MAY / JUNE 2022 # 84

# the Pathologist®

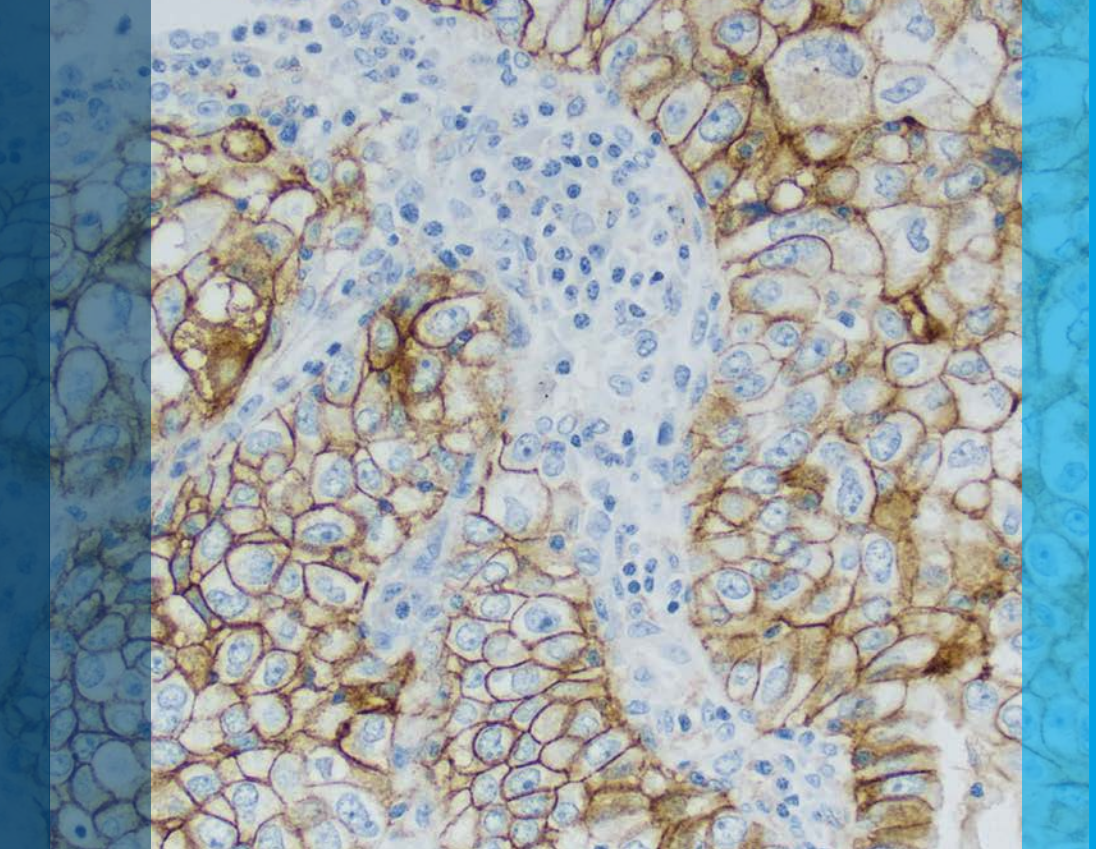
*Official society partner of The Pathologist*



## Gurus of Omics

Four leading minds weigh in on our ever-expanding molecular universe





Agilent

Trusted Answers

# PD-L1 CPS Training Utilizing the Atlas of Stains

## Build Confidence in PD-L1 Scoring using PD-L1 IHC 22C3 pharmDx Atlas of Stains

| | |
|---|---|
| July 12 | 1:00 -4:00 EDT/ 10:00- 1:00 PDT |
| August 30 | 1:00 -4:00 EDT/ 10:00- 1:00 PDT |
| September 29 | 1:00 -4:00 EDT/ 10:00- 1:00 PDT |

Key learning outcomes:

- Understand how to navigate the PD-L1 IHC 22C3 Atlas of Stains
- View (H&E, NCR, PD-L1) of each case
- Learn to filter for tissue type and staining characteristics
- Read full case descriptions including scores
- Create notes or make comments on areas of interest
- Save as PDF or email cases of interest to colleagues

Register with your smart device:

**Speaker: Allen Gown**
Director and Chief Pathologist,
PhenoPath Laboratories

Dr. Allen M. Gown is a pathologist-scientist recognized as one of the world's leading experts in the diagnostic and research applications of immunohistochemistry (IHC). His Pathology career started at the University of Washington in Seattle, where he developed many monoclonal antibodies still in use in diagnostic immunohistochemistry, and contributed extensively to the expanding horizons of immunohistochemistry. He then became the founder of PhenoPath, a national consultative reference laboratory specializing in IHC, flow cytometry, FISH, PCR, and cytogenetics testing. He is currently the Clinical Professor of Pathology at the University of British Columbia in Vancouver, BC. Dr. Gown has well over 300 peer-reviewed journal publications and numerous book chapters.

D66594/01/2021MAY28

# Behind the Mask

*As a non-laboratorian in pathology, does self-doubt ever go away?*



If I asked how many of you had ever suffered from impostor syndrome, would you raise your hand? It's frequently talked about in the modern world – particularly among millennials – but why does it always feel like you're the only one who has these thoughts of self-doubt?

In January 2022, I was promoted to Deputy Editor of The Pathologist – an achievement I had worked hard for over the past year, but one that came with new responsibilities and higher expectations. As the first promotion of my career, it was met with joy sprinkled with an ounce of dread – what if I didn't perform as well as I had in a more junior position?

Impostor syndrome is the kind of feeling that leads you to a fork in the road; you can either let it paralyze you and hinder your progress or you can sit with the anxious feeling and carry on regardless. For me, it's a feeling that comes and goes depending on the task at hand – such as when we launched our podcast, The Pathology Grand Tour (listen, rate, and subscribe, if you haven't already!). The show is a 12-episode tour around the different subspecialties of the lab but, as someone who has never set foot in a wet lab (my background lies in psychology and neuroimaging), who was I to host a podcast about life in the lab? "But then again…" I'd think to myself. "That's what my guests are for!" Sometimes all it takes to gain confidence is a few positive comments from others – but we all need to become advocates for ourselves before the hard shell of self-doubt can truly crumble away.

Since starting at The Pathologist, I've learned how pathologists can be forgotten as members of the clinical care team – working behind the scenes as the "doctor's doctor." I used to think my primary care provider issued my diagnosis from blood tests; now, I rave to anyone willing to lend an ear that it is, in fact, lab medicine professionals who spot, identify, and stop your disease in its tracks. It's easy to see how impostor syndrome might arise in pathologists when their work often goes unrecognized by patients and fellow clinicians despite being the backbone of patient care.

Does impostor syndrome ever go away? Honestly, I hope not. Experiencing that semi-dreaded feeling means we're pushing ourselves out of our comfort zone and creating a seat for ourselves at the table – and that helps us grow. Personally (and professionally), I've found that the only way to get over it is to go through it – and go through it I will because I've come to realize that no one knows what they're doing 100 percent of the time; we're all often just winging this crazy thing called life!

**Liv Gaskill**
*Deputy Editor*


When interpreting HER2 in metastatic breast cancer

# The full spectrum of HER2 expression deserves more recognition

Identifying each level of HER2 expression, including low levels, may have a meaningful impact on clinical decision-making for patients with metastatic breast cancer.

Daiichi-Sankyo | AstraZeneca



Take a deeper look into HER2 expression at **ScoreHER2.com**

# Contents

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**Upfront**

*Research*
*Innovation*
*Trends*

# Epigenetics of Early Arrivals

## Epigenetic testing could identify those at risk of preterm birth

The World Health Organization estimates that 15 million babies – over 10 percent – are born prematurely every year, risking major health problems for survivors both immediately and later in life. But what if you could identify pregnant individuals at risk of giving birth prematurely and put a clinical management plan in place to delay or prevent it? "The problem is that there are currently no efficient clinical biomarkers for preterm birth susceptibility, prompting me and my team to explore a noninvasive option with epigenetic biomarkers," says Michael Skinner, senior author of a new study that aimed to identify markers of preterm birth (1).

In the study, the team collected buccal cells from cheek swabs of the mother, father, and child in both full and preterm births, then analyzed them to identify differential DNA methylation regions (DMRs). They identified 165 epigenetic DMRs in mothers of preterm babies that were different to those in full-term mothers, as well as 136 such DMRs in female preterm children, suggesting that epigenetic inheritance may play a role in preterm birth. Fathers of preterm births had fewer DMRs than mothers and children, but the epigenetic signature was still sufficient to indicate a potential paternal role. The signature was not found in male preterm infants. The results demonstrate that DNA methylation changes in the mother, father, and female children could act as biomarkers of preterm birth.

The research is a proof-of-concept study, so a larger clinical trial is now needed to improve and validate the epigenetic signatures. "Our findings have significant potential to improve population health and reduce disease burden in later life, while saving many children's lives after severe early preterm birth," says Skinner. "For mothers, epigenetic testing could help to avoid the stress and complications caused by preterm birth – a major problem in perinatal health – and improve preventative options for preterm birth treatment."

*Reference*

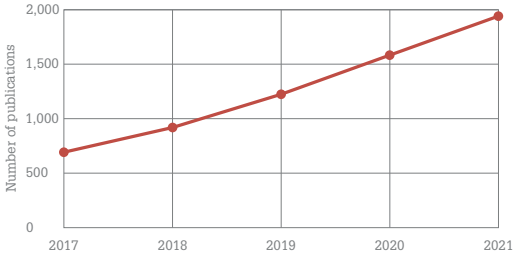
1. *P Winchester et al., Sci Rep, 12, 3361 (2022). PMID: 35232984.*

INFOGRAPHIC

# Benchmarking... Liquid Biopsy

## A look at the last five years of publishing on liquid biopsy

### Publications per year




### Key phrases

1. *lung cancer*
2. *breast cancer*
3. *cell-free DNA*
4. *sensitivity and specificity*
5. *extracellular vesicles*
6. *prostate cancer*
7. *circulating tumor cells*
8. *tissue biopsy*
9. *tumor tissue*
10. *cancer patients*

Credit: Image from Shutterstock.com

QUICK HITS

**A speedy overview of the latest in pathology and laboratory medicine research**

### Battling Resistant Bacteria

A new study has developed a novel and precise method for detecting drug-resistant bacteria (1). Researchers applied a neural network for patch classification against transmission electron microscopy (TEM) patches, allowing them to identify bacterial strains based on classification results and highlight genes strongly associated with resistant cells. With an accuracy rate of 0.94, the approach identified enoxacin-resistant *Escherichia coli* cells in images without antibiotics.

### Annotation Automation

Manual annotations for gigapixel whole-slide images (WSIs) are labor intensive – but automated alternatives, such as deep convolutional neural networks, usually require trained supervision. To remedy this, scientists developed a histopathology image workflow capable of tissue classification without annotation (2). Results showed similar accuracy to that of standard supervised methods and higher accuracy than previous unsupervised baselines.

### TKI Tok

Tyrosine kinase inhibitors (TKIs) are valuable in treating chronic myeloid leukemia (CML), but the drugs' high cost and side effects reduce patients' quality of life. A new study investigates the proportion of patients who experience molecular recurrence (>0.1 percent BCR-ABL$^{IS}$) after discontinuing TKI therapy (3). The Life After Stopping TKIs (LAST) study will run for three years and regularly monitor patients using RQ-PCR and digital PCR. The goal? To evaluate the safety of TKI discontinuation and identify an optimal follow-up schedule.

### Experimental Electrode

Urinalysis underpins the diagnosis and monitoring of numerous conditions, including diabetes and some cancers. Co-detection of multiple substances is often needed, but sometimes tricky - for instance, when the presence of ascorbic acid prevents co-detection of uric acid and dopamine. A novel, graphene-based, ternary composite may overcome this limitation (4), detecting tiny amounts of uric acid (5 mM) and dopamine (1 mM) even in the presence of high levels of ascorbic acid.

*See references online at: tp.txp.to/rsch-r-up*

# Splitting Hairs for HIV

**Can hair glucocorticoid levels provide an indication of HIV progression?**

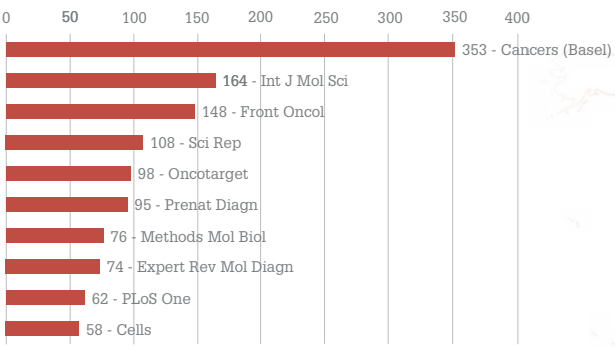
HIV has shifted from a terminal diagnosis to a chronic disease whose patients can, with the help of treatment, live a long life. But HIV progression can vary widely between people. High glucocorticoid levels have previously been implicated, but research into the association between cortisol and HIV progression has been inconsistent. Could hair offer a better estimate of this relationship?

Recently, researchers investigated the relationship between hair glucocorticoid levels and two indicators of HIV progression – CD4 count and viral load – in people with HIV treated with antiretroviral therapy (1). Although hair glucocorticoid levels were negatively associated with CD4 count, surprisingly, an even stronger association emerged between hair cortisone levels and CD4 count. This relationship did not extend to viral load in people with HIV.

Longitudinal research is now needed to determine hair glucocorticoid levels' predictive capacity for HIV progression, taking into account external factors that could influence cortisol levels, CD4 count, and viral load.

*See references online at: tp.txp.to/split-hairs*

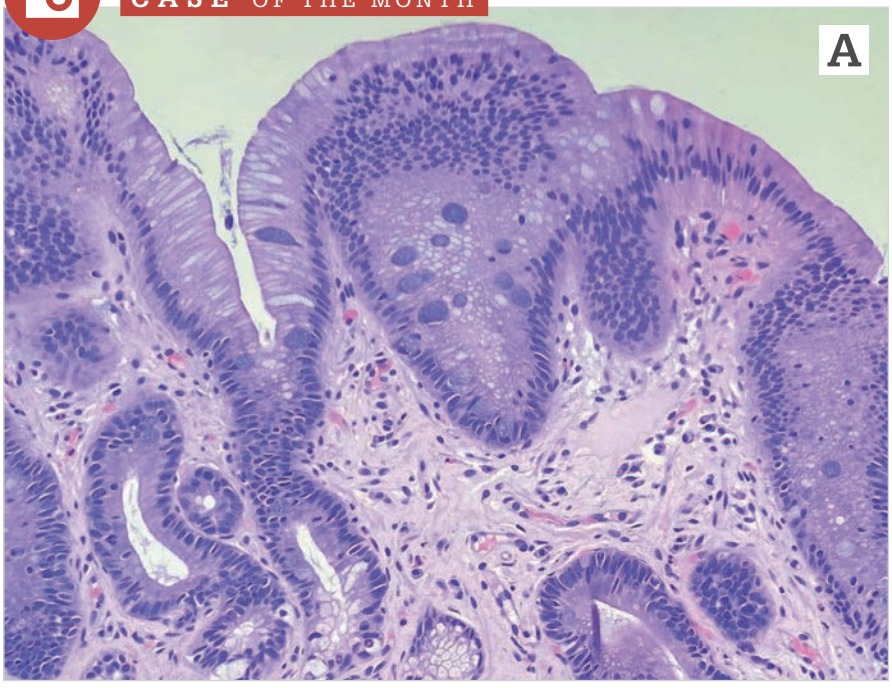
## Top journals

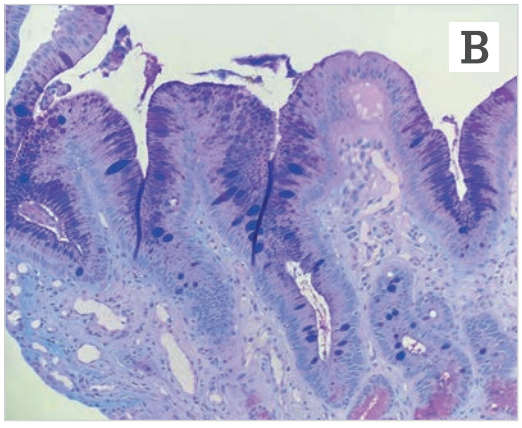


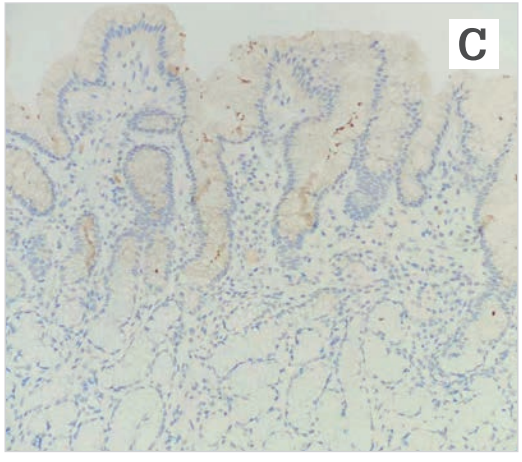

## Top countries

CASE OF THE MONTH

Antral biopsy. A) Hematoxylin and eosin 40x; B) periodic Acid-Schiff-Alcian Blue 40x; C) positive immunochemistry for microorganism. Courtesy of Gang He.




