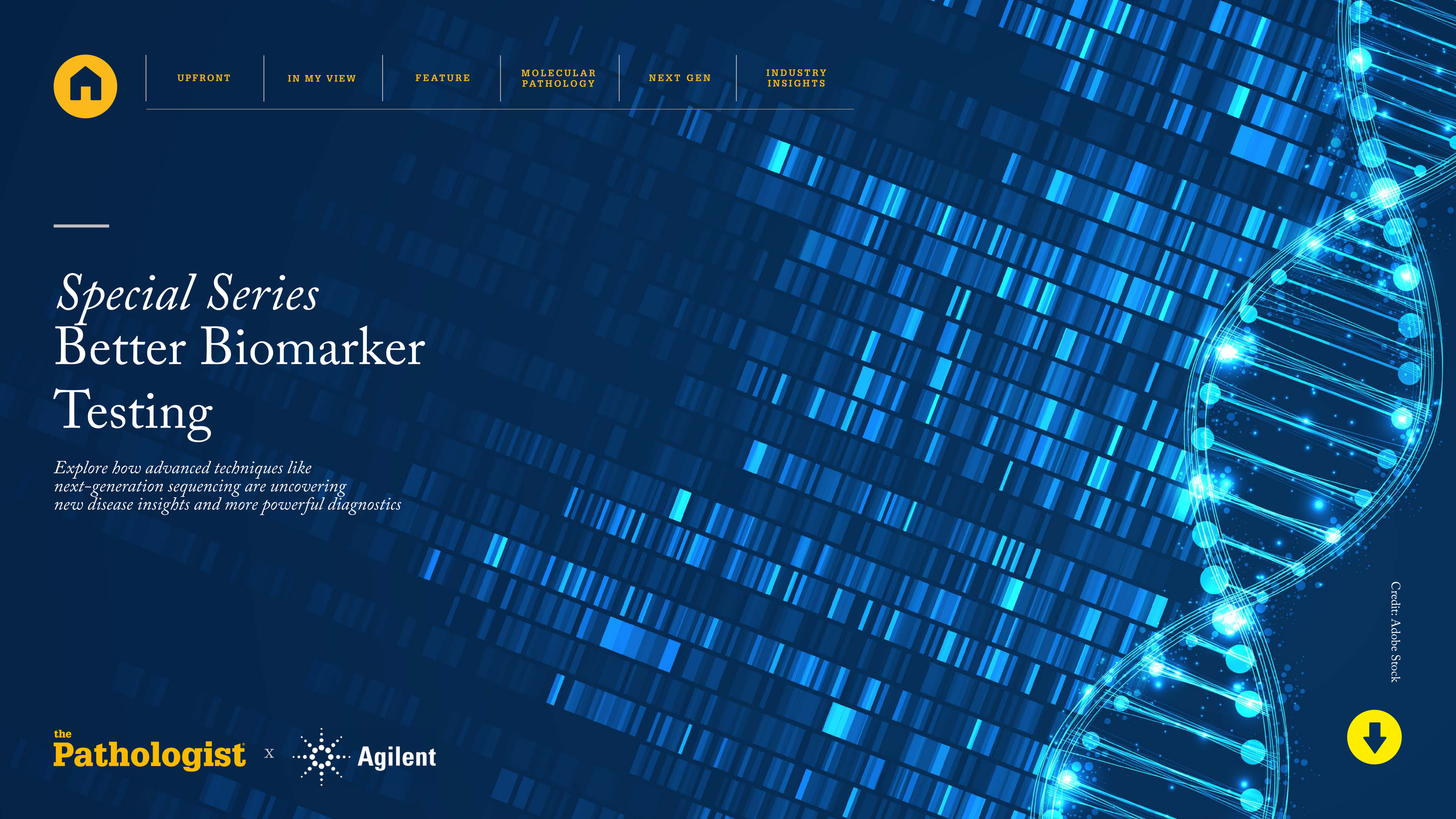
Special Series Better Biomarker Testing

Explore how advanced techniques like next-generation sequencing are uncovering new disease insights and more powerful diagnostics



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Unlocking Hidden RNA Signals

Targeted sequencing aids rare disease diagnosis

A targeted long-read RNA sequencing approach may help improve the interpretation of genetic variants and support diagnosis in patients with rare diseases, according to a study published in *Science Advances*.

