improvement Amendments (CLIA) certification. Subsequently, Walgreens terminated its partnership with Theranos and sued the company for breach of contract in federal court.

Despite all of these reports and red flags, Theranos could have harnessed several opportunities for redemption, but it ignored them. At an American Association for Clinical Chemistry meeting in August 2016, the CEO delivered a 90-minute presentation during which the public had been expecting to see the data behind the company's robust claims. However, the presentation failed to meet expectations. After the presentation, the moderator commented, "The evidence you presented fell far short…." The data shown (which were neither independently validated nor confirmed) were primarily from conventional blood draws, taken from arms in larger volumes, and not from single drops of blood from finger sticks. Then as the company was nearing bankruptcy in 2017, it was able to secure a loan from Fortress Investment Group (FIG) for $100 million, using its patents as collateral. In the conditions of the load, FIG required Theranos to produce an independently audited financial statement. Ironically, it was the monetary scandal and not the shady science that led to the ultimate demise of Theranos. The SEC charged both the CEO and COO in early 2018 with "massive fraud." There was a hasty search for a potential buyer of the company, but that too fizzled, and the company finally dissolved in September 2018. Theranos was ultimately required to turn over the ownership of its patents to FIG.

uBiome, a microbiome diagnostics company, was "once compared to Theranos" and hailed to be another example of an "up-and-coming" biotech company that exponentially grew and shined brightly in the limelight, only to be rapidly excreted from public favor. Founded not even a decade ago, the company initially provided a "Gut Explorer" service directly to the public. An individual would "submit" a stool sample to uBiome for analysis in its labs. uBiome then provided a report and analysis of the individual's microbiome to those of other consumers who had also sent in stool samples to the company, all for around $100. The company's founders subsequently expanded the scope of the company to develop "clinical tests" for both vaginal and gut microbiome analysis. According to the DOJ's indictment release, "uBiome would seek reimbursement from health insurance providers in amounts up to nearly $3000." The SEC brought fraud charges against the company's co-founders in March 2021. The SEC alleges that the founders "touted uBiome as a successful and fast-growing biotech pioneer while hiding the fact that the company's purported success depended on deceit."

> *Other companies have exploited the current COVID-19 pandemic. The sheer number of products and tests that have flooded the market during the COVID-19 pandemic has been astounding.*

These products come in a plethora of categories, from supplements, vitamins and other foods to products claiming to be tests, drugs, medical devices or vaccines. A couple of examples that have come under scrutiny and recent investigation during the COVID-19 pandemic are Arrayit and Decision Diagnostics. Arrayit claimed that it could detect the coronavirus in dried blood samples. According to the Department of Justice (DOJ), the company's president declared it could test for COVID-19 and allergies with a blood smear placed on an index card that would then be mailed to Arrayit's lab. Similarly, Decision Diagnostics told its investors that the company had developed a rapid, 15-second blood test to detect COVID-19 at home using an automated device. However, this test was still in the idea phase and nowhere near the development of a prototype. Both of these cases are still pending adjudication by the DOJ.

The FDA and other government agencies have been working around the clock with retailers to remove "misleading products" from shelves and online marketplaces. As appropriate, the FDA can send "warning letters, or pursue seizures or injunctions against people, products, or companies that violate the law." Additionally, many public health officials continually sound the alarm and raise awareness of these fraudulent and misleading products. Education and engagement with the public remain integral parts of their understanding. Also, consumers need to pay attention to products that offer a disclaimer, such as, "These claims have not been approved or supported by the FDA." If a consumer does not purchase or buy into the hype of these products, then there will no longer be a market for them.

Fraudulent products and claims can have a long-lasting negative stigma on the broader scientific community and the public's faith in science. The misguided "anti-vaccination movement" was largely built upon a now-retracted paper. Because its fraudulent conclusions continue to be perpetuated by some, its effects still ripple through the scientific community and beyond. A present-day outcome is that many individuals remain hesitant to receive one of the available COVID-19 vaccines, which have received Emergency Use Authorization (EUA) by the FDA. EUA allows the FDA to approve the use of "unapproved medical products … in an emergency to diagnose, treat, or prevent serious or life-threatening diseases or conditions." The FDA requires the EUA application to include the comprehensive safety data from clinical studies of both phases 1 and 2, along with an "expectation that phase 3 data will include a median follow-up … after completion of the full vaccination regimen." The COVID-19 vaccines were carefully tested and evaluated through multiple extensive clinical trials with "tens of thousands of study participants." Further, the FDA will continue to monitor the data and follow-up analyses with the individuals from the trials for the evaluation of safety and effectiveness for consideration during the subsequent approval and potential licensure process. Ultimately, vaccination remains key to getting the current pandemic under control.

Here are some warning signs to watch out for when considering a new product:

- Products that claim to be effective for treating a wide range of health conditions and/or diseases.
- Products that don't provide preliminary results from clinical trials, but rather rely on personal testimonials of the product.
- Products marketed as "quick fixes" or "miracle cures," or that offer a "revolutionary change" from current products.
- Products that claim 100% accuracy or effectiveness. (If it sounds too good to be true, it almost certainly is!)

As a consumer or potential investor, it is imperative to educate oneself on any product. Be mindful of the hype and the "one-pill magical cures," especially when there are not any data or trials to back it up. Similarly, it is important for the scientific community (from governmental agencies to academia, nonprofits and even industry) to remain as unbiased as possible and lean on moral guidance. Scientists play a pivotal role in evaluating the underlying data objectively of a company's claims. It also is important to help friends, family and the broader community distinguish between justified claims and "magical cures."