**Chronic active gastritis**

The changes in these images obtained from the antrum in a patient with chronic active gastritis are typically caused by *Helicobacter pylori*.

Do biopsies with these metaplastic findings commonly stain positive for the associated microorganism?

*a) Yes*
*b) No*

Answer to last issue's Case of the Month…
*c) Grease*

Our patient presented with this puncture wound following a suspected injection injury from a hydraulic line the previous day. Representative sections of the fingertip showed necrosis of the epidermis, dermis, and subcutis (Figure 2A) and the presence of intravascular and interstitial foreign material (Figure 2B–D).

High-pressure injection injury is a medical emergency that must be addressed quickly to avoid extensive tissue damage. This type of injury is associated with deep infiltration of soft tissues; thus, debridement is required over a much larger area compared to the injury site, often requiring multiple revisions. Outcomes differ depending on the injected material; water generally has the best prognosis, whereas paint solvents usually require amputation (1–4). Injuries with hydraulic fluid and grease tend to cause less inflammatory response (5).

Though this type of injury is not uncommon, we present only the second case in literature that focuses on the histologic features (6).

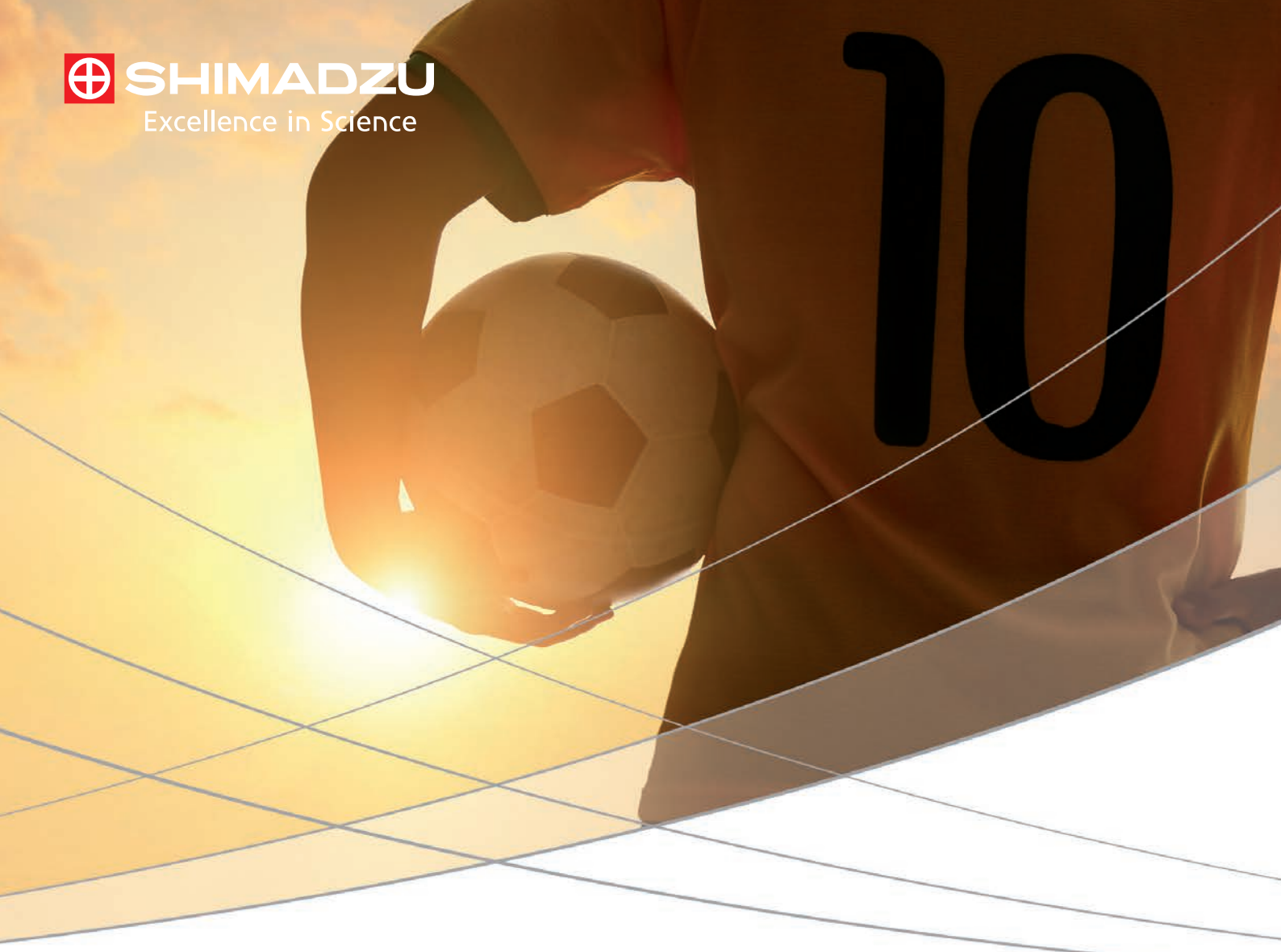
*Submitted by Rand Abou Shaar, Sameer Chhetri Aryal, and Jason Pimentel, Department of Pathology and Laboratory Medicine, Henry Ford Health System, Detroit, Michigan, USA.*

*See references online at: tp.txp.to/CotM-0622*

**To register your guess, please go to http://tp.txp.to/0622/case-of-the-month**
**We will reveal the answer in next month's issue!**

*Case of the Month is curated by Anamarija M. Perry, University of Michigan, USA.*

SHIMADZU
Excellence in Science



# Rookie of the year

## Small size. Massive impact.

The MALDI-8020 is the newcomer in the Shimadzu family of MALDI products. This linear MALDI-TOF mass spectrometer combines talents and skills such as outstanding speed, accuracy and performance. It targets researchers developing MALDI-based diagnostic methods as well as labs where quality control methods or rapid screening of intact samples are routine.

**Small size**
due to benchtop design with a compact footprint

**Massive impact**
through performance similar to larger, more expensive devices

**Multi-talent system**
for analysis of proteins, peptides, polymers and other analytes

**Additional 'Rookie of the year' talents**
such as TrueClean automated cleaning source, barcode reader and MALDI Solutions software for Pharma quality control labs

**www.shimadzu.eu**/rookie-of-the-year

**In My View**

*Experts from across the world share a single strongly held opinion or key idea.*

# Job Killer or Collaborator?

## Digital pathology is here to stay – and we need to embrace it

*By Martin Potash, Managing Director of AmeriPath Denver, Quest Diagnostics, Colorado, USA.*

The microscope is the cornerstone of surgical pathology and, though the field has seen significant changes over time, the microscope has only recently been reimagined for the computer-centric world we live in today. I cannot imagine a scenario in which the microscope is completely replaced – but I am confident that digital pathology and artificial intelligence (AI) will play an even larger role in anatomic pathology's future. Digital pathology and AI are now FDA-approved for primary diagnosis – and together they can revolutionize surgical pathology. Digital platforms can serve as a re-envisioned microscope, a logistics solution, and a medium to allow pathologists to take advantage of AI tools.

Most of my practice's pathologists work at hospitals throughout the state, remote from our technical laboratory – so it is fortunate that we have already had the opportunity to work with new FDA-approved software. Digital platforms allow us to share material or collaborate with our colleagues instantaneously, negating the need to commute or wait for slide delivery. I am currently based in the Denver, Colorado metro area, but I have been able to get secondary consultations from a dermatopathologist in Indianapolis within minutes. These platforms have also enabled remote collaboration and sharing at multidisciplinary tumor boards with more meaningful pathology involvement than traditional static photomicrographs allow.

AI, on the other hand, has the potential to add significant value to pathologists' work. It has immediate applications for technical laboratories using digitized slides, improving digital workflows and scan validations. For our patients, AI enables modules for enhanced detection of some of the most common cancer types, such as prostate and breast. It is easy to see the benefits this approach has for pathologists and patients, particularly when it comes to personalized diagnostics. With the promise of so much added value, digital and computational pathology have me excited about the future of our field.

A recent study found that the number of pathologists in the US decreased by nearly 18 percent between 2007 and 2017 (1). Given the average age of most pathologists, many have predicted that retirement will cause a workforce shortage over the next 10 years (2) – and this doesn't even account for the lack of pathologists in developing countries. By enhancing the role of the pathologist and enabling easy remote work, digital pathology and AI have the potential to increase access to critical subspecialized expertise – improving accuracy and quality of diagnosis and, ultimately, patient care. Surgical pathology remains a field based on interpretation, requiring clinical, radiographic, and laboratory correlation. AI will find its place in aiding the pathologist to perform qualitative and quantitative tasks and allowing them to focus on the art of the practice.

Developments in healthcare and an increased adoption of molecular testing have elevated the critical role of the pathologist. Emerging markets outside the US have a need for pathologists, too; digital services allow for connections to be made without physical presence. Access to efficient tools that allow pathologists to add value in these key areas can help move healthcare forward. Though it may be cost-prohibitive for many organizations to adopt digital technologies, forward thinking in adopting cutting-edge technology will pay off in the future. With medicine only growing more complex, any technology that can elevate the role of the lab is needed and, by refocusing on quality and innovation, pathologists can leverage digital pathology and AI to improve diagnostic quality and deliver the best possible patient outcomes.

*References*

1. *DM Metter et al., JAMA Netw Open, 2, e194337 (2019). PMID: 31150073.*
2. *SJ Robboy et al., Arch Pathol Lab Med, 137, 1723 (2013). PMID: 23738764.*

# A Century of Excellence

## Celebrating 100 years of growth and advancement in pathology and laboratory medicine

*By E. Blair Holladay*

In 1922, at the American Medical Association (AMA) meeting in St. Louis, a group of 39 physicians established what would become the American Society of Clinical Pathology (ASCP). Fourteen years later, ASCP pathologists worked with the AMA to create the American Board of Pathology to offer certification in anatomic and clinical pathology. You must expect that, when these physicians collaborated to establish the structure of modern pathology and laboratory medicine, they knew they were changing the way medicine was practiced. Did they understand, however, just how intrinsically they would reshape the way patient care is delivered?

In the past century, innovations in medicine have changed the way we provide treatment and care for our patients. Diseases that once ravaged populations are now nearly, if not completely, eradicated. The way we provide care has shifted, putting the patient at the center and collaborating across multidisciplinary teams to ensure patients receive the right care at the right time. In the past 100 years, we have reached well beyond our own borders and improved patient care in the farthest corners of the world, because we have learned that global health is local health. High-quality patient care does not end in our own backyards. Rather, providing that care to the areas that need it most strengthens and supports healthcare overall.

ASCP is celebrating its 100th anniversary this year. For our organization to reach such a milestone brings us an incredible feeling of pride in our achievements and hope for the discoveries yet to come. Throughout this year, we are celebrating the century of innovation, education, advocacy, and research our members have accomplished. Medical discoveries in pathology and laboratory medicine have changed the face of medicine over the past 100 years and there is no doubt that continued research and practice will further that change. In more recent decades, the amount of transformation the laboratory has experienced feels almost like we are just getting started – but, knowing our history and the dedication and determination of the pathologists and medical laboratory scientists who came before us, we understand that we are building on the solid foundation that was laid for us.

As we move into the next century of ASCP's history, we do so knowing the challenges in front of us: raising the laboratory's visibility so that patients and other healthcare providers understand the critical knowledge we provide; continued advocacy on behalf of our patients and members to create a better working community for the lab; and developing a pipeline of future pathologists and medical laboratory scientists to continue providing high-quality care for patients, as well as continuing to search paths of discovery in medicine.

We are excited to celebrate our 100-year anniversary and we hope you will join us throughout this year, but especially in Chicago at the ASCP Annual Meeting from September 7–9. Without our members and others within the laboratory community, the past 100 years of growth and advancement in pathology and laboratory medicine would not have been possible – and that is truly something to celebrate.

# Seeking the Perfect Fit

## Demystifying the discussion of sequencing panel size in oncology genetic testing

*By Cecília Durães, Carla Pereira Gomes, Jose Luis Costa, and Luca Quagliata*

Clinical laboratories worldwide are implementing next-generation sequencing (NGS) to identify cancer genomic variants and ultimately improve patient outcomes. But different sizes of NGS panels have different advantages and drawbacks for tumor molecular profiling – and their clinical applicability Also varies. Here, we explore how a variety of panel sizes address key aspects of clinical testing…

### Diagnostic yield and clinical utility

The diagnostic yield is an important selection criterion for determining the performance of any assay. It is primarily defined as "the likelihood that a test will provide the required information for a genetic diagnosis." In a study aimed at understanding the appropriate size of a solid tumor sequencing panel to identify clinically actionable variants (27), researchers directly compared the results of a large gene panel (315 genes) with those of a medium-sized panel (161 genes) and a small hotspot panel (50 genes). Although the larger panel detected more variants, the additional variants beyond those included in the medium panel had no impact on patient management. Even more remarkably, 88.5 percent of those variants would have been identified by the 50-gene panel. Overall, these results indicate that small and medium-sized optimized gene panels are as informative as larger panels when the primary goal is to identify clinically actionable mutations.

### Turnaround time and cost-effectiveness

One of the most critical components of clinical testing for rapid decision-making is the turnaround time of the test. Larger gene panels are more time-consuming because they often require a more complex data analysis workflow. In contrast, small hotspot panels (<50 genes) or medium-sized panels are best-suited to obtaining faster results because they are less "sequencing-intensive" and their analysis is based on a limited number of clinically valuable targets (16).

Another important component of diagnostic testing is cost, which is directly influenced by several components, including the size of the genome targeted and the labor and equipment required for data generation and analysis (19). The cost of library preparation and overall sequencing also depends on sample batching. Sequencing solutions are now available that allow cost compression to combine small- to medium-sized targeted panels with optimized sample batching capabilities (31).

### Sample quality and quantity requirements

Regardless of the NGS approach and methodology used, the feasibility of molecular profiling depends on the quality and quantity of the sample to be tested. The use of NGS to detect low-allele-frequency somatic variants in nucleic acids extracted from formalin-fixed paraffin-embedded tumor tissue is challenging for clinical molecular diagnostic laboratories because these types of samples often yield low quantities of degraded, poor-quality genetic material (10,11). It is estimated that molecular profiling fails in 5–30 percent of tested patients due to insufficient material or poor sample quality (12,13), with the hybridization capture method being the most affected by this issue (5). Additionally,

*"Regardless of the NGS approach and methodology used, the feasibility of molecular profiling depends on the quality and quantity of the sample to be tested."*

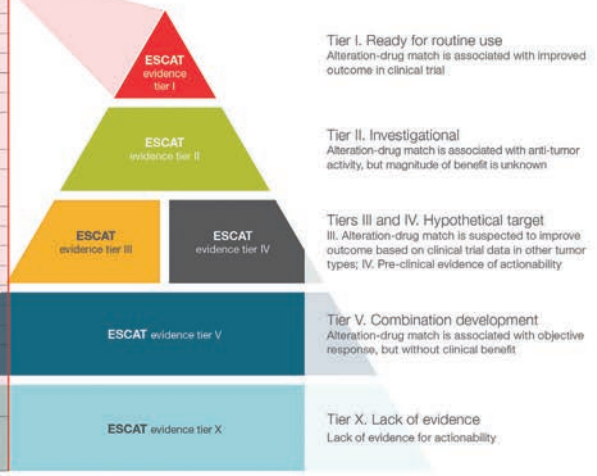
many patients with cancer are only diagnosed at advanced stages, when the available sample material is often limited (16). This hampers the use of large gene panels, which may require a large amount of sample material to provide reliable results. The desired limit of detection must also be considered to determine the minimum amount of DNA or RNA a test requires and the lowest frequency of mutant alleles it can detect (18).

### Bioinformatics and variant interpretation

The large amount of raw data NGS-based assays generate requires a bioinformatics pipeline capable of converting nucleotide sequences into meaningful biological and clinically actionable results. In addition, such an analysis must meet several analytical requirements and ensure the accuracy and reproducibility of the results. A typical pipeline for analyzing NGS data

**European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT)[24]**

| | | | |
|---|---|---|---|
| I-A: evidence from prospective, randomized clinical trials | *ALK* | Fusions | Non-small cell lung cancer (NSCLC) |
| | *EGFR* | Common mutations and T790M | NSCLC |
| | *ERBB2* | Amplifications | Metastatic breast cancer, metastatic gastric cancer |
| | *BRAF* | V600E mutations | Metastatic colorectal cancer |
| | *PIK3CA* | Mutations | Metastatic breast cancer |
| | *BRCA1/2** | Somatic and/or germline | Metastatic breast cancer, advanced prostate cancer, advanced pancreatic ductal adenocarcinoma |
| | *IDH1* | Mutations | Advanced cholangiocarcinoma |
| | | Microsatellite instability-high (MSI-H) | Metastatic colorectal cancer |
| I-B: evidence from prospective nonrandomized clinical trials | *BRAF* | V600E | NSCLC |
| | *MET* | Exon 14 skipping | NSCLC |
| | *ROS1* | Fusions | NSCLC |
| | *FGFR2* | Fusions | Advanced cholangiocarcinoma |
| | *EGFR* | Uncommon mutations | NSCLC |
| I-C: evidence from clinical trials across tumor types or basket clinical trials | *MET* | Fusions | NSCLC |
| | *RET* | Fusions | NSCLC |
| | *NTRK1/2/3* | Fusions | NSCLC, metastatic gastric cancer, metastatic colorectal cancer, metastatic breast cancer, advanced pancreatic ductal adenocarcinoma, advanced hepatocellular carcinoma, advanced cholangiocarinoma |
| | | MSI-H | Metastatic breast cancer, advanced prostate cancer, advanced pancreatic ductal adenocarcinoma, advanced hepatocellular carcinoma, advanced cholangiocarinoma |




*"Careful cost-effectiveness analysis is needed because testing all patients with late-stage cancer using large panels is not affordable for most healthcare systems."*

can be divided into four main operations: base calling, read alignment, variant identification, and variant annotation (20).