Current genetic testing methods, including exome and genome sequencing, identify the cause of disease in around 20 to 50 percent of cases. This leaves many patients without a confirmed molecular diagnosis. RNA sequencing can provide additional information by showing how genetic variants affect gene expression and splicing.

In this study, researchers developed a workflow called STRIPE (Sequencing Targeted RNAs Identifies Pathogenic Events). The method focuses sequencing on selected disease-related genes and uses long-read sequencing to analyze full-length RNA transcripts. This allows variants and their effects to be assessed together and linked to specific parental alleles.

The researchers applied the method to skin fibroblast samples from 88 individuals, including patients with congenital disorders of glycosylation and primary mitochondrial diseases. In patients with known genetic diagnoses, the approach identified all previously reported pathogenic variants and provided additional information about how these variants affected RNA processing.

Compared with short-read sequencing, long-read sequencing enabled analysis across larger regions of DNA, helping to determine whether variants occur on the same or different alleles. This can be difficult with standard methods, particularly when variants are far apart.

The study also showed that variants affecting splice sites can lead to a wider range of RNA changes than expected. In more than half of the splice site variants examined, the effects were more complex than simple exon skipping. Some variants were associated with early termination of RNA transcripts due to activation of alternative polyadenylation sites.

Importantly, the method helped identify disease-causing variants in several patients who had not previously received a genetic diagnosis, including cases where standard RNA sequencing approaches were inconclusive.

Because the approach targets specific genes, it can achieve greater sequencing depth and improve detection of low-level transcripts in clinically accessible tissues such as fibroblasts. However, it is limited to the genes included in the panel and may not detect variants in genes outside the selected set.

The authors note that further work will be needed before the method can be implemented in clinical laboratories, including standardizing workflows and integrating the approach into existing diagnostic pathways.

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From Molecules to Meaning

How molecular pathology translates into real patient impact

By Sharjeel Chaudhry

Pathology has always been the silent backbone of clinical medicine. Yet, as diagnostics move beyond morphology and into the molecular realm, the role of the pathologist is no longer confined to naming a disease. It is increasingly about shaping the patient's entire clinical journey.

Molecular pathology sits at the intersection of biology, technology, and patient care. While its value is well understood within laboratories and multidisciplinary meetings, its real-world impact is not always clearly articulated outside these spaces. This article reflects on how molecular pathology meaningfully "lands" with patients and why communicating this impact matters more than ever.

From diagnosis to decision-making

Traditional pathology answers a foundational question: What is the disease? Molecular pathology goes further: Why is this disease behaving the way it is, and how can we intervene effectively?

In head and neck oncology, molecular testing can distinguish tumors that appear morphologically similar but behave very differently at a biological and clinical level. Human papillomavirus (HPV)–associated oropharyngeal squamous cell carcinoma, for example, represents a distinct molecular entity with improved prognosis and treatment responsiveness compared with HPV-negative disease. p16 immunohistochemistry, widely used as a surrogate marker for active HPV infection, carries strong prognostic significance and directly influences treatment intensity and patient counseling.

Similarly, immune biomarkers, such as PD-L1 expression, play a central role in selecting patients for immune checkpoint inhibitors, influencing therapeutic decisions and survival outcomes in recurrent or metastatic head and neck cancers.

For patients, this translates into clarity about prognosis and treatment expectations. The clarity helps reduce overtreatment, sparing patients from unnecessary toxicity. It means that patients are more likely to receive therapies with a higher likelihood of response.

Molecular pathology quietly transforms uncertainty into informed clinical action.

Impact we rarely see but always shape

Patients rarely meet the molecular pathologist. Yet molecular reports often carry more weight than any single imaging study or clinical examination. A single molecular result can:

- Confirm or exclude a hereditary cancer syndrome.
- Explain primary or acquired treatment resistance.
- Identify minimal residual disease.
- Determine eligibility for targeted therapy or immunotherapy.

Sharjeel Chaudhry

"Technologies such as NGS, multiplex immunohistochemistry, and integrated multi-omic platforms have improved diagnostic accuracy, refined risk stratification, and expanded access to targeted treatment and clinical trials."