Lastly, the focus should be on the treatment, cure or prevention of disease for the patient, not on the profits. Fraud can be aided by a charismatic CEO overselling the technology, investors who fail to request the preliminary data and the financials, or the fact that there was no data ever shown for the product as it was intended in the form of accurate numerous diagnostic tests. Fraud in biomedical diagnostics is rare, but the implications and stakes are high, because people's health, wellness and even lives may be on the line. Detecting fraud demands the attentiveness of and proactive response from scientists who care and continuously work to preserve the integrity of the life sciences and biomedical industries.

Leadership Update

BY VICTOR DIRITA, PH.D., ASM PRESIDENT

Farewell and Updates from June Board of Directors Meeting

It has been a privilege to serve as ASM President this past year. Although it was a challenging year, there have been signs of hope. Building on years of fundamental research scientists developed new vaccines for COVID-19; the scientific and global health communities came together to distribute those vaccines and ensure equitable access; and just last week ASM convened 9 societies, including experts and thought leaders from more than 120 countries, to discuss and solve some of the world's most pressing challenges at World Microbe Forum. There have also been many signs of progress within ASM, as the Board of Directors (BOD) continues to work on several initiatives – these include the nominations process, our DEI efforts, and an ongoing program review – all with the goal of making ASM the most inclusive, transparent, and accountable scientific society in the world.

NOMINATIONS PROCESS

Toward ASM's commitment to ensuring a more diverse and inclusive ASM, the Board approved a pilot project for volunteer leadership appointments and nominations, based on recommendations presented to the ASM Board by the DEI Taskforce at the December 2020 Board meeting. This effort, led by our Past-President Robin Patel, will start this year. We will populate slates for elected and appointed volunteer leaders based on specific profiles that describe core criteria, including competencies, skills, and behavior expected for the given positions.

ASM will conduct inclusive, thorough searches for candidates, with the goal of identifying the best candidates and expanding inclusion and representation from historically underrepresented scientists in the microbial sciences. This is how we will aim to improve inclusion and access to ASM volunteer opportunities. As part of this reform, ASM is realigning all elections to fall on the same timeline, November 1 to December 1.

DIVERSITY, EQUITY & INCLUSION

The DEI taskforce presented their report to the ASM Board at the December 2020 Board meeting. At the March Board meeting, the Board approved a 3–5-year implementation plan, developed by an ad hoc cross-organizational staff task group and dedicated to the implementation of the DEI taskforce report.

The plans we have laid out so far are aggressive and encompass a rapid and clear response to some of the most pressing recommendations made in the DEI taskforce report. However, this work is only the beginning of society-wide action in making ASM, and the microbial sciences, more diverse and inclusive.

To meet these goals, the Board decided that it is essential for ASM to form a high-level, integrated, organization-wide Committee that will coordinate all DEI activities and ensure that DEI is the lens through which we look at every ASM activity. To this end, the Board approved a new Committee of the Board (the highest Committee level in ASM) fully dedicated to Inclusive Diversity with Equity, Access and Accountability (IDEAA). Recognizing the urgency for acting quickly, the Board approved an interim committee roster with Board Members Greetchen Diaz as Interim Chair, and Julie Segre and me as interim members. The committee will work together with our professional staff led by Kim Shankle, ASM's Director of Human Resources and Administration. Permanent Committee members will be appointed following our new nomination processes described above.

ASM will also begin to collect enhanced demographic data on Race, Ethnicity and Gender. This will establish a baseline in order to measure progress toward embodying our ideals of inclusion, equity and access.

ASM PROGRAM REVIEW

As mentioned in the last Board update, the ASM Board also approved putting every ASM program on a permanent cyclical assessment review to ensure proper alignment of ASM's programs to its mission, strategy and sustainability. The program review will ensure that ASM programs and offerings are scientifically updated, relevant to our communities, and provide maximum value in promoting and advancing the microbial sciences. To put this plan into action, the Board has established two committees, loosely modeling the NIH peer review system.

Thank you for all that you do to promote and advance the microbial sciences. It has been my privilege to serve as your ASM President this past year. Among the most satisfying aspects of the job has been the opportunity to work closely with CEO, Dr. Stefano Bertuzzi, and the incredibly professional staff within ASM. As microbial scientists, we benefit tremendously by having professional partners who can work with us to extend our scientific interests to advocacy, communications and publishing. I look forward to seeing this partnership and progress continue as Dr. Steven Finkel takes the office of ASM President on July 1. I am grateful for your support this year.

What's New at ASM

BRANCH SPOTLIGHT: MARYLAND BRANCH

The Maryland Branch formed from the Maryland Society of Bacteriology in Baltimore in 1931. The Maryland Society of Bacteriology was initially independent of the Society of American Bacteriologists (SAB), which became ASM in 1960. The first president of the Maryland Branch was William W. Ford of Johns Hopkins University School of Hygiene and Public Health.

In 1934, a provision was made by the SAB for local Branches to be represented on the SAB council. This ruling stimulated the growth of local Branches. In 1935, under the leadership of President Clinton L. Ewing of the Baltimore City Health Department, 11 members of the Maryland Society of Bacteriology petitioned the SAB council for local Branch status. In 1936, Branch status was granted for the Maryland Society of Bacteriology, along with six additional regional organizations. The number of local Branches increased to 12 nationwide. J. Howard Brown was appointed the first councilor of the Maryland Branch.

Currently, the Maryland Branch has an active community of 64 members from local academic, industry and government institutions. The Maryland Branch holds four dinner meetings per year, three of which feature a talk by a renowned expert regarding current topics in microbiology. Although the Branch does not currently have a Student Chapter, the fourth meeting is a student-focused poster session and talks, with awards presented for the most outstanding undergraduate and graduate student presentations. These sit-down dinner meetings include a preceding social hour, are well attended and provide participants with excellent opportunities for networking.