The larger the region of the sequenced genome, the greater the likelihood of encountering rare or novel variants that require complex interpretation.

Another challenge is deciding which genes to test in a given clinical scenario. Although there are guidelines that define the most common mutations or genes of interest (tests that are usually reimbursed), the literature and clinician interest may propose other genes (tests that are usually not reimbursed) that may be medically useful (19,23). To standardize the reporting and interpretation of clinically relevant genomic data in the management of patients with cancer, the European Society for Medical Oncology (ESMO), led by the ESMO Translational Research and Precision Medicine Working Group, developed the Scale for Clinical Actionability of Molecular Targets (ESCAT) ranking system (24).

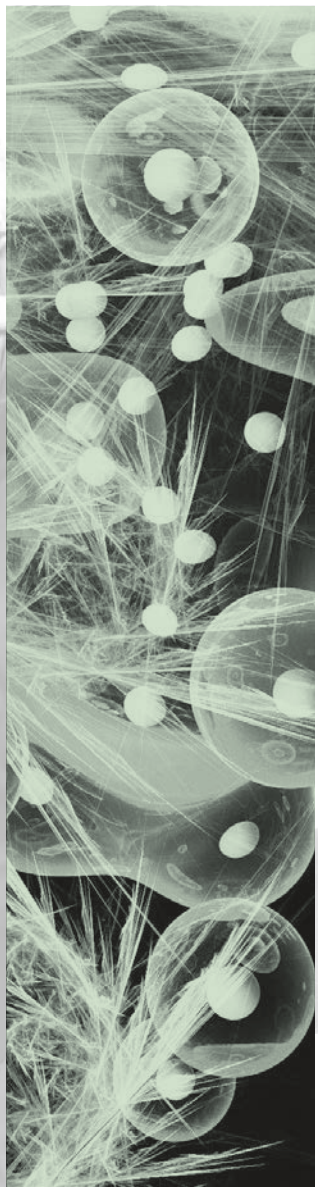
In summary, choosing the best panel size for clinical practice has sparked intense debate among researchers and clinicians. For routine patient testing, diagnostic yield and clinical utility – along with technical aspects such as sample availability and turnaround time – should be the guiding principles in making decisions regarding the most appropriate sequencing method and panel size. Careful cost-effectiveness analysis is needed, because testing all patients with late-stage cancer using large panels is not affordable for most healthcare systems, nor does it currently provide substantial clinical benefit for all patients. The need to advance our understanding of cancer biology and provide patients with the opportunity to participate in clinical trials while keeping the financial burden reasonable requires thorough consideration of what panel size will best serve the target patient population.

*Read the whole article and see references online at www.oncomine.com/content-center*

*Cecília Durães, Carla Pereira Gomes, Jose Luis Costa, and Luca Quagliata are from the Clinical Next-Generation Sequencing Division, Thermo Fisher Scientific.*

# GURUS *of Omics*

LEADING MINDS FROM CORE OMICS DISCIPLINES SHARE THEIR THOUGHTS ON WHERE WE ARE NOW – AND WHAT WE CAN EXPECT FROM TOMORROW



# QUICK INTRODUCTIONS

**Claire Eyers,**
representing proteomics
*Professor, Institute of Integrative Biology, University of Liverpool, UK*

I develop proteomics strategies to enhance our understanding of protein post-translational modifications, largely phosphorylation, in cellular regulation. We think we understand a great deal about the roles of phosphorylation, but studies in vertebrates have focused almost exclusively on the phosphorylation of serine, threonine, and tyrosine. We recently found that at least six more of the 20 common amino acids are also phosphorylated in humans. Considering this, it's clear that our job defining the functions of protein phosphorylation is far from over. There really isn't an area of biological and clinical science that won't benefit from proteomics and I feel privileged to play a role in its progress.

**Gary Siuzdak,**
representing metabolomics
*Professor and Director of The Scripps Center for Metabolomics, The Scripps Research Institute, San Diego, California, USA*

We aim to create technologies that facilitate identification of metabolites that can alter biological systems (hydrocortisone's immunosuppressive function is a great example) – I like to call such efforts "activity metabolomics." From a technical perspective, we have achieved this through our development of XCMS and METLIN. XCMS was the first chromatographic mass spectrometry (MS) platform that allowed correction of the nonlinear alignment of chromatographic and MS data; METLIN was the first database of tandem MS data and now hosts over 850,000 molecular standards.

**Michal Holčapek,**
representing lipidomics
*Professor, Faculty of Chemical Technology, University of Pardubice, Czech Republic*

I always seek the best analytical performance possible. In our laboratory, we don't like to use "features" or "tentatively identified molecules." We strongly prefer to report lipid species confidently – identified based on retention times, accurately determined masses, and fragmentation behavior. The result? False identification rates close to 0 percent. Our approach goes against the mainstream in omics, but is a powerful force in my team's application of lipidomics to solving problems in human disease and beyond.

**Alejandro Cifuentes,**
representing foodomics
*Professor, Laboratory of Foodomics, Spanish National Research Council, Madrid, Spain*

My research has a core objective: to demonstrate that food can benefit our health in countless ways. A great aim in this is the investigation of revalorizing food byproducts. For some years, we have been working on how these could be applied to the treatment of colon cancer; my group was the first to combine transcriptomics, proteomics, and metabolomics to investigate bioactive food compounds in this context. Currently, we're working on the effect of these compounds from natural sources on Alzheimer disease – another serious "pandemic" – covering oxidation, inflammation, neurotransmitter depletion, and beta amyloid plaque formation.

## *We've come a long way since the Human Genome Project…*

The mapping of our genetic self was an incredible milestone in scientific history. And, for omics, it was the Big Bang that gave birth to an ever-expanding universe of holistic molecular analyses. Today, that universe is almost unrecognizable from the one we inhabited at the turn of the millennium – and its associated applications are booming, too.

As for the use of the suffix "-omics," there has been an equally impressive explosion – epigenomics, microbiomics, lipidomics, foodomics, interactomics, even CRAPomics – as countless scientists find their niches and dedicate their labs and their lives to digging ever deeper into nature's molecular mysteries. We invited four such scientists, each a leading mind in their own omics discipline, to talk about the state of their field today.

How did we get here? What can we achieve with the tools available to us? And are we nearing a Big Yawn in the expansion we've witnessed thus far? Let's see what our gurus had to say…

### WHAT DREW YOU TO OMICS?

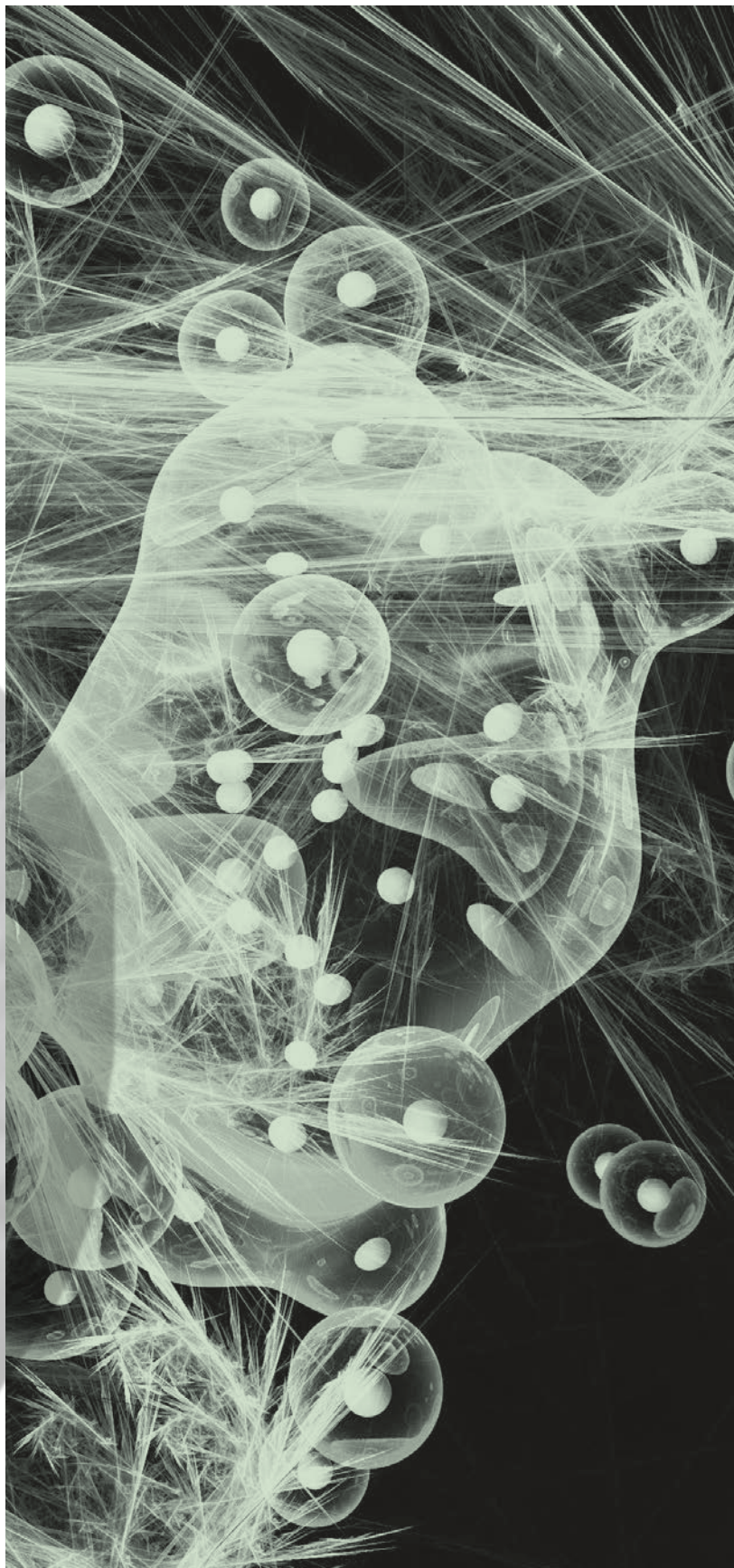
*Claire Eyers:* My interest in proteomics started as a PhD student working on phosphorylation-mediated cell signaling in Dundee. I believed (and still do) that proteomics can help us better delineate and understand complex biological systems and the ways in which proteins act – both individually and in concert – to regulate biological function.

*Michal Holčapek:* Like Claire, it all started with my PhD. I joined Pavel Jandera's lab in 1995 and we were fortunate to have the first benchtop liquid chromatography-mass spectrometry (LC-MS) system in the Czech Republic. It was equipped with a single quadrupole mass analyzer, which is almost laughable today. One of my first tasks was to analyze reaction mixtures from biodiesel production (these complex samples contain tri-, di-, and monoacylglycerols). It was then that I realized the beauty of lipid analysis. These species have regular increments per their individual classes, resulting in regular retention patterns and predictable m/z values. This is part of the reason that I decided to focus on lipids in biological systems, and – 25 years and a new name (lipidomics!) later – I still consider this the best choice I ever made.

*Alejandro Cifuentes:* Other areas, such as biotech and pharma, have typically been the first to benefit from omics – that's where the big money is, after all. Food science was always left behind. I found this hard to stomach (if you'll pardon the pun), especially considering the gigantic impact food has on our lives. I introduced the concept of foodomics in 2009 with the aim of applying omics technologies to studies of food and nutrition. The ultimate aim is to improve food quality and safety, and to explore the relationship between food bioactivity and human health.

*Gary Siuzdak:* Unlike my friends above, I was never drawn to "omics" per se, but instead focused on the lack of information available regarding the role that metabolites play in so many areas of biology. Metabolomics, in my view, has turned out to be an ideal technology to address this problem.

*"In 20 years, we will still be trying to comprehend the huge complexity of interactions between food ingredients and our body!"*



## WHAT'S THE STATE OF YOUR FIELD TODAY, AND HOW HAS THIS CHANGED DURING YOUR CAREER?

*AC:* I would say foodomics is still in its infancy. As I said before, it was only defined 13 years ago, so there's much room for growth. Though foodomics itself hasn't changed much throughout my career, the methods we rely on certainly have. For example, early proteomics investigations relied on two-dimensional electrophoresis followed by image comparison, protein cutting from gels, trypsin hydrolysis, and sequencing by MALDI-TOF tandem MS. Now, we can analyze the proteome of a biological system with a single injection by combining nano-LC and high-resolution MS with isotopic probes bound to hydrolyzed proteins. And it's now possible to use several high-resolution MS analyzers in metabolomics, either alone or coupled to a separation technique. This adds even more punch to the discipline.

*CE:* Proteomics has changed considerably since I started in the field 20 years ago. Advances in chromatography instrumentation and column chemistry have dramatically improved sensitivity, partly because chromatographic resolution has focused MS instrument time. Combined with vast improvements in the speed and sensitivity of mass spectrometers, we are now able to identify and quantify changes in protein abundance in very small sample amounts – even to the single-cell level in some systems. Routine implementation of different forms of peptide fragmentation during tandem MS, ion mobility separation to complement (or replace!) chromatographic peptide separation, and better understanding of the chemical mechanisms driving peptide fragmentation have all improved the automated analysis of peptides and their post-translational modifications.

*GS:* Metabolomics is on a tremendous growth streak. The trajectory of this growth today is higher than ever before. This is largely due to many different biological and therapeutic fields realizing the wealth of information that can be gleaned from metabolomic analyses. Whether it is fundamental biology, the microbiome, the exposome, therapeutics, or any of myriad other areas, metabolomics can add both mechanistic and functional information – or even identify metabolites that can alter phenotype. Lots has changed since I first got involved in LC-MS-based metabolomics 27 years ago!

*MH:* Scientific knowledge evolves constantly and dramatically. Lipids were initially known for their roles as energy stores and building blocks of cellular and intracellular membranes. Today, however, we know that they also play roles in countless other biological processes, including cell signaling.

The lipidomics toolbox has needed to expand rapidly to study the crucial roles of lipids. We have, for example, benefited from enormous improvements in analytical instruments and bioinformatics. I was happy with HPLC coupled to a single quad spectrometer 25 years ago. Today, ultra-high, ultra-fast performance is ubiquitous. Powerful instrument configurations quickly generate tremendous amounts of data, putting pressure on bioinformatics processing and statistical evaluation. Indeed, bottlenecks tend to present themselves on either side of such impressive systems, with both sample preparation and data analysis demanding further attention.

## WHAT'S THE GREATEST FEAT ACHIEVED IN YOUR FIELD THUS FAR?

*GS:* The sheer number of tools that have been created to support metabolomics is likely the most impressive collective feat. A new technological (typically informatic) tool comes out almost every week and these developments are helping the field accelerate at an incredible rate. I am personally fond of my own team's success in developing the XCMS/METLIN platform, which facilitates metabolite and chemical identification. METLIN now boasts over 850,000 molecular standards, each with experimental tandem MS data. This is (I hope) helping move the field forward, especially combined with other technological achievements.

*CE:* Scientists outside of omics may think of proteomics as a "plug and play" technology – put the sample into a (black) box and out comes a list of identified and quantified proteins. Though this isn't really the case (much expertise is required for good sample preparation, instrument setup and maintenance, experimental design, data handling, and so on), the fact that many people perceive proteomics as routine indicates that the technology has come of age!

As for specific points of excitement, I don't think I could highlight just one, but single-cell proteomics is up there. Our ability to quantify proteins at this level allows us to understand heterogeneity – a crucial factor in studying, for example, signal transmission across cell populations and disease. We can also explore proteins from species for which we don't have a genome and examine combinations of post-translational modifications via top-down proteomics approaches. "Native"

MS (to understand protein complexes and the conformational dynamics of proteins subject to ligand binding or protein modification) can be used to conduct particularly powerful protein analyses.

*AC:* The greatest achievement in foodomics is a tricky question because we are still building our relatively new discipline. We have a long way to go to catch up with more established omics fields such as genomics and proteomics. For now, I would say, "Let's wait and see!"

Some great examples of our field's potential can be found in foodomics studies looking at the antiproliferative activity of certain food ingredients against colon cancer both in vitro and in vivo; for example, my group found that the green extraction and concentration of specific compounds (and the elimination of other not-so-positive natural compounds) from rosemary creates a product with antiproliferative effects. There is also huge interest in searching for new bioactive compounds with neuroprotective activities that can help treat Alzheimer's disease. We've found some very interesting compounds from food byproducts (in the orange juice and olive oil industries) and microalgae with promising neuroprotective activities. This is a huge area of interest in foodomics today. Foodomics also helps corroborate the work that goes into ensuring the safety and quality of food commodities.

## AND WHAT ABOUT THE PROUDEST MOMENT IN YOUR OWN CAREER?

*MH:* I am proud of my group's achievements in oncolipidomics. We found that the lipid profiles (in blood) of subjects with various types of cancer are distinct – and multivariate analyses could differentiate cases from controls with over 90 percent accuracy. Our most convincing results concerned a large set of pancreatic cancer samples – we were recently granted a European patent for our pancreatic cancer diagnostic method – but similar dysregulation is also apparent in many other cancers. We are now working on translating our diagnostic method into clinical practice. If successful, it would be a real breakthrough in pancreatic cancer screening and, hopefully, screening for other cancer types as well!