The emergence of liquid biopsy approaches, particularly circulating tumor DNA (ctDNA), has further expanded the reach of molecular pathology. ctDNA allows non-invasive tumor profiling, real-time monitoring of treatment response, and early detection of recurrence, which is especially valuable in head and neck cancers where repeat tissue sampling may be challenging.

When a therapy succeeds or fails it is often because of molecular information generated long before treatment begins.

Bridging the communication gap

A persistent gap exists between what molecular pathologists do and what patients understand. Even within multidisciplinary teams, molecular findings may be framed as "technical" or "confirmatory," rather than as central determinants of care.

This situation presents a number of opportunities for molecular pathologists. By actively contributing to tumor boards and multidisciplinary decision-making, they can reframe the significance of molecular test results in the care pathway. Furthermore, their expertise should be channelled to communicate molecular findings clearly and clinically in diagnostic reports. This, in turn, will support clinicians in translating molecular data into patient-relevant language.

When patients understand why a molecular test was performed and how it influences their care, trust in diagnostics and confidence in treatment decisions are strengthened.

Precision medicine is not a buzzword

Precision medicine is often portrayed as futuristic or restricted to high-resource settings. In reality, molecular pathology already enables precision care in routine clinical practice. The identification and validation of predictive and prognostic biomarkers have transformed oncology by aligning therapies with tumor biology rather than anatomy alone.

Technologies such as NGS, multiplex immunohistochemistry, and integrated multi-omic platforms have improved diagnostic accuracy, refined risk stratification, and expanded access to targeted treatments and clinical trials.

The challenge is not whether molecular pathology works but how equitably and thoughtfully it is integrated into healthcare systems worldwide.

Reclaiming the narrative of pathology

Pathology is no longer limited to slides and stains. It is about molecular mechanisms, predictive biomarkers, and personalized care. Molecular pathologists are not ancillary specialists, but central contributors to modern medicine.

By clearly articulating the patient-facing value of molecular diagnostics, we can both strengthen interdisciplinary collaboration and improve patient understanding and engagement.

Ultimately, molecular pathology gives disease a language and patients a clearer path forward. Our responsibility now is to make that impact visible.

Sharjeel Chaudhry is a pathologist at Dow University Hospital, Karachi, Pakistan.

Advancing Better Biomarker Testing

Connecting precision, efficiency, flexibility, and scale in genomics today

Biomarkers play an increasingly important role across the healthcare continuum, from disease discovery and diagnosis to therapy selection and disease monitoring. As next-generation sequencing (NGS) technologies continue to evolve, laboratories are balancing the need for broader genomic insights with the practical demands of routine testing. To explore how biomarker testing is changing, we spoke with Edward Jan, Product Manager, NGS Solutions, and Danielle Fletcher, Field Application Scientist, Bioinformatics, both from Agilent Technologies, about biomarker discovery, panel design, sequencing technologies, and the future of precision medicine.

How has NGS changed biomarker testing?
EJ: Previously, most testing relied on single-gene assays, which meant running separate tests for each DNA or RNA biomarker. The adoption of NGS transformed that approach by enabling multiplexed biomarker testing, where many genomic targets can be analyzed simultaneously from a single sample.

As sequencing accuracy, target enrichment technologies, and bioinformatics tools improved, it became more practical for routine and translational workflows. This allowed broader adoption for applications such as oncology and inherited disease testing in precision medicine.

DF: In particular, targeted sequencing enables focused, high-depth interrogation of genomic regions linked to disease. Target enrichment technologies offer the flexibility to choose from fixed panels or to take a more flexible approach, including customizable designs and exome sequencing. These options support the detection of somatic variants associated with cancer and germline variants linked to inherited diseases. Combined with increasing automation, biomarker testing can be more scalable, efficient, and comprehensive to align better with complex diseases.

How are new biomarkers being discovered today?
EJ: Biomarker discovery has expanded well beyond targeted gene studies. While exome sequencing drove many early discoveries, researchers are increasingly using whole genome sequencing to explore previously uncharacterized regions of the genome.