The Maryland Branch has meetings in February/March, October and December. Microbiology topics that have been covered in recent Branch meetings include water purity and Legionnaire's disease, influenza and flu vaccines, microbial pathogenesis, and the effects of the microbiome on human diseases. The October meetings are typically hosted at the University of Maryland Baltimore Country (UMBC) Albin O. Kuhn Library, which also houses the ASM Archives. Every year since 2015, ASM Archivist Jeff Karr has arranged a special display from the archives for the attendees. This meeting is held just prior to the annual ASM Board of Directors meeting in Washington, D.C., and invitations are extended to the ASM National Board members and other staff at ASM headquarters. The current ASM president normally speaks at the October meeting. At the December meeting, the Branch typically invites a local expert to present the keynote talk. At the February/March meeting, one of the ASM Distinguished Lecturers gives the keynote presentation. Following the March 2020 shutdown due to COVID-19, the Branch quickly organized two virtual Zoom meetings on COVID-19 topics and has continued with virtual meetings in 2020-2021.

More Information About the Maryland Branch

SAEED KHAN, PH.D.

ASM'S COUNTRY AMBASSADOR TO PAKISTAN

Dr. Saeed Khan is a professor of pathology and the head of the Molecular Pathology section at Dow International Medical College and the additional director of the Institute of Basic Medical Sciences (IBMS). He received a Ph.D. in microbiology from the University of Karachi in collaboration with Aga Khan University Karachi-Pakistan, followed by post-doctoral training in molecular virology from the Mullins Molecular Retrovirology Laboratory at the University of Washington in Seattle. Dr. Khan has been ASM's Country Ambassador to Pakistan since 2020.

Describe your experience working as the head of the Molecular Pathology section at Dow Diagnostic Reference and Research Laboratory.

My experience as head of the Molecular Pathology section at Dow Diagnostic Reference and Research Laboratory is interesting and challenging. When I started in 2011, there were hardly any resources available for testing complex diseases. I used my expertise and knowledge during my graduate and post-graduate studies in the United States to design new technologies and assays and to increase the list of parameters particularly for transplants. We developed different tests for liver, kidney and bone marrow transplants, as well as testing for virology and different cancers. It was a very rewarding experience to utilize my skills and abilities to serve the community. I am very proud to make this contribution to the society. However, working in a resource-limited setting is very challenging. The technology isn't advanced, and even when financial resources are available, ordering supplies from abroad can be an extremely time-consuming process. Therefore, we have managed to improvise and follow this main principle: Things can be done no matter how complex or difficult; if you have commitment and willpower, anything is possible.

How has working on the frontlines in Pakistan during the COVID-19 pandemic impacted your career?

I will never forget January 25, 2020, when I thought of an idea to develop diagnostic tests for COVID-19. I sent a text message to my vice chancellor that COVID-19 is hitting China. At that point, only 3,000 COVID-19 cases were reported in China and nowhere else in the world. Although the cases were restricted to China at the time, I predicted that we would need diagnostic tests in Pakistan immediately. The COVID-19 pandemic had not yet been declared, so there were no kits available commercially. I am thankful that my vice chancellor gave me his approval to order raw reagents and design our own testing kits. With limited resources, we were able to make our kits available in early February. My prediction was correct because we started receiving samples at the end of February. We were so proud for taking a proactive approach and having all the required reagents and consumables, kits and PPEs ahead of time.

Those initial days of the COVID-19 pandemic were very difficult because everyone was afraid of death. So many patients were afraid of receiving a negative diagnosis. It is so remarkable to see the relief on a patient's face after receiving good news that they are no longer positive for COVID-19. We have faced a lot of issues along the way, but have succeeded due to trial and error. I have used my experience to provide valuable suggestions and guidelines to different policy-making committees of the Sindh government, NGOs, WHO and federal government. As molecular biologists and microbiologists on the frontlines, we continue to make numerous contributions in our roles toward the betterment of society and to fight this pandemic.

Tell us more about your research in molecular virology and your work with the Mullins Molecular Retrovirology Laboratory.

Those were the golden days in Seattle! I love the city of Seattle, and I had a wonderful experience at the Mullins Molecular Retrovirology Laboratory. Dr. James Mullins is extremely skilled. He trained me in virology, particularly in the bioinformatic area to sequence HIV and to read inside the sequences to make consensus regions for vaccine production. We worked on numerous projects, including learning different PCR techniques, RTPCR and sequencing in collaboration with the Fred Hutchinson Cancer Research Center. When I returned to Pakistan, I used all the techniques that I learned at Mullins Lab and taught my students. We have published several research articles. I am grateful that Dr. Mullins is my mentor and that we still have a strong collaborative relationship.

Why did you become an ASM Country Ambassador, and what has been your most rewarding experience thus far?

I have been affiliated with ASM for nearly a decade. I started attending the ASM Annual Meetings, now ASM Microbe, and developed relationships with ASM ambassadors Dr. Shahana and Dr. Irum. When the ASM Ambassador to Pakistan opening was announced, I applied because I also wanted to contribute to ASM. I was appointed as the ASM Ambassador to Pakistan in 2020, but unfortunately due to COVID-19, there were very few opportunities to get involved. However, I was able to train hundreds of hospital and laboratory workers under the ASM umbrella during this time. I trained people with the government of Sindh and WHO on COVID-19 protocols such as biosafety and biosecurity, processing samples safely, properly caring for COVID-19 patients and how to discard waste. I felt so proud to be part of the ASM Ambassador program. I'm excited about our upcoming webinar series for health care heroes leading the frontlines to combat COVID-19. We are inviting health care workers from Pakistan, Khyber Pakhtunkhwa, Punjab, Balochistan, Gilgit-Baltistan and Azad-Jammu-Kashmir to join this webinar. We want to connect with every microbiologist across the country to spread our message about ASM and encourage them to be part of the ASM family.