*AC:* Coining the term "foodomics" was certainly a proud moment. And yet, nothing would have come of it without its positive reception among colleagues – and the incredible work they conduct in this area. It also makes me very happy to think about how many (mostly young) researchers have been prepared for life as capable professionals following time in our lab!

*GS:* I feel the same way, Alejandro. My answer is simply the number of individuals who came to my lab and left for exciting metabolomic careers across academia and industry!

Recently, two separate technologies that have come out of our lab are especially compelling. The METLIN Neutral Loss (METLIN-NL) database contains neutral loss spectra on 867,000 molecular standards. METLIN-NL is the first neutral loss database of its kind and it provides a unique dimension in molecular identification of unknowns. Previously, people relied largely on MS/MS data – but the information NL data provide is very complementary and very underappreciated.

A second development was Q-MRM, a technique that mimics triple quadrupole quantitative analysis (QqQ-MRM) using a single quadrupole with in-source fragmentation. The performance is very similar to the triple quadrupole, but it can be accomplished on a much less expensive instrument. We recently applied to a common clinical biomarker and the results were excellent.

*CE:* Demonstrating the true extent of "non-canonical" phosphorylation in human cells (my team used a novel analytical pipeline to show that protein phosphorylation in human cells is much more diverse that anybody previously thought) and the excitement that this has created within the cell signaling community is one of my proudest proteomics moments. It took a number of years to get a working method, given how unstable atypical phosphorylation events are under standard proteomics conditions, and I am incredibly proud of the students and postdocs that contributed to this project.

Also, working with other members of the COVID-19 MS coalition, we have developed robust MS assays for the simultaneous screening of both COVID-19 and winter flu in saliva samples. These are currently being evaluated in clinical hospital labs. Exploiting the sensitivity and specificity of targeted MS assays that can be multiplexed for rapid screening of diverse markers of infection or disease in this way will have significant societal benefit.

## WHERE DO YOU SEE THE FIELD IN 20 YEARS' TIME? WHAT CHALLENGES WILL NEED TO BE OVERCOME?

*CE:* We have this discussion quite often in our lab. I personally think that proteomics in 20 years will probably not be conducted using

*"Proteomics has changed considerably since I started in this field 20 years ago."*

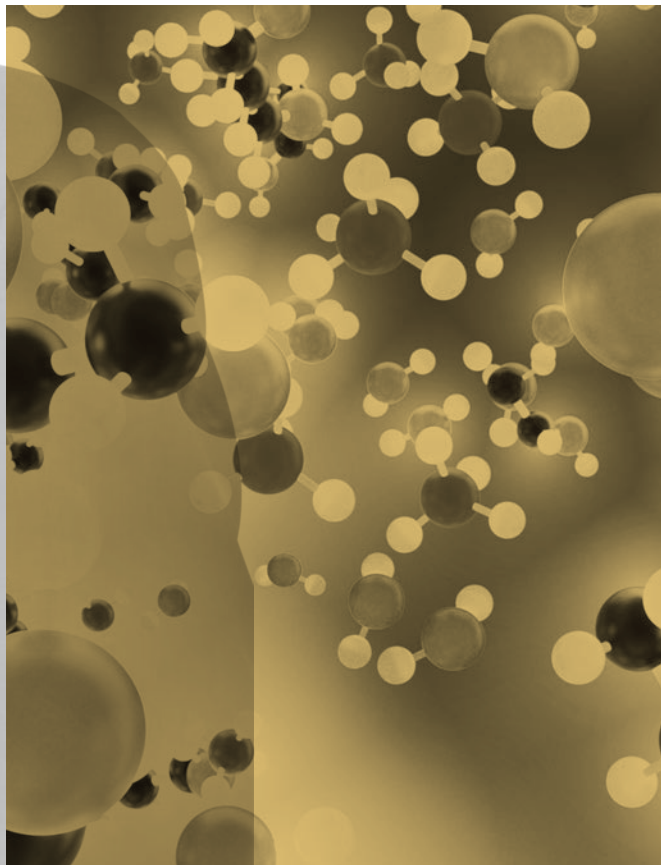
*"I am proud of my group's achievements in oncolipidomics."*

today's classical LC-MS workflows. Proteomics (or rather, bottom-up proteomics) has an issue that stems from the inference of protein identities from peptide identification using tandem MS.

Problems with "missing peptides" and our inability to fully characterize sites of covalent modifications mean that we don't currently define the large numbers of proteoforms present in systems. Current instrumentation also struggles with the robust separation of large (50 kDa or bigger) proteins that may differ only by the addition of a number of small chemical entities (methyl, phosphate, and sulfate groups, for example) and we are still unable to completely fragment these proteins inside the mass spectrometer and interpret these data. The sequential way in which we typically analyze samples means that high-throughput "complete" protein characterization remains outside our current capabilities. Overcoming this limitation will be a great focus for the next 20 years and will likely require radical new ways of thinking about our technology and data analysis tools.

*GS:* If the question is, "Where would I like to see the field in 20 years' time?" then the answer is pretty straightforward; I'd like to see metabolomic tools with a dynamic range sufficient to comprehensively quantify and identify all metabolites – from the most ubiquitous to least common.

The instrumental challenges are rather daunting, though. A dynamic range improvement from the current state of the art (six orders of magnitude) to ~12 orders of magnitude would be needed. And 20 years may not be long enough!



The identification challenge is also daunting. PubChem lists 93 million molecules in its existing chemical space; identifying these rapidly and with stereoisomer precision is almost impossible. Major leaps will be needed regarding the size of our own METLIN database and technologies for high sensitivity stereoisomer assessment. Again, 20 years may not be enough…

*AC:* In 20 years, we will still be trying to comprehend the huge complexity of interactions between food ingredients and our bodies! I anticipate a deeper understanding of the microbiome by then, but so-called "personalized nutrition" will take longer still. I would say that overcoming current limitations in data treatment is the main challenge ahead, including integration of the huge amounts of data generated at different levels of expression (genomics, transcriptomics, proteomics, metabolomics) by existing analytical techniques and subsequent transformation of this data into useful biological information.

Imagine if we understood exactly how food could impact our health – knowing the mechanisms behind the way different ingredients impact our bodily homeostasis, positively or negatively, based on a range of factors: our individual genome, our population group, or our specific answer to different food ingredients (allergies, intolerances, and so on). I believe we will eventually know exactly which ingredients can slow the development of pandemic-scale noncommunicable illnesses such as Alzheimer's or cancer.

*MH:* We have made significant progress in lipidomic quantitation, but we aren't perfect. In the words of my coworker, Denise Wolrab, "It seems that all labs have troubles and, even for the so-called leading groups in lipidomics, it is far from perfect. Improvements are necessary." Harmonizing our protocols so that different labs can report comparable results with high confidence and structural detail represents a crucial step. Other hurdles we must overcome are the identification of specific lipids' roles in metabolic pathways and ability to merge information from separate omics disciplines in a systems biology approach – a huge challenge for bioinformaticians.

In addition, the alterations in lipid metabolism occurring across numerous diseases (cardiovascular and liver diseases, cancer, Alzheimer's, and so on) remain poorly understood. There is much work to be done by the lipidomic community on this front! As methodological and instrumental improvements materialize, we will also be able to dig deeper into structural details with MS and ion mobility approaches.

# Biomarkers: The Bigger Picture

**To unlock patient care, pathologists must focus on molecular pathology and ensure efficiency and effectiveness to improve testing. With this testing, patients can benefit from the best treatment to combat their disease.**

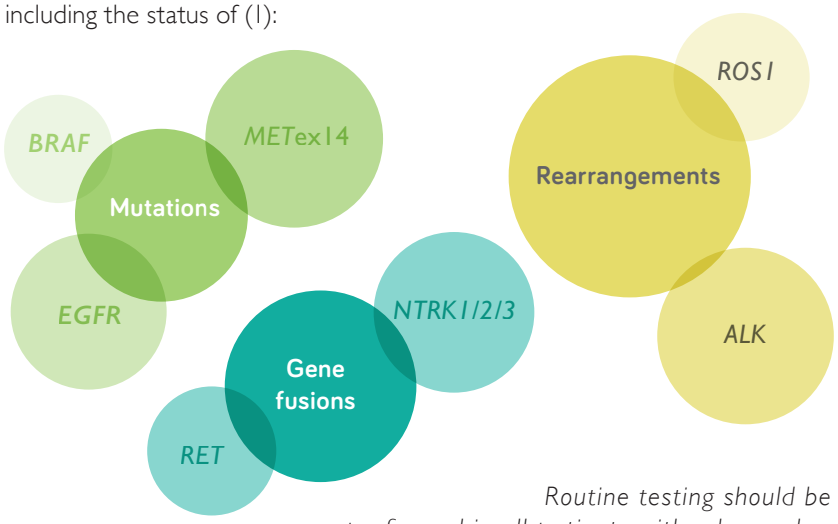
## European Society for Medical Oncology (ESMO) Recommendations

ESMO recommendations for NGS in patients with metastatic cancers (2)

- it is recommended that a tumor (or plasma) sample from a patient with advanced non-squamous NSCLC is profiled using NGS technology to detect alterations ready for routine use
- use large panels of genes only if they yield an acceptable overall cost increase
- use tumor multigene NGS panel in patients presenting with advanced non-squamous NSCLC, prostate or ovarian cancers, and cholangiocarcinoma
- use NGS as an alternative to PCR-based tests in colorectal cancers only if not associated with extra cost
- research centers to perform multi-gene sequencing to speed up cancer research, drug development, wider access to treatment, and data collection

## International Guidelines for Molecular Biomarkers

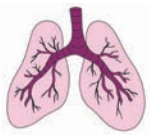
Before any treatment decisions, minimum testing is required, including the status of (1):




*Routine testing should be performed in all patients with advanced or metastatic NSCLC non-squamous cell carcinoma.*

## Filling the Gap

RNA testing can detect gene fusions missed by DNA testing (3)



**2,522**

**lung adenocarcinomas were profiled using the MSK-IMPACT large hybrid-capture, DNA-based NGS assay**

**31%**

**of DNAseq driver-negative patients had a previously undetected fusion on RNAseq**

**275**

**driver-negative cases using DNAseq with available tissue**

**254**

(92.4%)

**suitable for RNAseq**

## RNA's real-world impact (3)

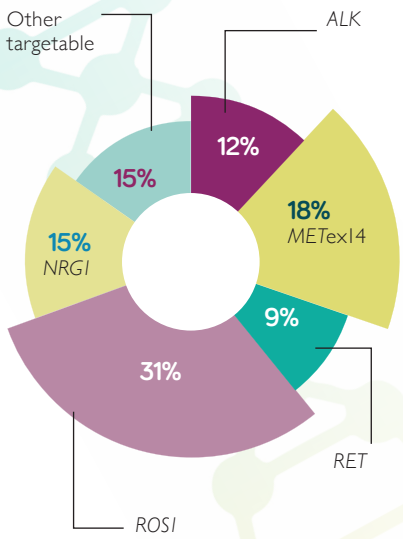
N = 36 cases

**33** actionable cases

**10** patients received matched targeted therapy

**80%** achieved clinical benefit from matched targeted therapies based on RNAseq

## RNAseq fusion-positive cases




## The Value of Anchored Multiplex PCR

**Anchored Multiplex PCR (AMP)**
a method of targeted NGS that allows users to detect fusions and identify partner genes from low amounts of RNA and DNA in FFPE samples.

**Why use AMP for gene fusion detection?**

- good diagnostic utility for detecting gene fusions, oncogenic isoforms, mutations, and expressions (4)
- detects known and novel gene fusions and oncogenic isoforms (5)
- targets RNA (5)

**Targeted RNAseq** in all driver-negative cases **offers improved detection** of actionable **gene arrangements (3).**

**RNA NGS with AMP technology is superior to other fusion detection methods – linking more patients to suitable therapies.**

## AMP for ctDNA: a good choice

**Liquid biopsies are**
- minimally invasive
- faster, safer, and more affordable
- better able to investigate heterogenous tumors

**AMP is good for detecting small cfDNA fragments (6)**
- requires only one primer-binding site within a fragment, increasing specificity of variant cells
- reduces background noise and increases sensitivity

**Invitae's ctDNA assays based on AMP may offer (internal data)**
- 100% detection sensitivity for 1% AF variants using 10 ng DNA input
- 100% sensitivity for 0.3% AF variants using 30 ng DNA input
- reduce false positive and to led to ≥99% accuracy after molecular barcode-enabled error correction and variant filtering

## Can plasma NGS improve mutation detection and identify more patients with targetable biomarkers? (7)

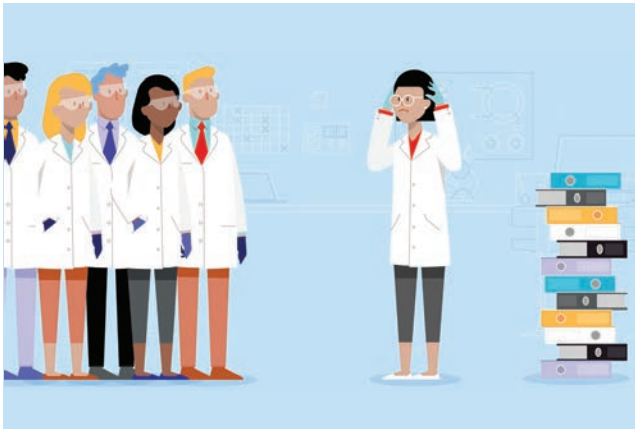
In **229** patients who received NGS testing, targetable mutations were found in…

**20.5%** using tissue alone

**35.8%** using plasma sequencing plus tissue

# The only true specimen-to-report NGS solution for genomic profiling

Worried NGS implementation in your lab means several new instruments and suppliers to navigate?



That you would need new staff with expertise and several new procedures to implement?

That you could not handle the complex bioinformatics?


No need to worry—NGS is easy with the Ion Torrent™ Genexus™ System.

**Benefits include:**

- A true end-to-end, specimen-to-report workflow with two instruments, operated by one software, all from one supplier
- Only 20 minutes of hands-on time to set up the system, which delivers results in as little as one day
- Over 70 variants across 50 key genes from FFPE tissue or liquid biopsy samples in precision oncology research with the Oncomine™ Precision Assay

It's NGS, but as fast and simple as IHC or PCR, enabling you to provide one complete report with key biomarkers in one day.

Find out more at **oncomine.com/genexus-oncology**

ThermoFisher SCIENTIFIC



# Managing Methods for MSI

## Seeking cancer diagnostic and therapeutic guidance with microsatellite instability testing

*By Richard Hamelin*

Microsatellite instability (MSI) in tumors is an approved biomarker for breakthrough immune checkpoint inhibitor therapy (1), with polymerase chain reaction (PCR) testing currently the most direct, accurate, and cost-effective measurement method. In 2017, the US FDA granted the first tissue-agnostic approval for pembrolizumab – a monoclonal antibody that blocks immune suppressing PD-1/PD-L1 receptor interactions, or "immune checkpoints" – for patients with unresectable or metastatic MSI or DNA mismatch repair-deficient (dMMR) solid tumors. Moreover, December 2020 saw the European Medicines Agency (EMA) adopt a new indication for pembrolizumab as a first-line treatment for metastatic colorectal cancer based on MSI or dMMR biomarker status (2).

These approvals were based on clinical trial data demonstrating that dMMR or MSI was able to predict treatment response across 12 different solid tumor types, including colorectal cancer (3–5). Given the compelling therapeutic rationale for measuring dMMR and MSI and the fact that MMR deficiency has been identified in up to 20 percent of different solid tumors (3), the benefits of detecting these biomarkers accurately and affordably are clear.

There's an indelible biological link between dMMR and MSI (6). The MMR system of proteins recognizes and repairs DNA base pair mistakes, insertion and deletion errors (indels), and DNA damage that occurs during replication and recombination. Mutations in genes encoding MMR proteins can cause DNA mismatch repair defects throughout the genome, including within microsatellites – widely distributed stretches of DNA composed of short (up to six base pairs) motifs repeated up to 50 times.

Just like gene coding sequences, microsatellites can accumulate errors from dysfunctional DNA repair, including base-base mismatches and small indels that differ from the inherited microsatellite (7). MSI – the accumulation of these errors – reflects overall tumor genetic instability, whereas indels in coding sequence microsatellites may lead to frameshift mutations. In tumors driven by dysfunctional DNA repair, genetic instability is responsible for the increased tumor mutation burden that drives immune cell infiltration into the tumor microenvironment (TME) (5,6). As a survival strategy, tumors can inhibit immune cell activation in the TME by engaging immune checkpoints. MSI and dMMR can signpost tumors that are more likely to respond to immune checkpoint inhibitor therapies (3).

The MMR system includes a number of proteins including MLH1, MSH2, MSH6, and PMS2 (8) – and dMMR is often assessed in tumor cells by the absence of immunohistochemical (IHC) staining for any one of these four major proteins (9,10). MSI is detected either by PCR amplification of tumor DNA or by next-generation sequencing (NGS) methods (8,11,12); however, neither approach is optimized for MSI detection and not all platforms use the most sensitive marker panels. NGS is also expensive and not all NGS biomarkers have been clinically validated. Concordance between IHC and MSI assessment of tumors is high (approximately 90 percent), tempting pathologists to rely on one method alone (9, 13) – but, if we only employ one method for detecting MSI and dMMR, it must be the most direct, accurate, and efficient method out there.