DF: Long-read sequencing is helping to drive those discoveries by resolving complex genomic regions, including structural variants, repeat expansions, and highly homologous genes. This is particularly valuable in inherited diseases such as Fragile X syndrome, as well as in cancer, where it can improve detection of gene fusions and other complex alterations.

How should laboratories view short-read versus long-read sequencing for biomarker testing?
EJ: Short-read sequencing remains the standard for routine biomarker testing because it is accurate, scalable, and cost-effective. Long-read sequencing complements it by providing better resolution of complex regions that are difficult to analyze with short reads alone.

The tradeoff is that long-read sequencing typically comes with higher costs and more complex data analysis requirements. As a result, the two technologies are increasingly used together. Short reads are often used for routine high-throughput testing, while long reads are applied to discovery projects and particularly challenging genomic regions where deeper insight is needed.

Once biomarkers are discovered, how are they translated into routine testing?
EJ: Biomarker discovery and routine testing have different goals. While discovery prioritizes breadth, routine testing requires sensitivity, reproducibility, and efficiency. Target enrichment helps bridges that gap with a more precise, scalable strategy, by directing

sequencing to disease-relevant regions using focused gene panels or expanded content such as exome sequencing.

That means sequencing power is concentrated where it matters most, improving detection of low-frequency and disease-relevant variants. This deeper coverage is especially important in oncology for profiling tumor-specific alterations, but also benefits germline applications in rare hereditary variant identification. By delivering more relevant data with high confidence, targeted approaches help laboratories improve performance while optimizing cost and scalability.

What role does automation currently play in biomarker testing?
EJ: Automation is becoming essential, particularly as testing volumes increase. By reducing manual steps and standardizing key processes for NGS library preparation and target enrichment, automated workflows improve reproducibility and minimize variability across samples and operators. For laboratories looking to scale testing capacity or standardize operations in multi-site collaborations, automation provides the consistency needed to enable reliable results at volume.

Just as importantly, automation frees highly trained personnel to focus on interpreting results to gain relevant insights rather than performing repetitive lab tasks. That elevates the overall value of an automated laboratory workflow.

As biomarker needs evolve, what should laboratories consider when designing custom targeted panels?
DF: Designing an effective target enrichment strategy starts with clearly defining the biological and clinical question being addressed—what needs to be detected, and why. There is growing demand for panels that can capture multiple types of biomarkers, such as SNVs, CNVs, gene fusions and other complex genomic features, into a single assay.

Once the targets are defined, labs must balance breadth and depth, selecting the right regions while ensuring sufficient coverage to detect relevant variants with confidence. Additional considerations include the type of application, the quality of the sample and the complexity of the target regions. Factors such as GC content, highly repetitive sequence and pseudogene content are important considerations during the design phase. Ultimately, a well-designed panel ensures consistent coverage, minimizes gaps, and delivers reliable, high-quality data that aligns with the intended use.

How do these approaches support both somatic and germline applications?
EJ: Targeted sequencing provides a versatile framework that supports both somatic and germline testing. In germline analysis, it enables highly accurate detection of inherited variants. In somatic applications, deep sequencing coverage helps identify low-frequency mutations in complex samples, making it particularly valuable for emerging applications such as minimal residual disease (MRD) testing.

DF: Target enrichment is also expanding beyond human genetics into infectious disease research and testing. It can selectively capture and sequence bacterial or viral genomes present at low levels, supporting pathogen detection, strain surveillance, and outbreak monitoring. Examples include HPV in cancer panels and respiratory panels that target multiple viral pathogens.

By using common workflows across applications, laboratories can improve efficiency while maintaining the performance and reliability needed for each use case.

In translational oncology research, how can better biomarker tests help move discoveries towards patient care?
DF: Advanced biomarker tests can help bring together research and clinical practice by enabling researchers to identify and validate biomarkers linked to therapy response, resistance, and disease progression. High-confidence detection of alterations in genes, such as *EGFR, ALK, ROS1, BRAF, HER2,* and *NTRK,* helps researchers evaluate targeted treatment strategies and generate evidence for future clinical use.