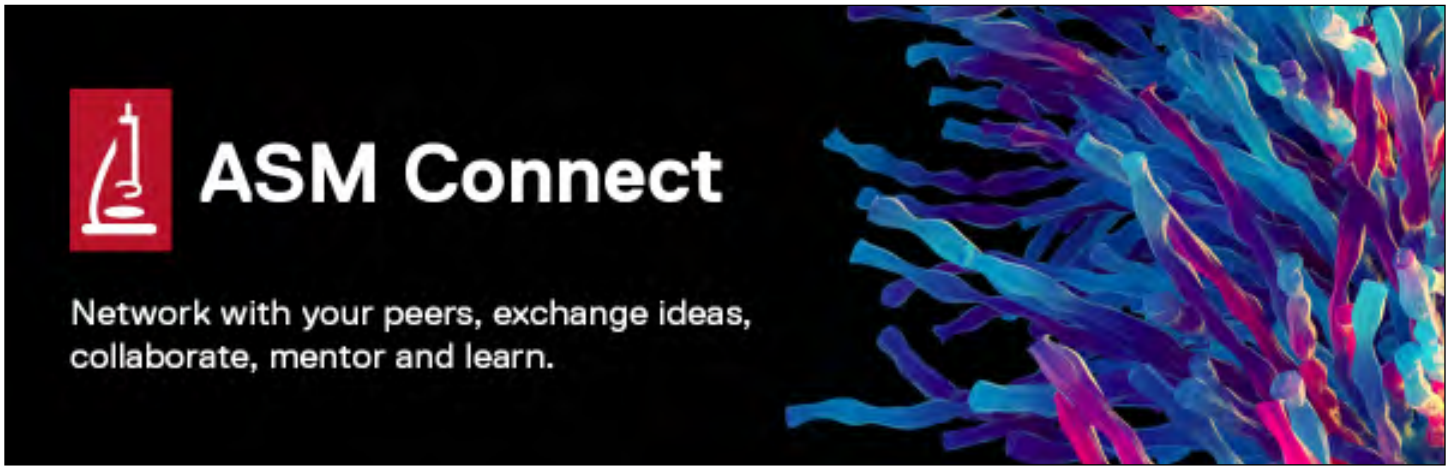
What are your plans and future goals as an ASM Country Ambassador?

As a country ambassador for ASM, I want to build a stronger membership network. I want us to be able to unite and utilize our resources to help each other. My goal is to expand the network in my country and then expand internationally. We currently have an internal network of 1,000 contacts, and it's growing. My plan is to continue to reach more like-minded people and help spread the mission of ASM. As our contribution to society, we should unite to identify and diagnose different infectious diseases and discover solutions for these pathogens in our roles as microbiologists and members of ASM.

MICROBIOLOGY IS ... YOUR MICROBIOME, YOUR HEALTH

Dr. Filipa Godoy-Vitorino, Ph.D. discusses the importance of a healthy microbiome and discusses ways we can keep our microbiomes functioning properly in order to prevent diseases, such as cervical cancer.

Watch the video



JOIN THE CONVERSATION ON ASM CONNECT

Collaborate and network with ASM leaders and members from around the globe on our new online community platform, ASM Connect. This exclusive and secure members-only community allows members to meet microbial science experts and make new connections.

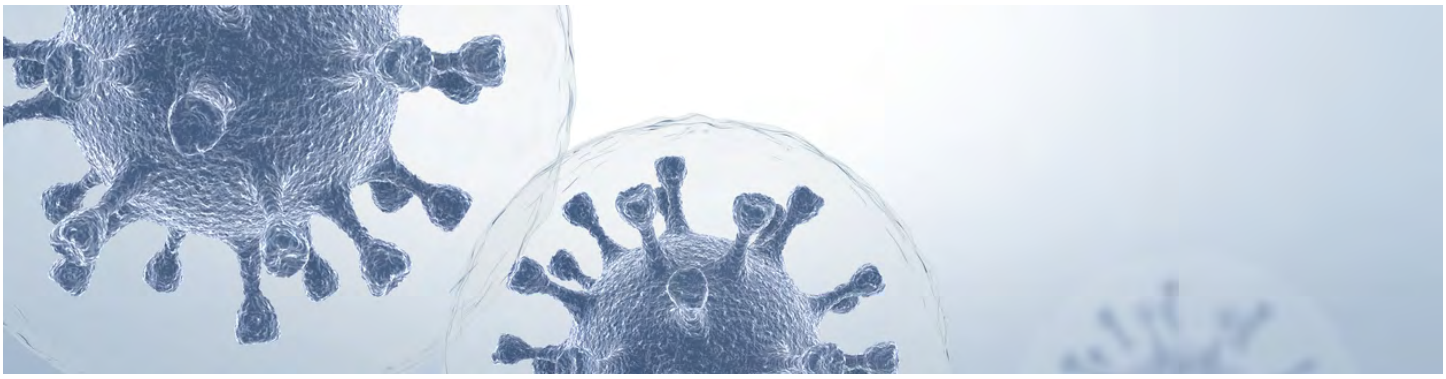
ASM Connect allows you to:

- Pose questions, participate in discussions about the latest research and share information with global members.
- Network and collaborate with leaders in your field.
- Attend member-exclusive events and discuss upcoming meetings.
- Get updates from peers straight to your inbox (receive posts in real time via email or opt for a daily or weekly digest).

The opportunities to connect with your peers are limitless. Log in to ASM Connect

What's Hot in the Microbial Sciences

BY ASHLEY HAGEN, M.S. & STANLEY MALOY, PH.D.