### IHC detection of dMMR

IHC detection of dMMR is based on specific antibody recognition of MLH1, MSH2, MSH6, and PMS2 in tumor cell nuclei (10), with the

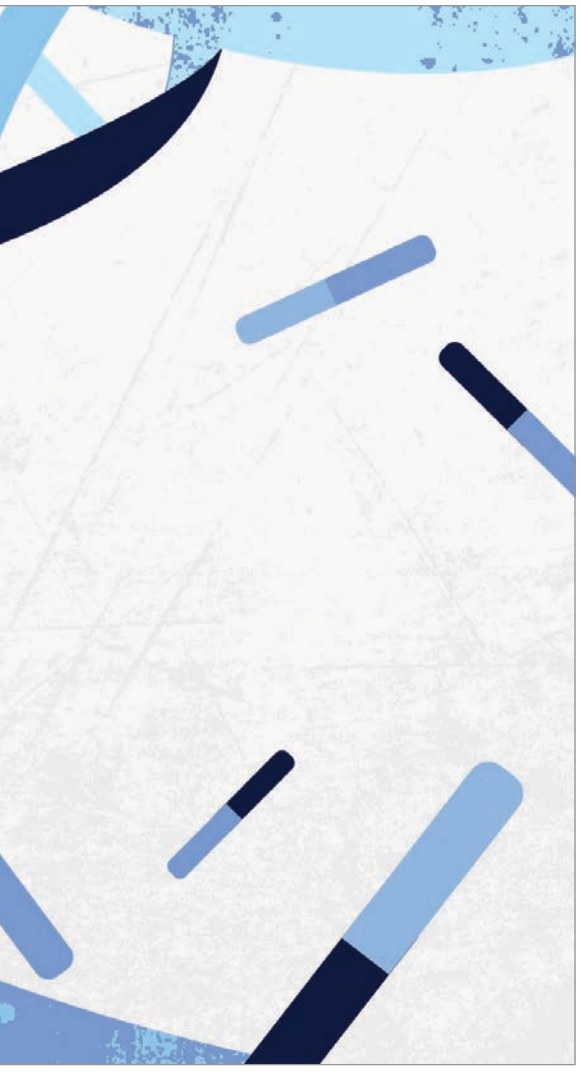
absence of an IHC signal indicating dMMR. IHC protocols are simple, rapid, inexpensive, and require minimal specialized instrumentation.

A distinct advantage of IHC for dMMR detection is that it reveals the identity of mutated MMR genes by lack of IHC staining – using specific antibodies against the wild-type proteins. However, IHC staining doesn't cover all MMR genes (12), requires a tissue sample large enough to perform four separate incubations, and may be unreliable (10). For example, tumors from patients exposed to preoperative chemotherapy or radiation therapy are more difficult to assess using IHC due to artifactual loss of MSH6 protein expression (14).

The major caveat with IHC dMMR detection is the potential disconnect between MMR protein antigenicity and function (9,10). Some missense mutations involving only a single nucleotide can lead to nonfunctional MMR proteins that are nevertheless recognized by antibodies, resulting in a false negative result (15). In addition, MMR gene mutations can code for unstable truncated proteins that can stain with the IHC test sample before degrading in the remaining tumor tissue. This leads to false negatives in up to 10 percent of samples (5,16). On the other hand, false positives can be caused by missense mutations that lead to the loss of antibody recognition without compromising protein function (9,10). Clearly, equivocal IHC results must be verified by follow-up or tangential PCR MSI testing.

## PCR detection of MSI

PCR MSI detection, which reports on the functional loss of MMR proteins, is a more direct biomarker of MMR function (10,17). Tumor DNA is extracted from a 1 ng sample shaved from a formalin-fixed, paraffin-embedded block of tumor tissue, then amplified using DNA primers that span specific marker regions (loci) in microsatellites. These PCR products (amplicons) are separated according to size by capillary electrophoresis and detected based on fluorescent primer pairs. In the analysis,



MSI shows up as novel peaks of DNA fragments not present in normal tissue (see Figure 1).

Microsatellite markers play a critical role in influencing MSI detection (18) and can significantly improve test sensitivity and specificity (8,11). In 1997, the National Cancer Institute recommended a reference panel of five microsatellite markers for MSI detection; known as the Bethesda panel, it consists of two mononucleotide (BAT-25 and BAT-26) and three dinucleotide (D2S123, D5S346, and D17S250) loci (19). Since then, mononucleotide microsatellite repeat sequences have proven particularly sensitive to transcription errors – making them optimal targets for measuring MSI (7,11,20).

A panel of five consensus mononucleotide repeats (BAT-25, BAT-26, NR-21, NR-24, and either NR-22 or NR-27) outperformed the NCI reference panel (11, 21). The development of a commercial multiplex assay (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) and the improved performance over the NCI reference panel has made this pentaplex panel the gold standard for MSI detection (7,8,20).

The quasi-monomorphic nature of these microsatellites facilitates their analysis. Their size is highly homogeneous in human populations (11,21,22), with rare allelic variants mostly in African populations (22). This makes the comparison of tumor and matching normal DNA optional in most cases, although highly recommended for ethnically diverse populations.

Historically, according to the proportion of unstable markers, MSI status was divided into three categories: MSI-High (MSI-H), MSI-Low (MSI-L), or MS-Stable (MSS). However, because no clinical differences were observed between MSS and MSI-L tumors, it is now recommended that tumors be classified into two categories: MSI (formerly MSI-H) and MSS (formerly MSI-L and MSS). ESMO guidelines recommend including MSI-L tumors with MSS tumors (23).

Using the pentaplex panel, a tumor is considered MSI if two or more markers are unstable (24); tumors with no instability, or one unstable marker, are classified as microsatellite stable (MSS). In cases where exactly two markers are unstable, a healthy matching tissue DNA analysis is critical to confirm or reject instability due to the possible presence of rare microsatellite polymorphisms in that individual. Because it is carried out in a single PCR, the pentaplex panel is simple to use and free of errors that arise from mixing samples. It also benefits from rapid turnaround times (two to three days) and results are highly reproducible (25,26).

Recently, alternative methods based on NGS technology to establish tumors' MSI status have been proposed (27). These methods require advanced technical capabilities and are more expensive than traditional PCR assays. Each method evaluates different microsatellites and there are few comparative studies available to fully evaluate their performance.

*"Microsatellite markers play a critical role in influencing MSI detection (18) and can significantly improve test sensitivity and specificity."*

## The clinical utility of MSI

Accurate MSI testing has had clinical significance in oncology prior to the checkpoint inhibitor indication. In a study investigating MSI prevalence across 32 different tumor types, 24 showed evidence of MSI – most commonly in early-stage disease (3). The highest incidence of MSI was in colorectal cancer (10 to 15 percent for sporadic cases) (3,24) and in endometrial cancer (17 percent) (3). For colorectal cancer, MSI is also correlated with better outcomes, indicating it as a strong prognostic factor for patient survival (28).

MSI also plays a preliminary role in

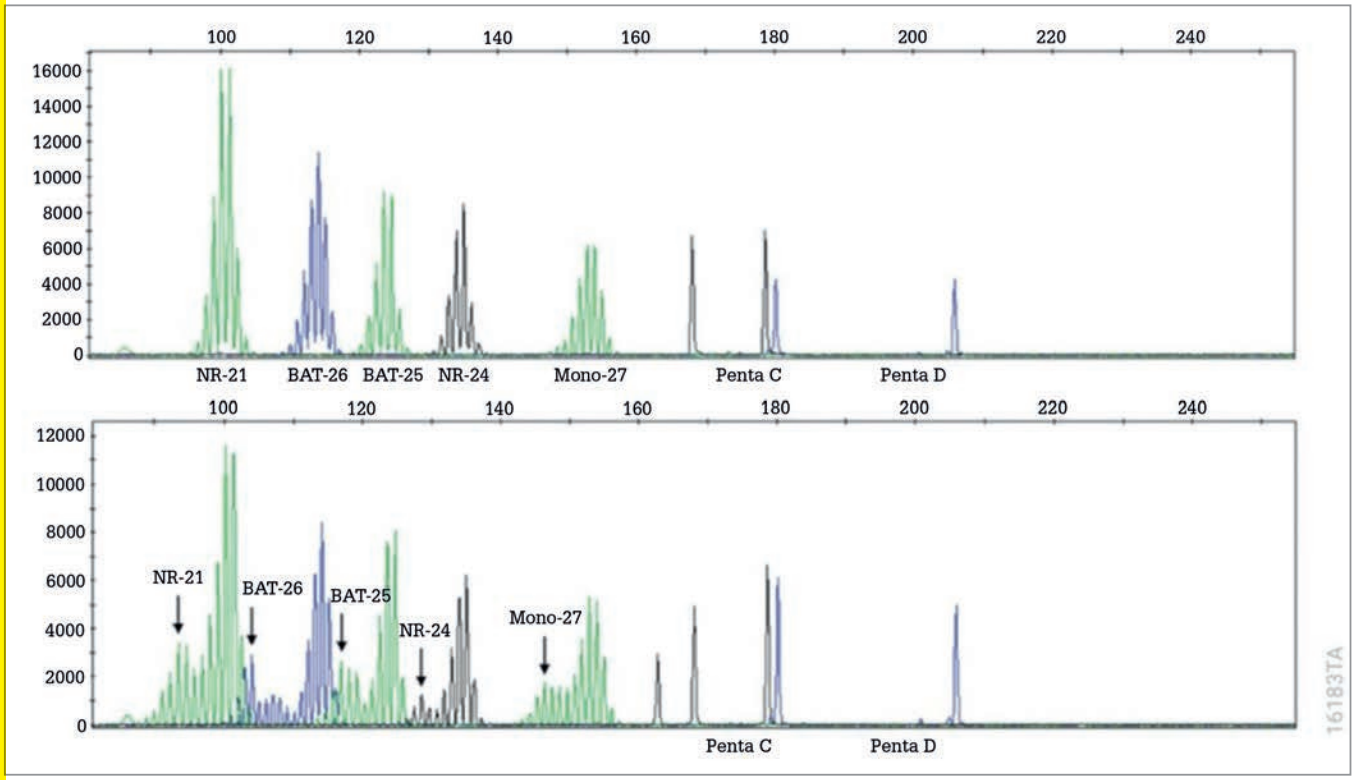



Figure 1. Example allelic profiles of NR-21, BAT-26, BAT-25, NR-24, and Mono-27 in DNA from normal tissue (top) or MSI-H tumor (bottom).

diagnosing Lynch syndrome (LS) (1) – an autosomal-dominant, multicancer disorder that accounts for 2–4 percent of all colorectal cancers and 2–5 percent of endometrial cancers in women. The underlying pathogenetic drivers of LS-related cancers are germline mutations of an MMR allele, as opposed to sporadic MSI cases due to methylation of the MLH1 gene promoter. Though germline MMR genetic mutation assessment is the definitive diagnostic marker for LS, MSI testing of patients with newly diagnosed colorectal cancer can be a convenient method to confirm whether a colorectal cancer is potentially attributable to LS – 16 percent of patients with MSI-H tumors have germline mutations in MMR genes (29,30).

Beyond diagnostics, MSI has also been proposed as a screening tool for all surgical specimens from patients newly diagnosed with colorectal or endometrial cancer. ESMO guidelines further support PCR as the gold standard method, recommending MSI testing by PCR alone or with IHC to assess dMMR in any cancer type belonging to the LS spectrum (colorectal, endometrial, small intestine, urothelial, central nervous system, or sebaceous gland) (23). Once genetic mutation assessment identifies patients with LS-related cancers, physicians may then consider whether relatives should be advised to undergo screening for LS. For individuals with LS, the lifetime risks for colorectal and endometrial cancer are 70–80 percent and 40–60 percent, respectively, compared with 2 percent for the general population (1).

## Consequences of inaccurate results

Though most tumors do not exhibit MSI, accurate testing provides important guidance for oncologists. It can help physicians avoid prescribing expensive immune checkpoint therapy for patients with tumors that are unlikely to respond, rule out hereditary LS and sporadic dMMR cancers in patients with a variety of LS-related malignancies, and identify their relatives' propensity for cancer (3). It is also an important first step in guiding patients toward novel and effective immunotherapies. A key example is the KEYNOTE-016 clinical trial investigating the effects of pembrolizumab across tumor types, which demonstrated response rates of up to 53 percent, with 64 percent of those responses lasting 12 months or longer (31). There are clear clinical benefits to MSI testing – and sufficiently compelling evidence to warrant the use of PCR as the most accurate and efficient method of detection.

*Richard Hamelin is Research Director (Retired) at Inserm, Paris, France.*

*See references online at: tp.txp.to/ideal-method*

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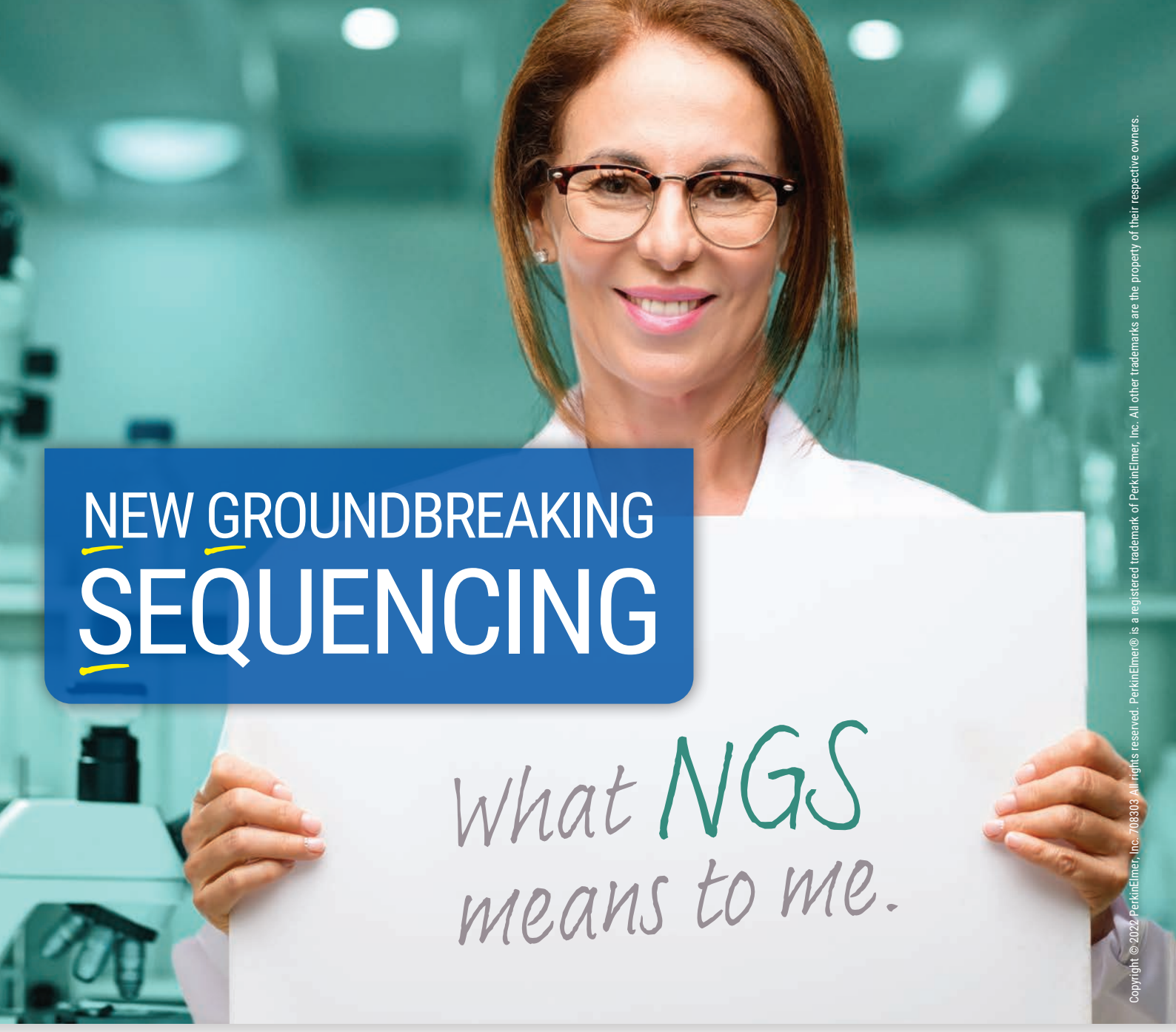




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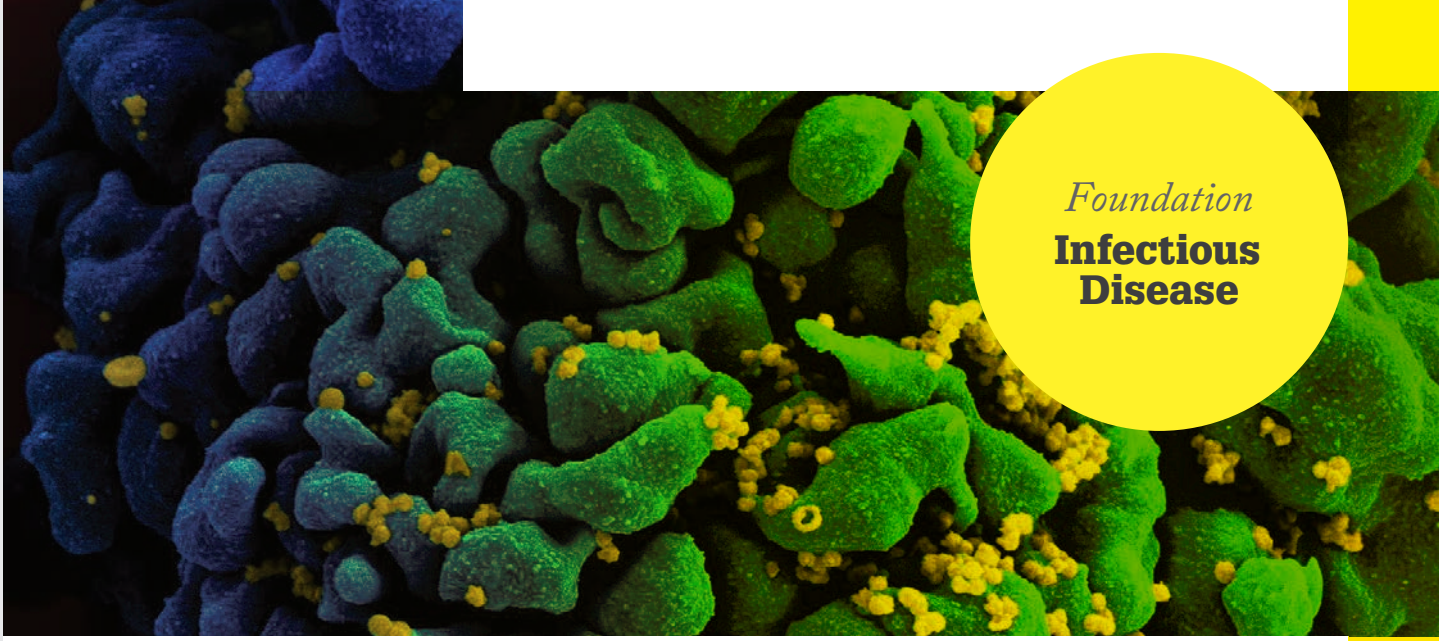

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**Trustworthy Testing.** Tuberculosis infection is not always easy to diagnose – especially in populations such as children or patients with HIV, who often have difficulty with sputum sample collection or provide samples with bacterial levels too low for reliable testing. A new CRISPR-based test that detects *Mycobacterium tuberculosis* cell-free DNA in blood is sensitive enough to diagnose tuberculosis in these populations – offering potential for earlier disease diagnosis and more rapid monitoring of treatment response (1).