Broader biomarkers, including microsatellite instability (MSI), mismatch repair deficiency (dMMR), homologous recombination deficiency (HRD), and tumor mutational burden (TMB), are also advancing research into immunotherapies and PARP inhibitors. Beyond therapy selection, robust biomarker assays support drug development, patient stratification in clinical trials, and emerging applications such as multi-cancer early detection.

Ultimately, better biomarker tests accelerate translational research by turning genomic insights into actionable findings that can improve future patient care.

LINK

Continue the conversation here

NGS Platforms, Pitfalls, and Progress

Experts reflect on the evolving role of next-generation sequencing in infectious disease diagnostics

Is next-generation sequencing (NGS) all it's cracked up to be? Following their packed panel session at ESCMID Global 2025, we sat down with Etienne Ruppe and Stefan Green to discuss NGS platform selection, challenges in application, and what the future holds.

Could you please give us a brief overview of your experience in NGS thus far?

Etienne Ruppe: I'm a professor of bacteriology at University Paris Cité and I also work as a clinical bacteriologist at Bichat–Claude Bernard Hospital and conduct research at the IAME Research Center in Paris. My experience with NGS started with studying antibiotic resistance. From there, I expanded into microbiota research and then clinical metagenomics. My focus is on analyzing genomes to track antibiotic resistance genes in complex environments, such as clinical and microbiota samples.

Stefan Green: I'm director of Core Laboratory Services at Rush University Medical Center in Chicago. I manage the DNA sequencing core and also hold a faculty position in the Division of Infectious Diseases. My background is in microbiology and microbial ecology, and I've been running core facilities for 14 years. My postdoctoral work involved bacterial whole genome sequencing. I started in environmental microbiology and now work on both environmental and host-associated microbiomes, including human studies – covering topics from Parkinson's disease to how spaceflight affects the gut microbiome.

What's your NGS "elevator pitch"?

ER: I think NGS brought a new level of scale and depth. Before, with techniques like PCR, we had to know exactly what genes we were looking for, and if we didn't, cloning was the only option – which was slow and difficult. Now, with just one experiment, NGS gives you broad, detailed data.

It's really about scale and accessibility. This has led to many new applications, like high-resolution strain tracking, identifying mobile genetic elements that used to be hard to detect, and sequencing clinical samples where unexpected findings can appear. NGS has also revolutionized microbiota research – it used to be limited and low-resolution, but now it's far more advanced. I think the microbiota field has benefited the most from NGS.

SG: Agreed – I always joke that during my PhD, I spent four years sequencing just 150 genes – and that was enough for a couple of papers and my degree. I was proud of it! Now, if a sequencing run doesn't yield 15 billion sequences, we think something went wrong. So yes, as Etienne said, the scale has massively increased.

One important point is that NGS can be an agnostic discovery tool – you don't need to know what you're looking for ahead of time. It can detect bacteria, viruses, and even eukaryotic pathogens all at once. And because of the increased scale and reduced cost, you can now run larger, more complex experiments that would have been impossible or too expensive before.

Also, sequencing costs have dropped faster than almost anything else in science or society over the last 20 years. That's opened the door to new uses. For example, in proteomics, scientists now attach DNA tags to antibodies and use sequencing to count proteins. The same idea is being explored for detecting metabolites. So we're likely to see sequencing used as a readout method for all kinds of biological questions – just because it's fast and affordable.

What are some of the primary factors guiding NGS platform decision making?

SG: There are a few key factors to consider, but a big one is cost.

Another is how stable the company behind a sequencing platform is – will it still be around in a few years? Many researchers stick with the reliable choice that's been stable for over a decade. But newer platforms offer different things, like cheaper instruments and reagents, longer read lengths, or different quality levels. It's a competitive and somewhat risky space right now, with many platforms to choose from.

So, yes, cost really matters. But so does the type of sequencing you need. Short reads work well for tasks like re-sequencing or counting things you already know are there. But if you're assembling a genome or trying to link genes – like for antibiotic resistance – then long reads are better.

Turnaround time is another factor: do you need results in an hour, a day, or a week? And lastly, availability plays a role. Some instruments are more accessible, have more reagent options, or are compatible with automation, which makes them easier to use. That tends to give established platforms an edge.