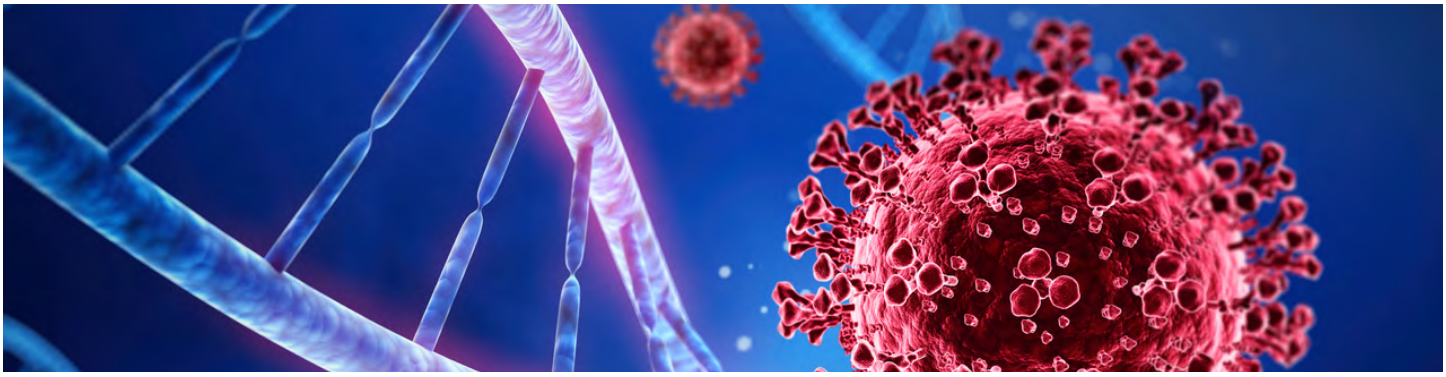


COVID-19 VARIANTS

The evolution of SARS-CoV-2 has posed the threat of new variants that may exhibit increased transmissibility or potential for antibody escape. This threat has amplified the importance of next-generation sequencing methods that can decipher and track how and when the virus is mutating. A study published in Nature demonstrated that chronic SARS-CoV-2 infection leads to the evolution of viruses with reduced sensitivity to neutralizing antibodies that are passively acquired through convalescent plasma treatment. Over the course of 101 days, researchers generated whole genome sequences from 23 time points, demonstrating the emergence of a dominant viral strain that contained a D796H substitution in the S2 subunit and a deletion (ΔH69/ΔV70) in the S1 N-terminal domain of the spike protein. Together, these mutations conferred a two-fold decrease in sensitivity to convalescent plasma in an immunocompromised host. However, as serum antibodies from the treatment diminished, so did the concentration of viruses with these escape mutations, suggesting that selective pressure in the host was driving viral adaptation.

Growing evidence indicates that many SARS-CoV-2 variants share similar combinations of mutations and might not be as highly variable as we once feared. In an article published in Scientific American, Dr. Vaughn Cooper, evolutionary biologist and ASM COMS-elected Board Director, describes how increased genome surveillance of the coronavirus has allowed several recent studies to identify signatures of convergent evolution, indicating that certain mutations that repeatedly arise in independent lineages with increased frequency over time may be linked to increased viral fitness. Dr. Cooper's lab has identified at least seven independent lineages that acquired a mutation at position 677 in the virus' spike gene, and the E484K mutation that has been correlated with antibody escape appears in multiple SARS-CoV-2 variants, including B.1.352, P.1 and B.1.526. Other mutations, including the ΔH69/ΔV70 deletion, as well as substitutions in the receptor-binding domain (at positions 417, 452 and 501) and near the furin cleavage site (at 681 and 701), have also been identified in multiple SARS-CoV-2 lineages.

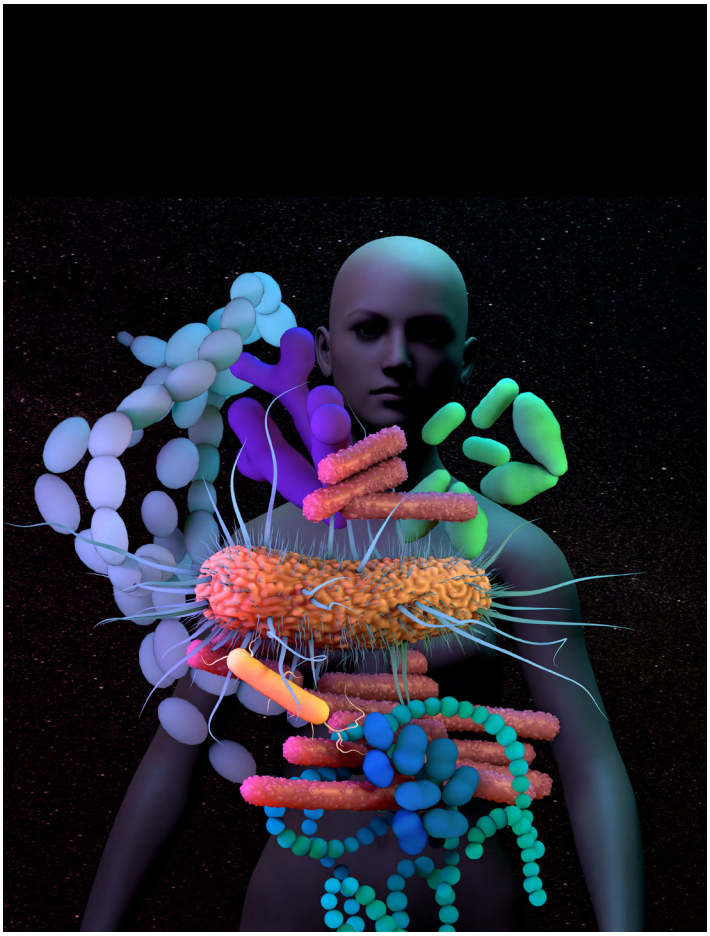
Although new major adaptations to the SARS-CoV-2 genome are relatively few and far between, Dr. Cooper warns that increased selective pressure, along with increased numbers of SARS-CoV-2 infections, facilitate continued virus evolution. In fact, a study in Nature Medicine describes the identification of 16 new lineages of SARS-CoV-2 isolated in South Africa between March 6 and August 26, 2020, many of which possess unique mutations that have not been identified elsewhere. Getting infection numbers under control and continuing to track SARS-CoV-2 variants are therefore key to managing the spread of the disease.