**Vaccine Validation.** Evaluating COVID-19 immunity is often difficult and labor-intensive – so researchers have developed an instrument-free microfluidic chip capable of directly visualizing SARS-CoV-2 antibodies (2). The chip has "sensitive" and "rapid" modes; the former offers a 13.3 ng/mL limit of detection and takes 70 minutes, whereas the latter has a 57.8 ng/mL limit of detection, but takes only 20 minutes. Testing showed higher antibody levels after mRNA vaccination than after inactivated vaccination; both showed significant decay at 45 days post-vaccine.

**Survival Stars.** Many drug-resistant bacteria evade antibiotics by reducing their accumulation – but how? Recent research reveals that fast-growing phenotypic variants of common pathogens exhibit higher expression of active ribosomes, allowing them to produce more pores and clear macrolide antibiotics more rapidly (3). Although previous studies have pointed to slow metabolism and dormancy as key tactics bacteria use to evade treatment, these new findings indicate a previously unexplored approach to antimicrobial resistance.

**Macrophage Modeling.** What happens to your lungs when COVID-19 hits? A lung and macrophage co-culture system reveals that different types of macrophages react differently to SARS-CoV-2, with both classically (M1) and alternatively (M2) polarized macrophages inhibiting infection (4). M1 macrophages also upregulate inflammatory factors and affect lung cells, suppressing their growth and enhancing apoptosis. Understanding these distinct responses could offer greater understanding of COVID-19 disease severity and allow better treatment of inflammation.


*References*

1. *Z Huang et al., Lancet Microbe, S2666-5247(22)00087-8 (2022). PMID: 35659882.*
2. *M Wu et al., Sci Adv, 8, eabn6064 (2022). PMID: 35658040.*
3. *U Łapińska et al., Elife, 11, e74062 (2022). PMID: 35670099.*
4. *Q Lian et al., Nat Commun, 13, 2028 (2022). PMID: 35440562.*
5. *J Omooja et al., BMC Infect Dis, 22, 474 (2022). PMID: 35581555.*
6. *Y Sato et al., Anal Chem, 94, 7814 (2022). PMID: 35604144.*
7. *F Obermeyer et al., Science, [Online ahead of print] (2022). PMID: 35608456.*


## IN OTHER NEWS

**Retro resistance.** *HIV genotyping is vital to treatment monitoring – but researchers have noted in-lab genotyping success rates of only 49.8 percent using dried blood spots, compared with 85.9 percent using plasma. To increase reliability, improvements are needed in sample handling and testing (5).*

**Imaging IAV.** *Researchers have developed a new class of triplex-forming peptide nucleic acid-based fluorogenic probes that selectively bind to the influenza A virus (IAV) RNA promoter region to allow IAV imaging and screening for new drug candidates (6).*

**Fit testing.** *Analysis of 6.4 million SARS-CoV-2 genomes reveals mutations associated with increased fitness, including correct identification of Omicron subvariant BA.2 as the existing lineage with the highest fitness (7). In combination with genomic surveillance efforts, such modeling could offer early warning of potentially important new variants.*

# Taking the Long View: Strategies for SARS-CoV-2 Surveillance

**Diagnosing and tracking SARS-CoV-2 and its variants requires an intelligent combination of platforms and technologies**

*By Angelica Olcott and Vanitha Margan*

Angelica Olcott

Although not the first virus to threaten the world in the 21st century, SARS-CoV-2's emergence in late 2019 gave rise to the most severe pandemic in recent history. And although mass vaccination and widespread diagnostic testing have reduced COVID-19 case rates, the disease continues to pose a significant threat of infection and death. This is largely due to its ability to evolve new variants, which in turn is related to its high worldwide prevalence (1). SARS-CoV-2 therefore remains a real concern for public health systems and for the global economy (2) – so how do we manage the risks?

The consensus is that governments should focus on readiness; public health systems should be able to quickly and effectively respond to the appearance of easily transmissible SARS-CoV-2 variants, such as Omicron, while remaining attentive to outbreaks caused by other pathogens. This implies that we should adopt surveillance methods designed to detect new variants – but successful surveillance requires a robust combination of technology platforms, including flexible antibody detection platforms for seroprevalence studies, real-time quantitative PCR (qRT-PCR) to monitor the prevalence of variant sequences, next-generation sequencing (NGS) to identify the emergence of new variants, and droplet digital PCR (ddPCR) for community-based surveillance through wastewater testing.

## Building the toolkit

*PCR methods: good, but not sufficient*

PCR testing is the gold standard for diagnosing COVID-19 in symptomatic individuals. qRT-PCR can detect viral RNA in nasal or salivary swabs with very high sensitivity and can therefore provide accurate diagnoses during early stages of infection. Relevant tests include assays specific for SARS-CoV-2 only and assays that test for a panel of respiratory viruses. The latter enables differentiation between COVID-19 and other infections – a useful

capability during flu season.

But PCR-based tests are not foolproof; in particular, the rate of false negatives varies during the course of an infection due to changes in viral load (3). The probability of accurate PCR-based SARS-CoV-2 detection is high (77 percent) four days post-infection, when there is a high viral load in the nasal cavity, but significantly lower thereafter (~50 percent at 10 days post-infection and ~0 percent at 30 days post-infection). The effectiveness of routine screening of asymptomatic individuals is therefore constrained by testing frequency and viral load effects (3). Finally, a diagnostic assay may not be as useful for new variants as it is for the variants for which it was designed, depending on the nature and site of mutations; for this reason, commercial kit vendors must continually monitor the sensitivity and specificity of their assays against emerging variants.

*Antibody methods: classic tests, still important*

Antigen and antibody assays detect SARS-CoV-2 antibodies regardless of infection status and can therefore confirm either current or historic COVID-19. Seroprevalence studies using these kinds of tools allow us to better understand true exposure rates across a population and characterize the spread of new variants. This is important; because four in five patients with COVID-19 are asymptomatic or only mildly symptomatic, restricting tests to symptomatic individuals alone does not allow us to track the true incidence of infection (4). Some serology assays also enable us to measure IgA, IgG, and IgM antibody responses to nucleocapsid, receptor binding domain (RBD), spike 1, and spike 2 SARS-CoV-2 antigens, respectively, and therefore provide excellent data on natural infection and vaccine-induced immunity (5). In addition, serology assays give information on both population exposure rate and – because each isotype is associated with a different stage of infection – the profile and duration of humoral responses (6,7). Data from antibody-based population surveillance therefore helps us understand both the rate of immunity and its persistence. This has obvious utility in determining the longevity of vaccine effectiveness following mass vaccination programs (5,8). Such tests can also help us understand how symptom severity correlates with antibody response during disease progression, which may help plan new therapies to control disease severity and improve patient outcomes.

| *Varients of Concern* | *Varients of Interest* |
|---|---|
| Alpha (B.1.1.7) | Lambda (C.37) |
| Beta (B.1.351) | Mu (B.1.621) |
| Gamma (P.1) | |
| Delta (B.1.617.2) | |
| Omicron (B.1.1.529) | |

Table 1. SARS-CoV-2 VOCs and VOIs. These variants contain mutations in the spike (S) protein, within the amino-terminal domain (NTD) or within the RBD. These alterations influence transmissibility, virulence, and/or evasion of immune responses (9).

## Using the toolkit

*Define your task: monitoring variants*

The rapid, global spread of SARS-CoV-2 has been accompanied by the emergence of numerous variants, categorized as variants of concern (VOCs) or variants of interest (VOIs) (see Table 1). We must monitor the circulation of these new subtypes, even within vaccinated populations, because accurate monitoring may identify variants that can evade immune responses. We need look no further than the Omicron variant, with its high transmissibility even among vaccinated individuals, to see that variant surveillance remains essential and may help prevent outbreaks caused by emerging VOCs (10).

Such surveillance should not be limited to measuring isotype-specific antibody responses; it must also assess antibody efficacy – that is, the extent to which vaccine-induced antibody responses remain effective against the new variant – by determining levels of neutralizing antibodies that protect against disease. Data from these kinds of tests are critical determinants of the level of concern public health systems attribute to arising variants.

*Combine your tools: variant monitoring with qRT-PCR, NGS, and ddPCR*

One of the most powerful ways to monitor changes in circulating SARS-CoV-2 sequences over time is to use ddPCR, qRT-PCR, and NGS technologies in combination, because each approach has different advantages. For example, qRT-PCR assays permit rapid, cost-effective assessment of the prevalence of known SARS-CoV-2 variants. In this context, commercial systems that assay multiple mutations in a single reaction are more cost-effective than single-mutation assays and provide more complete mutation profiles per sample. Furthermore, qRT-PCR assays are compatible with samples containing

Vanitha Margan

low levels of viral RNA (Cq values of 31–40), enabling mutation profiling of samples that cannot be reliably evaluated by NGS, which requires less challenging Cq values (11). NGS methods, however, can confirm variants indicated by qRT-PCR – and, indeed, qRT-PCR permits rapid prioritization of samples for subsequent NGS-mediated variant confirmation. Another advantage of qRT-PCR assays is their simplicity, which allows them to be rapidly deployed in laboratories around the world to enhance global surveillance measures (12). Finally, some qRT-PCR assays can be applied to ddPCR protocols. This is important because the ddPCR approach has several advantages, including lower rates of false positives, less variation between replicates across dilution levels, and maintenance of sensitivity and specificity even in very low abundance samples, such as wastewater (13).

In summary, continued surveillance using a combination of technologies will allow us to study new VOCs and VOIs, improve our understanding of variant transmission characteristics, and better monitor the effectiveness of vaccines. This knowledge will then guide key decisions, including whether and when to incorporate antigens from emerging VOCs into new vaccines or boosters.

*Use tools wisely: variant surveillance by population and wastewater testing*

Although PCR and antibody assays help determine whether an individual is or has been infected, they do not give a complete overview of a community's susceptibility to SARS-CoV-2 outbreaks. A better approach is to track SARS-CoV-2 incidence at the community level in real time with wastewater-based epidemiology (WBE) using ddPCR (14). There is a clear correlation between the number of COVID-19 cases in a population and the SARS-CoV-2 gene concentration in that population's wastewater (15) and, given that individuals shed SARS-CoV-2 virus into wastewater earlier and for longer periods than is suggested by respiratory samples, WBE permits more accurate monitoring of infections in a local population than conventional methods. Also, use of ddPCR protocols can mitigate the issues that make wastewater treatment plant influent notoriously difficult to work with: the large number of contaminants, the presence of PCR inhibitors, and low levels of virus. Furthermore, ddPCR offers levels of sensitivity and precision beyond those associated with qRT-PCR assays and is therefore particularly useful for low-abundance targets, for targets in complex backgrounds, or for monitoring subtle changes in analyte abundance. In particular, ddPCR's high sensitivity makes it well suited for population screening through pooled testing (detecting one infected individual in 10,000) and for confirming negative results suggested by other methods. In addition,

the ability of WBE-ddPCR to estimate the abundance of a specific variant in a sample and its independence of people's propensity to opt for a PCR test allow indirect monitoring of variant incidence. Indeed, recent studies of WBE for early identification of infection hotspots indicate that it effectively identifies incidence peaks and predicts infection outbreaks (16). Finally, some ddPCR assays can accurately discriminate and quantify multiple variants in a sample using a single-well assay (17) and, in comparison with multiplexing approaches based on other technologies, ddPCR multiplexing provides data that are less prone to artifacts and easier to analyze.

Thus, ddPCR-based WBE is a powerful surveillance tool that accurately measures copy numbers of both wild-type and variant genomes. It is encouraging that fully quantitative WBE surveillance is now globally available; this approach should be recognized as complementary to clinical testing and academic research and as a key tool in the management of COVID-19 and other pandemics.

## Looking ahead: surveillance for new variants

Now that vaccination programs have been rolled out, pandemic management has moved to a different phase; today's priority is timely detection of emerging SARS-CoV-2 variants. This requires reliable, population-level assays that identify new variants and estimate their prevalence and transmission rates. WBE is now a key component of these surveillance strategies (18); nonetheless, other tools remain critically important for identifying novel VOCs or VOIs and determining their prevalence. Used in combination, these various assay methods employed in prevalence studies will not only help manage near-term SARS-CoV-2 outbreaks, but also ensure that we are prepared for future pandemics. In brief, development and intelligent use of a comprehensive surveillance toolkit enables us to detect SARS-CoV-2, track carriers, and inform public health strategies. This will help us to slow the spread of the virus and allow the public to live their lives in safety.

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*See references online at: tp.txp.to/the-long-view*


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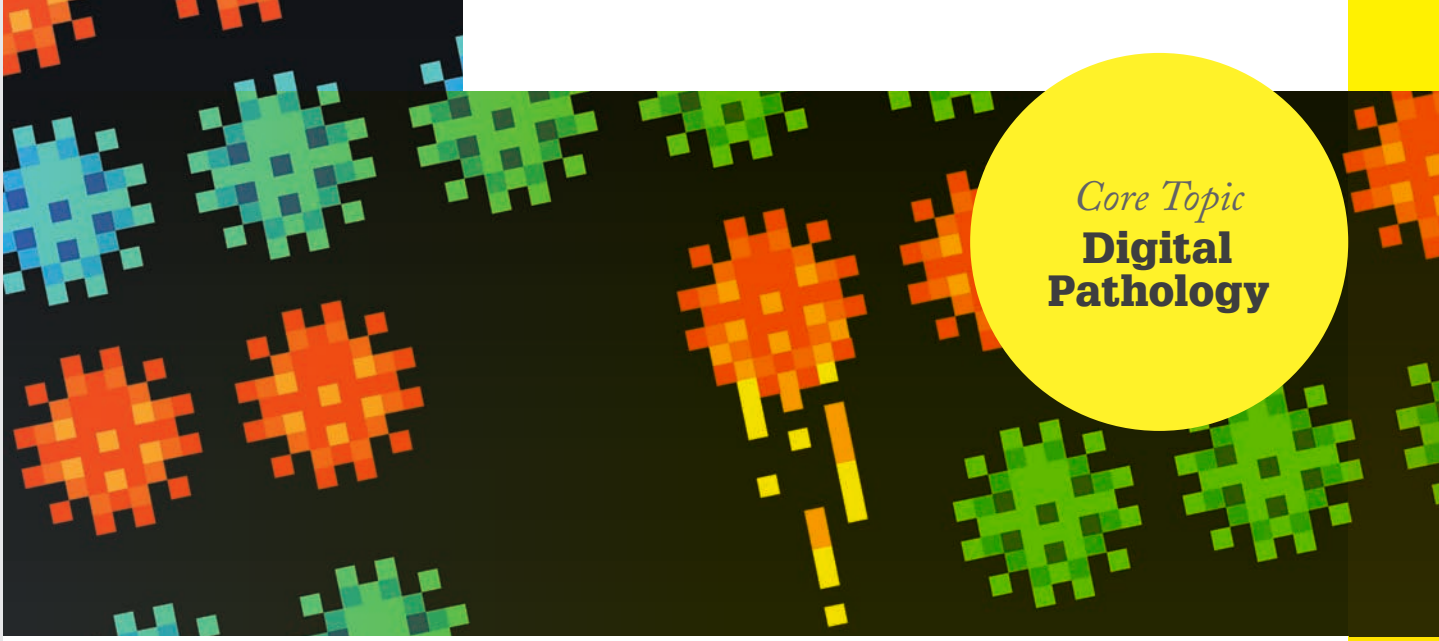


*Core Topic*
**Digital Pathology**

# Learning to ADAPT

## New machine learning tool designs sensitive viral diagnostics

Over the past two years, the COVID-19 pandemic has highlighted the importance of effective diagnostics for infectious diseases – not to mention the vital role testing has played in curbing the spread of SARS-CoV-2. Now, researchers have developed a fully automated system to predict the effectiveness of viral diagnostics and design optimal tests (1). Using CRISPR-based diagnostics, the team screened 19,209 diagnostic-target pairs and trained a deep neural network to predict the diagnosis. They then used combinatorial optimization to improve the model's sensitivity to viral genomic variation – arriving at the Activity-informed Design with All-inclusive Patrolling of Targets (ADAPT) system.