ER: Yes, as Stefan mentioned in our presentation at ESCMID Global 2025, it really depends on your specific needs. For example, in our lab, we mainly work with *E. coli*, so we don't need a large, high-throughput platform – just something suited to genome-scale work. But when we need to do something more complex, like metagenomic sequencing, we find the most appropriate platform for that and often outsource it instead of buying expensive equipment.

I think a key takeaway from our discussions is that today, with so many options available, you can match the platform to your needs. Ten years ago, it was the other way around – you had to adapt your work to the limitations of the available platforms.

SG: Just to add one more point – no single sequencing platform can do everything. Different applications need different types of sequencers. Shared resource facilities are a great way to access the technology you need without buying the equipment yourself. For example, your lab might already have a device, but if you need to sequence 10 human genomes, it wouldn't make sense to buy a million-dollar machine – you'd outsource it.

This kind of shared setup helps the whole research ecosystem. Why invest in a machine that costs a lot to maintain if you're not using it all the time? Plus, the technology changes fast – what you buy today could be outdated in four years. It's smarter and more flexible to use shared resources when needed.

How much weight should labs give to read length and throughput vs ease of integration into existing workflows?
SG: As we've been emphasizing, it really depends on the application. For example, if you're working with a new isolate and need to fully close its genome, you must use long-read sequencing – short reads just won't work for that. We also do a lot of 16S amplicon sequencing, where long reads can help improve species-level identification. Short reads can still work, but the results aren't as accurate.

In general, short-read sequencing is more cost-effective, easier to use, and better supported with automation. So unless there's a clear need for long reads, we prefer short-read platforms. Some researchers want to do everything themselves, and they can get a long-read sequencing instrument for about $1,000. This does also necessitate ancillary equipment and bioinformatics, so not everyone wants to run this in their own lab. But when someone comes to us, we always assess whether long reads are truly necessary – otherwise, we recommend short reads for simplicity, speed, and cost.

What are the key considerations for optimizing turnaround times?
SG: I sat in on the clinical metagenomics session before ours at ESCMID Global, and nearly everyone there was using the same type of sequencing platform. The main reason was speed – they needed same-day results. In clinical settings where you have only a few samples and need rapid answers, certain technologies that offer fast library preparation and real-time data output are a strong choice. You can get usable data within a few hours, often with minimal automation.

Other platforms are improving their turnaround time but still aren't as fast. Some generate data in real time as DNA passes through the sequencing system, while others build up data more slowly, base by base, and don't yield results until the entire run is complete.

So in clinical metagenomics, the priority is often speed rather than read length. That rapid turnaround makes certain sequencing platforms particularly appealing. Of course, the broader question

remains: is faster always better, or is it sometimes worth waiting longer for more detailed and comprehensive results?

ER: In cases like sepsis or severe infections in intensive care – such as pneumonia – we need results as quickly as possible. Multiplex PCR is currently a strong option; it's not perfect, but it's fast and reliable. We're hoping that metagenomic sequencing will be the next big step, but right now the turnaround time is still about six to seven hours. That means if you take a sample in the morning, you get results by the afternoon – still a bit too slow to compete with current methods.

Another challenge with real-time sequencing is timing. You may get early results – say, you detect Klebsiella – but only later pick up important resistance genes. That delay means we can't act too quickly on early findings without risking missing critical information. We might need to define time points in future studies, such as confirming that results after one hour are reliable enough to identify pathogens, while resistance genes might require more time.

So, while the technology shows a lot of promise in urgent cases, it's not yet fast or sensitive enough to fully replace existing methods.

SG: It's exciting to think about how rapidly sequencing technology is evolving. For example, there's an upcoming high-throughput nanopore sequencer, which – if it delivers on expectations – could combine the speed of nanopore sequencing with the data volume and quality of short-read platforms. The idea is that you could have a two-hour sequencing run and get something like 10 gigabytes of data per sample – enough to be confident in your results without needing to extend the run for 24 hours to see what else might appear. That could eliminate the current uncertainty that comes with early, incomplete results.

This kind of hybrid system – offering fast turnaround and high-quality, high-volume data – could be a perfect fit for clinical applications. Of course, this instrument hasn't been released yet, so everything is still speculative: we don't know its cost, performance, or even availability. But it's clear that we're approaching a point where these capabilities will be possible, and that's a game-changer for the field.