ROLE OF VIRUS AND HOST IN HUMAN DISEASE

Ever since it became feasible, genomic sequencing has been used to understand host susceptibility to a variety of infectious diseases. Likewise, it was expected that genetic risk factors play a role in the progression of COVID-19. A genetic association study aiming to better explain the wide variation in clinical manifestations, ranging from asymptomatic to mild to severe disease and death, was published in Nature in September 2020. In this study, scientists identified a haplotype, located on chromosome 3, that is associated with increased respiratory failure and a 60% higher risk of being hospitalized with COVID-19. Phylogenetic analysis revealed that the gene cluster is inherited from Neanderthals and found in approximately 50% of people in south Asia and 16% of people in Europe.

The same team of scientists recently published a study in PNAS that identified a different Neanderthal haplotype, located on chromosome 12, with the opposite effect. According to the report, each copy of this OAS1 variant reduces the risk of developing severe COVID-19 by approximately 22%. This region encodes oligoadenylate synthetases, enzymes that are involved in host immune responses that degrade RNA viruses and activate additional antiviral mechanisms. Genes on the OAS locus have proved to be protective against at least three additional viruses, including West Nile virus, Hepatitis C and SARS-CoV. There is still much to be learned about how genetic factors impact disease severity and host immune responses to COVID-19, but these studies have begun to unravel the mystery.



MICROBIOME ASSOCIATION

The gut microbiome is an important regulator of adaptive immunity. It has been shown to influence tumor development and modulate host responses to chemotherapy and immunotherapy. An article published in Nature Microbiology describes the identification of specific microbial signatures in patients with non-small-cell lung cancer (NSCLC) and how the bacteria identified impact the therapeutic efficacy of cancer drugs.

Linear discriminant analysis Effect Size (LEfSe analysis) of 16S ribosomal (rRNA) sequencing data from 96 NSCLC patient stool samples and 139 healthy controls was used to determine how cancer therapeutics affect microbiome composition. *Bifidobacterium bifidum* was significantly enriched in those who responded to treatment, and qPCR confirmed these results. Interestingly, previous studies have demonstrated that *Bifidobacterium spp.* enhances therapeutic efficacy of PD-1 blockades through dendritic cell maturation.

Next, in order to evaluate the therapeutic potential of the bacterium, syngeneic mouse tumors were treated with commercial strains of *B. bifidum*. Scientists found that only specific *B. bifidum* strains worked synergistically with PD-1 blockade or oxaliplatin treatment to reduce tumor burdens in mice. It was therefore proposed that *B. bifidum* modulates antitumor immune responses through the biosynthesis of immune-stimulating molecules and metabolites that potentiate interferon-γ production.

ONE HEALTH

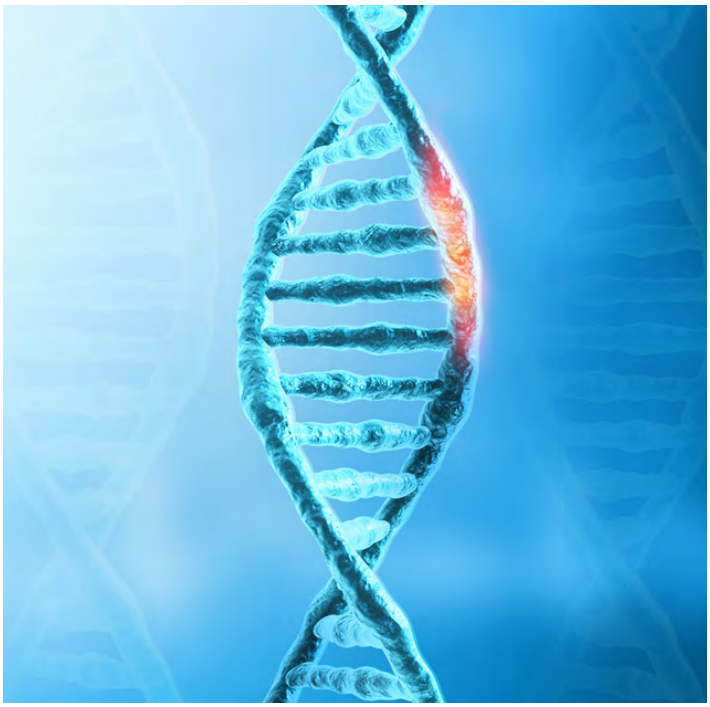
In what some are calling an impressive case of scientific detective work, researchers have finally identified the cause of vacuolar myelinopathy (VM), the neurological disease linked to mass eagle deaths across the southeastern U.S. for nearly two decades. VM is known to cause lesions on the brains of waterfowl and birds of prey, and has recently been found to also affect fish, worms, amphibians and reptiles.

In 2014/2015, the occurrence of VM was tied to the presence of a cyanobacterium, *Aetokthonos hydrillicola*, which was discovered growing on an invasive plant called *Hydrilla verticillate* in manmade bodies of water. Since the discovery of *A. hydrillicola*, researchers have suspected a cyanobacteria-produced neurotoxin to be at the root of VM. They were able to confirm the toxicity of crude extracts of *A. hydrillicola* taken from VM sites; however, laboratory cultures were not found to be neurotoxic.