"ADAPT is really about developing countermeasures that target the virus that's circulating right now and being prepared to move with the virus as it changes," said Pardis Sabeti, senior author of the study (2). "As we've watched SARS-CoV-2 adapt in real time, we've learned just how much we need to change with it and other viruses."

"When we concentrated on COVID-19 in mid-January 2020, it was remarkable how quickly the global community was generating genomic data on the virus, with 20 genomes at the time and that number growing exponentially," said lead author Hayden Metsky (2).

The team used ADAPT to design diagnostics for 1,933 vertebrate-infecting viral species, detecting most species within two hours and all but three within 24 hours. Though the ADAPT deep neural network was trained for CRISPR-based diagnostics, the team highlights that it can also be applied to models for other sequence-based tests, such as qPCR. "At the core of building good diagnostics is knowing what to target and how to target it," Sabeti said (2). "We spend a lot of time building technologies to do that, but we've shown that, with thoughtful algorithmic work, we can get these methods to work much, much better."

As COVID-19 shifts towards endemicity, there will no doubt be a shift in the number and type of tests required in healthcare and community settings – with a focus on identifying a wider variety of circulating respiratory viruses as they evolve. Recognizing this, ADAPT predicts which guide RNAs will yield strong signals in tests applied to different viral strains and variants, meaning that it would be able to detect different lineages throughout a virus' evolution. The researchers also built ADAPT using the latest viral genomes from public databases – and the system is fully automated, allowing it to stay up to date as new variants emerge.

*"We spend a lot of time building technologies to do that, but we've shown that, with thoughtful algorithmic work, we can get these methods to work much, much better."*


*References*

1. *HC Metsky et al., Nat Biotechnol, [Online ahead of print] (2022). PMID: 35241837.*
2. *Allessandra DiCorato (2022). Available at: https://bit.ly/3wiuDXW.*

# A Newer Frontier

**You've all heard of "going digital," but what about "going AI" – and what does the move toward AI actually mean for labs?**

*By Joseph Mossel*

Digital pathology is no longer a "new frontier" in the field – in fact, it's growing increasingly mainstream. But even for those who have readily adopted the digital side of the discipline, artificial intelligence (AI) often remains a mystery. Why should laboratories consider "going AI?" What benefits does it offer? And what about laboratories with fewer resources, higher case volumes, or less digital infrastructure? At first glance, AI may not seem accessible or equitable – but the reality might surprise you.

AI at Dr Lal PathLabs, India.

## Pathology's biggest challenges

Many places currently face pathologist shortages – with too few staff members to tackle the laboratory's volume of work. That's not necessarily the case everywhere but, even in places with sufficient staff, labs are under increasing pressure due to not just the growing volume of cases, but also their complexity. That's why a lot of labs are now looking to get into digital and computational pathology – but that, too, raises a lot of questions. How do I evaluate AI algorithms? How do I choose the right digital pathology vendor for my work? What IT infrastructure do I need? It's easy to talk about getting started, but harder to actually do it.

Conversations about digitization often compare pathology to radiology – but I think AI will play somewhat of a different role for pathologists than it does for radiologists. That's partly because, when radiology went digital, they simply didn't have the AI capabilities we have today. AI and pathology are growing and learning together, which means we have an opportunity to really add value by incorporating AI into our workflows. That has led to a reluctance among pathologists to just "go digital and then figure out AI later." They want to incorporate both at the same time because they feel that, without AI, they won't see the full benefits of digitization.

AI brings with it three main value propositions:

### 1. *Improved diagnostic accuracy*

Pathologists are often busy and overworked. Every day, we see AI catching potential diagnostic errors and alerting pathologists to their presence, giving them the opportunity to revisit the diagnosis and reduce the likelihood of error.

### 2. *Increased efficiency*

There are two ways in which AI can make diagnosis more efficient. The first is at the individual level; AI is a decision support tool that can help pathologists make diagnoses faster; you can think of it as a "thought assistant." The second is at the lab level – using AI to optimize the lab workflow. It can help you ensure that you address the most urgent cases first, assign specific cases to specific pathologists, or even order stains in advance, all of which can have a significant impact on turnaround time.

### 3. *A new frontier in cancer diagnosis*

Given a new ability to treat pathology slides quantitatively, what new insights can we glean from the data? That opens up huge opportunities for AI to support us in broadening our horizons. Computers can see things our eyes cannot – and that means they can allow us to make use of diagnostic information we could never access before.

## Taking the plunge

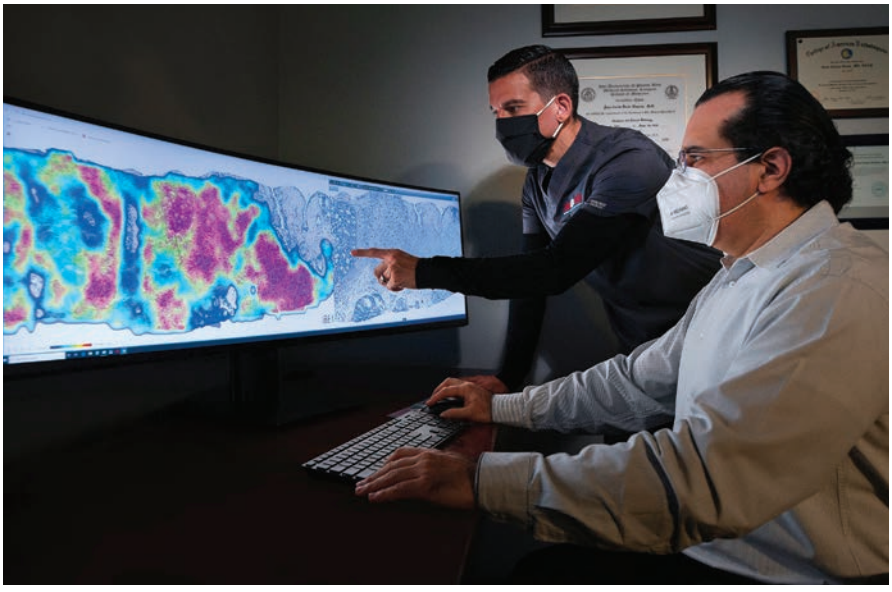
"Artificial intelligence" is a big – and

potentially scary – concept. It puts in mind the idea that technology might one day replace pathologists – a misconception not shared by most machine learning experts. But AI is really just another set of tools – one that happens to be digital. So what are the challenges you might face if your laboratory wants to implement AI?

First of all, you need digital pathology infrastructure, which goes beyond purchasing the right hardware and software products. You need a way to embed AI within your existing workflow. And you need to overcome potential resistance from your clinicians.

Some take that first step by implementing AI for case review. Basically, the algorithm performs an additional review – a second read - of all the cases going through the lab and raises an alert if there is a possibility of diagnostic error. It's easy to bring on board because it doesn't require any changes to the primary diagnostic process; it's simply an add-on step at the end that acts as a "safety net." Clinicians also really like this application because they tell us it "helps them sleep a bit better at night." Within this framework, the subsequent expansion of the AI platform toward assisting in primary diagnosis – which may lead to additional gains – would become more natural.

Every day, I see misdiagnoses – cases in which samples have been reported as benign until our system alerts on a potential false-negative error, at which point they are revisited and diagnosed as cancer. Recently, I encountered a situation in which the cancerous cells were not in the area of the biopsy the pathologists were examining; as a result, they were missed until the AI algorithm alerted to their presence. We took the case to an advisor – Stuart Schnitt, a breast pathologist at Brigham and Women's Hospital – who confirmed a diagnosis of breast cancer, but emphasized that it was a very subtle case. For us, that was incredible. Thanks to AI, the patient was able to avoid misdiagnosis and potentially a severely worsened outcome.



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## Thinking ahead

The transition to remote working has definitely accelerated the adoption of digital pathology – and of AI as well. The downside, though, is that many labs have been overwhelmed by the volume of COVID-19 testing they've been asked to do, making it a less-than-ideal time to deploy a new technology. My hope is that the acceleration of pathology's digitization will help the discipline move more quickly and efficiently toward its future.

And what is that future?

It comes in two parts: the adoption of digital pathology and AI into diagnostic practice and our increasing ability to extract data from slides. The first is already happening around the world – we're past the point of early adoption. There will be an inflection point where AI suddenly becomes a standard part of routine diagnostic practice. The second will be another modality that joins our existing troves of data – clinical data, genomic data, and now quantitative features extracted from slides by AI. Ultimately, having access to this kind of data, which enables new insights, will allow us to develop newer and better tests for not just diagnosis, but also prognosis and treatment selection.

There's one other important aspect to this – and that's the equity it brings to diagnostics around the world. Right now, if you're a patient in a developed country with access to top hospitals and world experts, you stand a high chance of getting the right diagnosis and treatment quickly. But not all patients are in these settings – and not all pathologists have the luxury of working in these environments. Many work under severe time and resource constraints and more pressure, which unfortunately creates more opportunities for things to go wrong. The beauty of AI is that it can be deployed everywhere. In fact, it brings the most value in labs with higher volumes and fewer resources, because that's where the likelihood of error is highest – so that's where an extra layer of oversight can yield the greatest benefit. All patients should have equitable access to diagnostics – and, even though it seems counterintuitive, new technologies can pave the way.

*Joseph Mossel is Co-Founder and CEO of Ibex Medical Analytics, Tel Aviv, Israel.*

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# Fake News!

## Combating misinformation in science and medicine

*Michael Schubert interviews Timothy Caulfield*

Timothy Caulfield's career began in general health and science policy research – but the more he investigated how matters of science and medicine are represented in the public sphere, the more aware he became of the growing problems of misinformation and disinformation. We asked him about the scale of the issue, the most common – and most difficult – misinformation he encounters, and what pathologists and laboratory medicine professionals can do to help combat it…

### Has the volume of misinformation increased in recent years?

I think the intensity and the impact have increased. This is largely because social media has increased the reach of misinformation – and its sway. I think we've seen that throughout the pandemic, but it was a problem even before COVID-19. The other factor is the role of pop culture in the spread of misinformation. Celebrities have played a major role in how we perceive health issues, which is something we saw starting to happen in the 1990s, but which accelerated throughout the pandemic.

### What are some of the most common pieces of misinformation you see?

This is a very important question right now, because we're starting to get a lot of revisionist history of what happened at the height of the pandemic, what the misinformation was like, and how wrong it really was. Weren't there a lot of contested issues that went unresolved? Why wasn't there more open debate?

That is all nonsense. The misinformation was pretty clear – it's things like:

- "The vaccines don't work."
- "The vaccines kill more people than they save."
- "The vaccines cause infertility."
- "Ivermectin and hydroxychloroquine are effective against COVID-19."
- "COVID-19 was caused by 5G technology."
- "COVID-19 is a hoax."

Those are just some examples; there was a constellation of entirely false narratives that did real harm. Before the pandemic, a lot of the same narratives existed within the anti-vaccine community – but we brushed them off. We laughed at health misinformation – that you can detox your body, that you need cleanses, that natural products are always better than pharmaceuticals – instead of paying attention. We shouldn't have, because ignoring or enabling misinformation facilitates its spread. We need to take pseudoscience seriously.

### What is the hardest misinformation to counter?

I think it gets really challenging when those who are spreading misinformation do certain things.

The first is when they leverage scientific uncertainty – something that has happened a lot during the pandemic. They say things like, "Oh, you know, public health experts didn't know about masks. Two years ago, they told us they didn't work." Of course, science evolves – and, of course, recommendations should evolve with it. But those who push misinformation effectively leverage that uncertainty to push more uncertainty and more misinformation. They create information chaos. And that works well, unfortunately, and can be difficult to counter.

The second is when they marry misinformation with ideology. That has been a trademark of misinformation in the pandemic era; in fact, there's an almost perfect correlation between ideology, belief in misinformation, and disagreement with public health measures, such as vaccination or judicious mask requirements. At this stage of the pandemic, at least in most developed countries, we're hearing less about issues such as equity, access, or even pain and needle phobias. Although

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those are all very real and important issues, we're largely left with individuals who are ideologically motivated to embrace misinformation.

That said, "anti-vaxxers" are not a homogeneous group. Many are ideologically opposed – but others are simply hesitant or under-informed. It's always important to listen and to have empathy.

You've touched on a common objection – "the science is always changing." Experts know why that is, but how do you explain it to people who don't?

Hopefully, this is a lesson we will learn from the pandemic. Scientists, science communicators, public health experts – we could have done better. A good example is the discussion surrounding masks early in the pandemic. We were too dogmatic. And that's partly because the first rule of public health communication during a crisis is clarity. We all erred on the side of too much clarity regarding the recommendations at the time, which meant that we weren't transparent enough about the scientific uncertainty that accompanied them. We didn't explain well enough that science is not a list of facts that never change; it's a fluid process. We should have said, "These recommendations are based on the best available evidence right now – so they may change as we learn more." We need to invite the public to join us on that uncertainty journey.

*"Research tells us that healthcare providers are among the most trusted voices out there – so you can do real good as a voice for science and truth."*

The other thing we need to tell people is that science is still the best tool we have. For example, let's say your neighbor thinks that the weather is caused by a machine in the sky and Big Weather produces rain to force us all to work harder. If a meteorologist (who bases his assumptions on data) tells us it's going to be sunny tomorrow and the neighbor tells us it's going to rain – and then it rains – that doesn't mean we should listen to conspiracy theorists. We should still listen to the science-informed voice. Unfortunately, we're seeing a lot of revisionist misinformation along the lines of, "Science said this and it was wrong, so that's proof that we shouldn't ever listen to the science." We've got to push back against that.

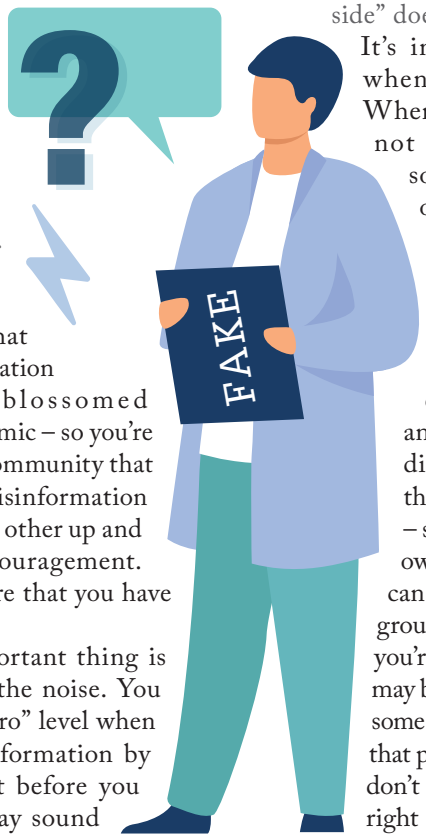
Healthcare professionals are in a unique position to fight misinformation. How can they help?

Healthcare providers should join the conversation. Engage with your patients and community. Call out misinformation when you see it. I know that can be difficult and not everyone wants to do it – but those who do need to know that they have the support of their colleagues and institutions, as well as legal support if things go sideways. Research tells us that healthcare providers are among the most trusted voices out there – so you can do real good as a voice for science and truth.

"Misinformation burnout" is prevalent among scientific and medical professionals. Do you have any tips for dealing with it?

The good news is that the science communication community has blossomed throughout the pandemic – so you're joining a wonderful community that can help you with misinformation fatigue. We hold each other up and give each other encouragement. You should also ensure that you have institutional support.

But the other important thing is to take breaks from the noise. You can do that on a "micro" level when you encounter misinformation by simply taking a beat before you engage with it. It may sound ridiculously straightforward and simple, but there's evidence that it makes you less susceptible to misinformation. You can also do it on a "macro" level. Step away from the noise in the evening or give yourself a weekend off – especially when it comes to things like social media. Too much exposure to negative news and misinformation can generate stress. It's important to take a break for the sake of not only your critical thinking skills, but also your mental health.




What do you do when the "other side" doesn't want to listen?

It's important to know when to step away. When you know you're not going to change someone's mind, it's okay to stop trying. But that doesn't mean you should lose your optimism. Your goal is to give people a path to credible information and there are a lot of different ways to do that. Narratives matter – so why not share your own experience? If you can find some common ground with the person you're speaking to, you may be able to break down some defenses and open up that path. People generally don't change their minds right in front of you, but

you may still make a difference – perhaps not today, but in the future.