It's a striking contrast from just a few years ago. Now, with sequencing costs dropping so drastically, it's often cheaper and easier to simply generate more data than to use data reduction strategies we used to use. The questions we ask today are completely different because we're no longer limited by cost or scale in the same way. Technology has changed not just the answers we can get – but the way we think about the questions themselves.

What are some of the limitations preventing routine implementation of NGS?

ER: In our ESCMID Global session, we ran a poll to discuss this very issue. One of the biggest challenges participants mentioned is the lack of bioinformatics expertise. Many users aren't sure what to do with the large amounts of data they get from sequencing. Bioinformatics can feel overwhelming because it often requires

coding, not just clicking through simple software. While user-friendly tools do exist – especially for genome analysis, where data volume is relatively small – not everyone is aware of them.

Things become more complex with metagenomic sequencing, like microbiota or clinical metagenomics, because the data volume is much larger and requires more computing power. Although private companies offer bioinformatics solutions, these often come with high costs. Keeping up with constantly evolving tools, classifiers, and databases is also a major hurdle – new software becomes outdated almost as soon as it's released. In many research labs, pipelines are still built in-house, which means hiring skilled bioinformaticians. Over time, this might get easier as more accessible tools emerge and AI becomes more helpful in analysis.

Another concern is cost – not just the machines and reagents, but also the staffing and storage. Fast turnaround requires dedicated personnel, which isn't always realistic given current staffing shortages and financial pressures. While sequencing costs have dropped significantly, data storage has become more expensive. Researchers are now debating whether it's better to store data or simply re-sequence samples when needed, which is a big shift from earlier days when generating even a single sequence was a major achievement.

Still, most participants felt that NGS clearly offers value over conventional methods, especially for diagnosing difficult infections where turnaround time isn't urgent. However, for critical cases where rapid decisions are needed, NGS may not yet be fast enough to fully replace existing tools.

SG: Those are great points. I also believe that soon the cost of bioinformatics will surpass the cost of sequencing itself – especially as the focus shifts toward getting accurate clinical answers. Some companies have already invested millions in building curated databases to support this. While you can run raw sequence data through public databases like NCBI, those aren't always curated, which can lead to incorrect results or misannotations. For clinical use, having reliable, up-to-date databases is essential – and that comes at a price.

The challenge is that people are generally willing to pay for sequencing, but not for bioinformatics. That mindset needs to change. Sequencing gives you a tangible output – the raw data – which feels like a product you're buying. But interpreting that data correctly is crucial. It's like buying a bicycle and expecting the instructions on how to ride it to come free – but in reality, expert guidance costs extra.

Ultimately, people will need to understand that the value of accurate data interpretation is worth paying for – especially in critical clinical settings. Changing that perception will be a major challenge.

Why is it so difficult to standardize this type of sequencing?
SG: I don't think standardization is impossible, but it's definitely more complicated for metagenomic sequencing than for targeted tests. In a typical lab test for one organism, like with real-time PCR, you use known standards to quantify results. But metagenomic sequencing is agnostic – it can detect anything, even organisms we didn't know existed. For example, there was no specific test for SARS-CoV-2 in December 2019, but shotgun metagenomics could still have detected it. That discovery aspect makes standardization harder.

That said, it is possible to build a standardized workflow: use a specific robot for DNA extraction, another for library prep, feed the sample into a sequencer, and process the data through a defined bioinformatics pipeline. You'd also need proper controls – positives and negatives – at every step, from extraction to sequencing. But this requires careful planning: for instance, deciding whether to spike in control organisms, and if so, which ones. It's tricky because different organisms – bacteria, viruses, fungi – have different properties and require different extraction methods.

In fact, I think nucleic acid extraction is one of the most overlooked steps in the whole workflow. If you don't consistently extract genetic material from all types of microbes, then it doesn't matter how good your sequencing or analysis is. Without standardized extraction protocols, the whole process can break down.

ER: Yes, I agree that for genome sequencing, standardization is definitely achievable. The real challenge lies in sequencing clinical (or "eco") samples. These samples vary widely in terms of the organisms present, the amount of human DNA, and how the sample was collected and stored – for example, whether it was frozen or not.