A study published in Science describes how mass spectrometry was used to expose the colocalization of VM-positive cyanobacteria samples with a brominated metabolite, subsequently named aetokthonotoxin (AETX). When researchers supplemented laboratory cultures with potassium bromide, *A. hydrillicola* readily produced AETX in the lab. Leghorn chickens that were gavaged with AETX developed VM brain lesions, while control chickens did not, confirming that AETX is the causative agent of VM. Furthermore, the metabolite is fat-soluble, suggesting that it has the potential to accumulate in tissues and pose a significant threat to the food web.

"One Health" emphasizes the relationship between environmental, animal and human health. This example demonstrates the role of environmental disruption in the transmission of neurological toxins and transfer through the food web. Understanding the ecological pressures and dynamics that drive this transfer can inform targeted mitigation strategies to protect both wildlife and human health.

Because AETX biosynthesis is dependent on the availability of bromide, an important step to controlling the spread of VM is identifying unnatural bromide sources and eliminating them from the environment. For example, the herbicide diquat bromide has been shown to accumulate in the leaves of the hydrilla plant upon which the cyanobacteriam feed. Rather than using herbicides to control this invasive species, stocking lakes with sterile fish that eat hydrilla could offer an alternative, chemical-free approach for containing this aggressive plant. Researchers suggest that coal-fired power plants, road salt, fracking fluids and brominated flame-retardants may also be sources of bromide in manmade bodies of water.



MOLECULAR MICROBIOLOGY

A fundamental question in molecular biology has to do with the coordination of bacterial replication: How do bacteria ensure that DNA replication and cell division proceed efficiently during metabolic conditions that are required for growth? A Journal of Bacteriology study characterized an essential role of the TusA protein that links translation efficiency to cell division in *E. coli*. TusA is a highly conserved and versatile protein. After transcription, TusA is responsible for inserting sulfur modifications into nucleosides of tRNAs that encode Lys, Gln and Glu at position 34. Posttranscriptional sulfur modifications, like the formation of 2-thiouridine, help ensure that translation proceeds accurately and efficiently. TusA-deficient mutants have been shown to exhibit poor viability, a defect in cell division and a filamentous morphology.

In this study, researchers discovered that lack of thiolation of wobble uridine (U34) nucleotides on Lys, Gln or Glu tRNAs deregulates the production of RpoS (Sigma S) and Fis (Factor for Inversion Stimulation) proteins. Together, these proteins are responsible for promoting, enhancing and regulating transcription.

The results indicate that limiting production of RpoS and Fis results in delayed filament formation by altering FtsZ regulation. Thus, the absence of TusA changes translation efficiency, disrupts the cellular regulatory network and ultimately causes major defects in cell division.

A study in mBio discovered that another *E. coli* protein, HolC, is involved in coordinating bacterial replication and transcription by overcoming conflicts between the replication fork and transcription elongation complexes. DNA polymerase III holoenzyme is the primary enzyme complex that catalyzes DNA replication in bacteria. It consists of multiple subunits, including a clamp-loader complex, which loads and unloads the processivity clamp, a ring-like structure that encircles the DNA and associates DNA polymerase with its template. The clamp-loader complex has two accessory proteins, HolC and HolD. Together, these proteins help assemble and stabilize the clamp-loader complex, but HolC is the only protein of the complex that binds with single-stranded binding proteins, an interaction that directs DNA polymerase to RNA primers and stabilizes the interaction.

Although HolC is not essential for viability, deletion mutants exhibit poor growth and acquire suppressor mutations. One of those suppressors reduces the stability of RNAP. Another duplicates the ssb gene. On the other hand, transcription factors, DksA and Rho termination factor NusA remain viable, even when HolC is absent, but loss of DksA and NusA leads to synthetic growth defects with HolC.

Transcription elongation complexes can impede the progress of the replication fork. In the absence of HolC, it appears that DNA replication is incomplete and Rho-dependent termination is critical to maintaining chromosome integrity. This suggests a new role for HolC in preventing collisions between transcription elongation complexes and the replication fork. However, the mechanism by which it accomplishes this task has yet to be fully defined.

VACCINE AND DRUG DEVELOPMENT

Tuberculosis (TB) is responsible for millions of deaths annually, presents a serious antimicrobial resistance (AMR) threat, and remains one of the most frequent causes of disease worldwide. Comparative genomics and protein analysis have become powerful tools for uncovering potential targets for effective vaccine and anti-mycobacterial drugs, both of which are desperately needed to manage this disease more effectively.

A study published in mSphere used publicly available sequence data across seven *Mycobacterium tuberculosis* (MTB) lineages to map 8,535 genome sequences against the H37Rv reference genome. From this data, single nucleotide polymorphisms (SNPs) and distribution frequency of nucleotide variants were identified, facilitating one of the largest-scale, most comprehensive analyses of MTB sequence variation to date. The researchers found that highly conserved genes were often associated with stress responses and the maintenance of redox balance, while highly variable genes were often associated with AMR. They also identified a number of highly conserved genes that could potentially be used as targets for novel vaccine candidates and antituberculous medications.

Efficacy of the *Mycobacterium bovis* BCG vaccine is geographically variable and is especially low in high-risk individuals. In an effort to identify new antigen targets for improvement or replacement of the BCG vaccine, a study published in Infection and Immunity describes a high-throughput proteome-wide protein-purification study that screened 1,781 proteins for antigenic activity. The study identified 49 antigens that induce antigen-specific gamma interferon (IFN-γ) release from the blood cells of TB patients and healthy donors. Of the three antigens that caused significant reduction in colony-forming unit (CFU) counts in the lungs of mice, two also induced protective T-cell immune response. Additional preclinical assessment of these two antigens (Rv1485 and Rv1705c) is needed to determine whether they have therapeutic potential.