### Do some ways of addressing misinformation work better than others?

The silver lining of the misinformation explosion is that there has been more research on what works and what doesn't to debunk misinformation. This is a complex phenomenon, so we have to come at it from every direction – responses from regulatory bodies, teaching critical thinking and media literacy at all stages of life, "prebunking" (warning people about misinformation before they encounter it), and debunking and correcting misinformation when we see it. How can you best do this?

- Talk about good science. Explain the scientific consensus, how it works, and why scientists have made the evidence-based decisions and recommendations they have.
- Speak out on social media. If you want the people who are seeing misinformation to also see the truth, you have to go where the misinformation resides – and, these days, it resides on social media.
- Make your content shareable. You want it to grow beyond the confines of your network.
- Be creative. I always like to say, "Creativity wins." Use humor; use stories; use art; use all of the strategies that the people pushing misinformation also use.
- Be empathetic. The research on tone is mixed (and, in fact, some even suggests that a little bit of snark can be okay) but, if you want to have a long-term conversation with an audience, it's important to be respectful – and to be upbeat.
- If possible, feature a diversity of voices to ensure that a variety of communities feel heard and addressed.
- Don't let the backfire effect scare you away. I hear a lot of people say, "We shouldn't try to counter misinformation, because it causes people to become more entrenched in their views." Most of the recent evidence tells us that the backfire effect is less common than people think (and is often context-specific) – so don't let it stop you from getting out there and engaging.

### Where can people find you on social media?

You can follow me at @caulfieldtim on Twitter and Instagram – but the initiative I really want to highlight is #ScienceUpFirst, which is @scienceupfirst on Twitter, Facebook, Instagram, and TikTok.

#ScienceUpFirst involves a wonderful and diverse team of science communicators, artists, and video producers who create content that we hope is shareable and relevant to a wide range of communities. We consult independent scientific experts to make sure that our information is accurate. And we try to tackle misinformation broadly, not just in the context of COVID-19, so that people know what to watch for and how to make sure they're not inadvertently spreading misinformation. The initiative has been incredibly successful, especially in Canada, but increasingly around the world. We would love for everyone to join the #ScienceUpFirst team and help it become a self-sustaining movement.

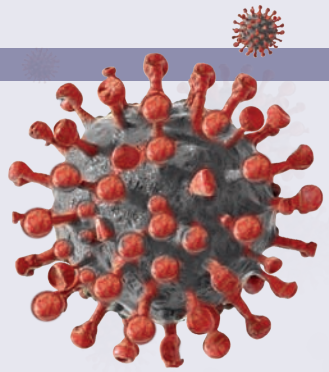
### What one take-home message do you want to give scientific and medical professionals about misinformation?

Get out there. Don't be afraid to counter misinformation. I know that I'm speaking to incredibly busy professionals who may not have the time – or the passion – to debunk misinformation. If that's you, what you can do to help the cause is go to sources that aggregate science in a responsible manner and share their content.

*"Unfortunately, we're seeing a lot of revisionist misinformation along the lines of, 'Science said this and it was wrong, so that's proof that we shouldn't ever listen to the science.' We've got to push back against that."*

People often ask me if there's one message that you should give the public about how to recognize misinformation – and I think it's fair to give healthcare professionals the same advice. Ask yourself, "What kind of evidence is being used to support this claim? Is it an anecdote? A testimonial? Primary research? Does it truly reflect the body of evidence at play? Is the source just cherry-picking data? What kind of science is being used?" Just asking those questions every time can go a long way to solving the problem of misinformation.

*Want to hear this article from Tim Caulfield himself? Check out the video interview at: tp.txp.to/vid-fake-news*

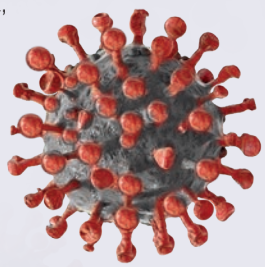


# Teamwork for COVID-19 Testing

**In 2021, in the height of the COVID-19 pandemic, Automata worked with the University Hospital Southampton (UHS) NHS Foundation Trust to automate reverse transcript loop-mediated isothermal amplification (RT-LAMP) testing.**

The University Hospital Southampton (UHS) NHS Foundation Trust was faced with a tremendous challenge: to distribute a newly developed, rapid form of asymptomatic COVID-19 testing in the UK to Hampshire and the Isle of Wight. The testing – called RT-LAMP – used a "no-swab" saliva test that benefits from higher sensitivity, faster turnaround, and less invasive collection than other methods (1). The issue, however, was that testing had to be not only fast, but also work on a massive scale; schools, colleges, and universities would be relying on this rollout to deliver results within 24–48 hours.

Expanding the use of RT-LAMP testing beyond the few schools involved in a local pilot study was not simple. Demand for testing exceeded thousands each day and, with mounting COVID-19 restrictions and limited technicians available, there were significant roadblocks in scaling the process to the necessary requirements. UHS soon realized that the laboratory needed to use machines – and, with the help of Automata, they set out to create an automated workflow for large-scale COVID-19 testing. This project was the first of its kind, meaning that each step would require bespoke equipment, processes, and systems to push the bounds of automation as far as possible.



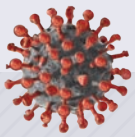
Success was no mean feat – and it was thanks to the flexible nature of Automata's designers and engineers that the project was even possible. UHS initially suffered from teething issues with other providers before ultimately settling on Automata, who had already been on board as a robotics supplier and had pitched a flexible, integrated platform. The partnership meant that Automata could supply custom-built machine solutions and marry its own robotic automation technology with that of other market leaders.

Though automation is bound to require cutting-edge technology, engineers were keen to integrate the lab's existing instruments into the new system to save costs and ease the acclimatization process for staff. This created a unique mixture of repurposed, new, and purpose-built laboratory solutions, including integrated barcode scanning, a process that tracks each vial that enters and exits the system. Machine-aided tracking eliminates the risk of human error in manual methods – a highly desirable and repeatable result that is ideal for a national clinical facility.

One of the project's main goals was to pair the quality and consistency of a standard operating procedure with the flexibility and speed of an automated system. Meanwhile, engineers were tasked with transforming a human-focused workflow to one designed for machines. This required a collaborative and dynamic approach. A service team intimately familiar with the technology was stationed on-site to make any necessary changes to the laboratory's requirements on an ad hoc basis. This proactive approach enabled engineers to actively participate in the implementation and development stages, minimizing wasted time.

Of course, technology is moot if lab technicians find the system cumbersome. Perhaps more impressive than the precision and speed of the automated system is the fact that it can all be controlled from a single smart tablet. The user experience is intuitive, functional, and practical for use by individual technicians – with great potential for labs across medical fields.

The successful six-month turnaround of an automated, mass-scale testing facility within the University Hospital Southampton (UHS) NHS Foundation Trust is a sign that diagnostics automation is not only our future, but also our present.



## Advantages of automation

- data traceability increases test turnaround speed
- improved scalability facilitates higher throughput goals and lowered potential transmission
- reduced reliance on manual interactions in lab processes

*Nikoletta Sidiropoulos is Associate Professor and Director of Molecular Pathology, Department of Pathology and Laboratory Medicine, University of Vermont Health Network, Burlington, Vermont, USA.*

*Reference*

1. *Automata (2022). Available at: https://bit.ly/3LSwwze.*

# Invitae Microsatellite Instability Detection

## A modular approach for determining microsatellite instability status in solid tumors

Invitae's VariantPlex® solid tumor research panels are four pan-solid tumor assays that use next-generation sequencing (NGS) powered by Anchored Multiplex PCR (AMP™) to identify microsatellite instability status with high sensitivity and specificity without a matched normal sample.

MSI status is typically stratified into three categories – MSS, MSI-high (MSI-H), and MSI-low (MSI-L) – based on the amount of instability observed. MSI testing is recommended across various cancer types and accurate and sensitive detection is critical in the management of cancer. The status can be detected through modalities including immunohistochemistry (IHC), PCR, and NGS. Compared with IHC and PCR, NGS has made it possible to identify a greater number of microsatellite loci to improve categorization of MSI status.

Invitae has developed a VariantPlex MSI module that uses NGS powered by AMP chemistry to examine the abundance of microsatellites repeated at 121 expertly curated loci. Built for NGS biomarker detection, AMP has unique advantages in detecting both simple and complex DNA variants, setting it apart from traditional primer-based techniques for preparing NGS libraries.

The VariantPlex MSI module includes molecularly barcoded adapters and 210 gene-specific primers for MSI loci amplification, permitting open-ended capture of DNA fragments from a single end. The stability of each microsatellite is determined by the distribution of repeat lengths after collapsing PCR duplicates using molecular barcodes.

*"MSI testing is recommended across various cancer types and accurate and sensitive detection is critical in the management of cancer."*

The accuracy of the VariantPlex MSI module to consistently make the correct MSI call for given samples was evaluated on the basis of concordance of MSI calls made on tumor samples with known MSI status. The VariantPlex MSI module was 97 percent sensitive (95% confidence interval [CI], 91.5–99.4%) and 100 percent specific (95% CI, 96.3–100%) at distinguishing between MSI-H and MSS. High concordance with gold standard MSI and dMMR detection was also observed in an early-access experiment in which MSI status was detected correctly in 98.3 percent of samples tested.

The VariantPlex MSI module was shown to be consistent across multiple instruments, including the Illumina NextSeq, NovaSeq, and MiSeq platforms. Additionally, experimental results showed consistent MSI status classification across a range of input masses. The VariantPlex MSI module

was also shown to be compatible with a variety of cancer types. FFPE tissue samples were derived from more than 20 source tissues with various MSI prevalence (e.g., colon, lung, and uterine). Concordance between VariantPlex MSI status and orthogonal MSI status across tumor types was 97.8 percent.

Invitae's VariantPlex assays are modular and can be easily customized, meaning that the VariantPlex MSI module can be used as a stand-alone solution or as part of a larger customized VariantPlex panel, meeting the needs of any lab as pan-tumor MSI testing becomes increasingly important. The VariantPlex assays and the VariantPlex MSI module are for research use only.

*Learn more about VariantPlex MSI testing at: assay-marketplace.archerdx.com.*

# Advocate for the Next Generation

**Sitting Down With… Jo Horne**, STP Training Programme Director and Midlands Healthcare Science Dean, National School of Healthcare Science, and Lead Practice Educator for the Southern Counties Pathology Network, NHS England, UK

### Did you always want to be a biomedical scientist?.

I always wanted to work in medicine, but I didn't want to go to medical school. Though I was a high achiever at school, science was my worst subject; I was terrible at physics, but I loved biology, math, and art. I chose to pursue biomedical science at university because I met a histology lecturer at the University of Portsmouth Open Day who showed me how I could combine science and art. After university, I was offered the first trainee biomedical scientist job I applied for in Southampton, which happened to be in histology. Almost 25 years later, I have gone from trainee to consultant – all within the same department!

### Tell us about the Scientists Training Programme (STP) – how did you come to work on it?

The STP was developed as a result of "Modernizing Scientific Careers" – a program aimed at connecting all the different healthcare science training programs in England and applying them to a single quality-assured framework that includes specialty and rotational workplace training, a Master's degree program, and leadership development. The three-year program is nationally funded and supports scientists who train while being paid to work. The curricula cover a variety of subjects, including histopathology, audiology, and imaging, but they all have the same overarching framework.

Like many people, the pandemic prompted me to reflect on what is most important to me professionally, my values, and what I want to achieve in the next stage of my career. I have faced many challenges, and my success has been a combination of hard work, determination, taking opportunities when they arise, and being lifted by the people who have inspired me to reach my goals.

In my work, I look after the training and development of many scientists and support staff and, though I have always valued this, I have come to realize just how important it is to me. I mostly support people working in pathology, but I have also been the lead healthcare scientist at my organization, which has given me the opportunity to meet and support the wider healthcare science workforce. I have also been working with colleagues from the National School of Healthcare Science who manage the STP – they have a supportive and collaborative working environment so, when a formal post came up in early 2021, I couldn't resist applying.

### What excites you most about the incoming generation of scientists-in-training?

The next generation of scientists will have so many more opportunities than I did at the same stage of my career! With the need for service and workforce transformation and a better awareness of healthcare science, we are now in a great position to offer and promote a wide variety of opportunities for people to get into the profession after school or college – and for them to progress into the most senior levels of expertise and leadership. I'm excited to be part of the team working at a regional and national level to ensure the voice of healthcare science is heard, that we develop and commission programs to support workforce transformation and service development, and that we provide opportunities and break down barriers for all healthcare scientists, no matter what specialty they choose.

### Have you had any key mentors in your career?

There have been several people who have shaped my career, though I may not have realized it at the time. The thread that links them all together is their assurance to me that I was good enough and their support for me to dream bigger. They inspired me to challenge myself and not take no for an answer from those who made me feel like I wasn't good enough. During challenging times, my mentors have been allies – standing shoulder to shoulder with me as I challenge the traditional way things are done.

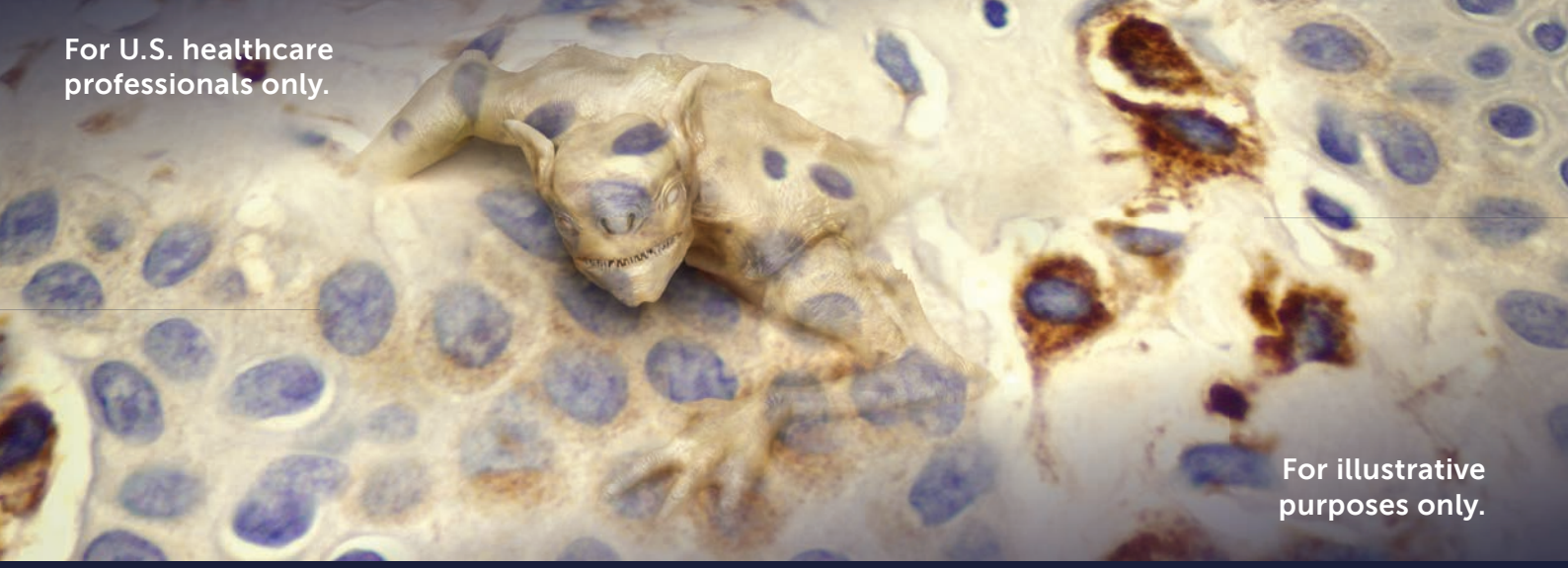
In the past few years, I have been fortunate enough to gain a mentor who has had an incredible impact on my career. She encourages me to challenge my own assumptions – guiding and supporting me to work things out for myself, rather than telling me what I should do. This relationship has inspired me to support others in the same way; I hope I can have as positive an impact on other people's careers as she has had on mine.

### What's the key thing pathologists and biomedical scientists should know about each other?

Pathologists should know that biomedical scientists are highly qualified experts in their field, often with more than one degree as well as the required experience to become a registered professional. They are often experts in quality, training, management, and leadership, as well as scientific study. On the other hand, biomedical scientists should know that pathologists are under constant pressure and should seek to understand their needs and challenges.

I believe we can all learn from each other. The key is to respect and value each other as individuals and be kind to each other – we must include everybody, listen to diverse opinions, and work together to find a common goal. To achieve this, we must seek to understand the responsibilities, needs, and expectations of different professional groups and the pressures upon all of us, which may not always look the same and may lead to uncomfortable conversations. When we work together as a true team, we provide psychological safety and look after each other better, which helps us to improve the services we provide to our patients.

*Read the full article online at: tp.txp.to/advocate-next-gen*

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**References: 1.** Gilreath JA, et al. *Clin Pharmacol*. 2019;11:77-92. **2.** Reiter A, et al. *Blood*. 2020;135(16):1365-1376. **3.** Garcia-Montero AC, et al. *Blood*. 2006;108(7):2366-2372. **4.** Verstovsek S. *Eur J Haematol*. 2013;90(2):89-98. **5.** Jennings SV, et al. *Immunol Allergy Clin North Am*. 2018;38(3):505-525.



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