BACTERIAL EVOLUTION: NICHE RESTRICTION AND THE PATH TO SPECIATION

Bacteria from the same species can exhibit wide genetic variations. In fact, only about half of the genes in any given strain of *E. coli* are shared by all other strains. As a species, *E. coli* has adapted the ability to survive in multiple niches, including human and animal hosts, as well as environmental habitats, and as it turns out, not all core genes are always necessary for reproduction and survival. A study in Nature Microbiology sought to determine how variations in environmental conditions and genetic backgrounds impact gene essentiality in *E. coli*. This study offers direct comparisons of the essential character of core *E. coli* genes from 18 strains that are representative of the genetic diversity of the species.

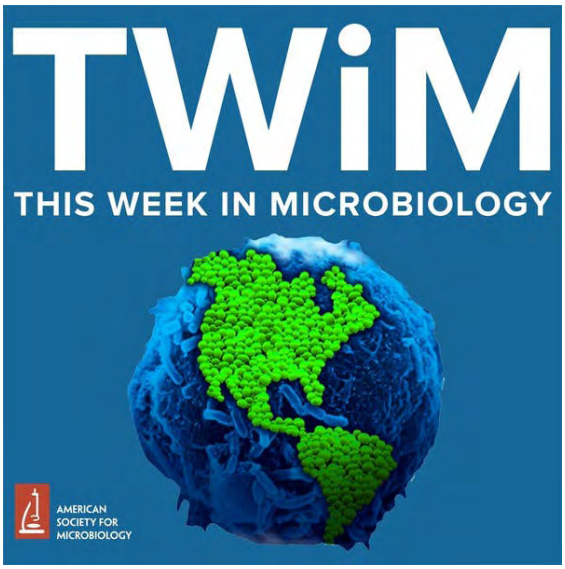
Using a CRISPR interference platform (CRISPRi) to bind and silence expression of target genes, a single-guide RNA (sgRNA) library was generated that allowed the analysis of gene essentiality in different genetic backgrounds. The results indicate that certain genes can become more or less essential in the presence or absence of environmental stressors, including toxins and prophages. In addition to providing evolutionary insights, identifying core essential genes could aid in the design of new antimicrobials and genome-reduction efforts.

MICROBIAL SURVIVAL

Can microbes survive in outer space? Thanks to a lot of patience, some ingenuity and the incredible utility of whole genome sequencing, researchers may be one step closer to finding out. According to a Frontiers in Microbiology article, four strains of bacteria in the *Methylobacteriaceae* family have been isolated from the International Space Station (ISS). Samples were collected during two consecutive flights (March 2015 and May 2015) from different locations on the ISS, including a research station, the cupola and the surface of a dining table. Whole genome sequencing, followed by phylogenetic analysis, revealed that three of the recovered strains, designated IF7SW-B2T, IIF1SW-B5 and IIF4SW-B5, belonged to the same previously unidentified species of bacteria and were most closely related to *Methylobacterium indicum*. Researchers proposed the name *Methylobacterium ajmalii sp. nov* for the novel species. The fourth recovered strain was identified as *Methylorubrum rhodesianum* and was discovered in an old HEPA air filter that returned to Earth in May 2011.

All four microbes belong to a family of bacteria that are commonly found in air, soil, freshwater and sediments. Members of the *Methylobacteriaceae* family are involved in nitrogen fixation and abiotic stress tolerance, as well as plant growth and protection from pathogens. Astronauts have been growing vegetables, such as red romaine lettuce, on the ISS for years. Now researchers are searching for genes in the newly identified species that may promote plant growth in low-gravity conditions. For example, IF7SW-B2T exhibited a higher number of stress-tolerance genes and contained a gene that is essential for cytokinin production, as well as multiple genes that are involved in regulating the cobalamin synthesis pathway. Characterization of this new species of bacteria is just beginning, and there is much to learn about the incredible diversity and adaptability of microbes by studying microbial life on spacecraft.

Another example of microbial survival involves the long-term asymptomatic survival of a virus in humans. Genomic analyses conducted by three independent research groups have revealed that the Ebola virus responsible for the current outbreak in Guinea is nearly identical to the strain that caused the 2013-2016 epidemic. Now scientists are questioning whether the virus has remained dormant in a survivor for over five years. A Science article recently addressed the plausibility and implications of such lengthy viral persistence. The genetic similarities between the two viruses make it highly unlikely that the source of the current outbreak was animal-to-human transmission. However, it is possible that the virus has remained in circulation through undocumented human-to-human transmission events over the years. In 2016, an Ebola resurgence, which also took place in Guinea, originated from a survivor who sexually transmitted the virus approximately 500 days after infection, but the evidence that Ebola may remain latent for at least half a decade is surprising. In addition to the public health implications, the potential for long-term persistence of this RNA virus may impact survivors who already face significant stigmatization because of the disease. Gaining a better understanding of how the virus behaves will help inform communities and guide public health practices, including vaccine distribution and the development of treatment options.



THIS WEEK IN MICROBIOLOGY (TWIM): 10-YEAR EPISODE

If you enjoy learning about a wide variety of different aspects of microbiology, we expect that you may already be familiar with the TWiM (This Week in Microbiology) podcast, hosted by Vincent Racaniello with co-hosts Elio Schaechter, Michael Schmidt and Michele Swanson. The March 11, 2021, edition of TWiM is the 10th anniversary of the podcast and includes a cast of microbiologists who peered into their personal crystal balls and predicted what the next 10 years of microbiology might bring.

We celebrate 10 years of wonderful, stimulating microbiology stories!