That's why DNA extraction is the hardest part. You're dealing with many different situations, and that makes it difficult to standardize. For microbiota specifically, it's a complex type of sample, but there has been progress. The International Human Microbiome Standards project has helped develop standard methods for these samples, so it's definitely feasible – at least for that sample type.

So, overall, I'd say microbiota sequencing is fairly well standardized. The bigger challenge is adapting to the variability in clinical samples – but even that is possible with effort.

What common mistakes or oversights do you see labs make when choosing or implementing an NGS platform?
ER: I would start by asking: What will I do with the data? What's the goal? One of the biggest mistakes in this field is failing

to communicate with the rest of the team involved in NGS. It's essential to bring everyone to the table – microbiologists, clinicians, clinical microbiologists, and bioinformaticians – so they all understand each other's needs and perspectives.

If there's no clear communication, especially between clinicians and bioinformaticians, misunderstandings can happen, leading to mistakes in how data is interpreted or used. Just like in a hospital, where different specialties must collaborate to give the best care, the same applies here. Bioinformaticians are now part of that team and should be fully integrated into the discussion.

SG: I completely agree with Etienne – you really need to involve your bioinformaticians from the start. You don't want to generate all your data and then realize it can't be properly analyzed, or that you didn't include the right controls or enough replicates. Planning ahead with the full team helps avoid these problems.

Also, you don't need to reinvent everything. There are well-established protocols and plenty of good literature available, especially for combined DNA and RNA workflows. Use those resources instead of starting from scratch.

Another important point: if you don't absolutely need to buy your own sequencer, don't. The technology is evolving fast, and shared resource facilities can meet most needs. Even a single sequencer in a hospital can be used for multiple purposes. Some instruments can handle more than one run at a time, and smaller platforms can be scaled with multiple devices. DNA and nucleic acids can be shipped easily, even internationally, so you can send samples to labs that have the tools you need. Unless you require rapid turnaround, you don't need to invest in every new machine yourself.

A common mistake is focusing too much on the sequencer itself and not enough on the upstream steps, like nucleic acid extraction and library preparation. These parts benefit the most from automation – even though they cost more, they're worth the investment. In fact, the sequencer is probably the least complex part now. What really matters is having consistent, automated prep that doesn't depend on which technician ran the sample.

In your talk at ESCMID Global, Stefan stated that by the end of the presentation, everything discussed was already out of date. With this in mind: are we fighting a losing battle?
SG: I don't think it's a bad thing that sequencing technology keeps changing – I actually see it as a sign that we're making real progress. Costs are dropping and capabilities are improving, which means we're winning, not falling behind. Think about it: you wouldn't celebrate using the same tech from 10 years ago. Change means advancement.

Yes, the constant updates can be frustrating. Sequencers become outdated, pricing models shift, and newer versions can make previous methods incompatible. But those changes also reflect big improvements in sequencing quality. So yes, the market is a bit chaotic right now, with many competing platforms and rapid change. But that competition is good – it drives innovation, improves performance, and lowers prices. It may feel overwhelming at times, but overall, the field is moving in the right direction.

ER: Agreed, at the end of the day, we win – as researchers, as clinicians – because we have more options to provide our patients with improved care.

Finally, if you had to summarize your advice for clinical professionals in a sentence or two, what would it be?
ER: In my view, NGS is a powerful tool – but it's just that, a tool, not a magic solution. Like any other test, it needs to be used in the right context and applied where it fits best. We once hoped metagenomic sequencing could be a catch-all method since it can detect almost anything, but it has its limitations too. Our job now is to understand those strengths and weaknesses, and use NGS where it truly adds the most value.

SG: To follow Etienne's comment, I'd emphasize that nucleic acid extraction is crucial – it's the starting point of the entire workflow. Even if everything else is done perfectly, poor extraction will affect your results.

More broadly, the key message is: you're not alone. You don't have to build everything from scratch. Talk to shared resource facilities, bioinformaticians, and others who've done this before. There's no one-size-fits-all solution, but there are reliable workflows you can follow and adapt. It might seem overwhelming at first, but with the right support and advice, it becomes much more manageable.

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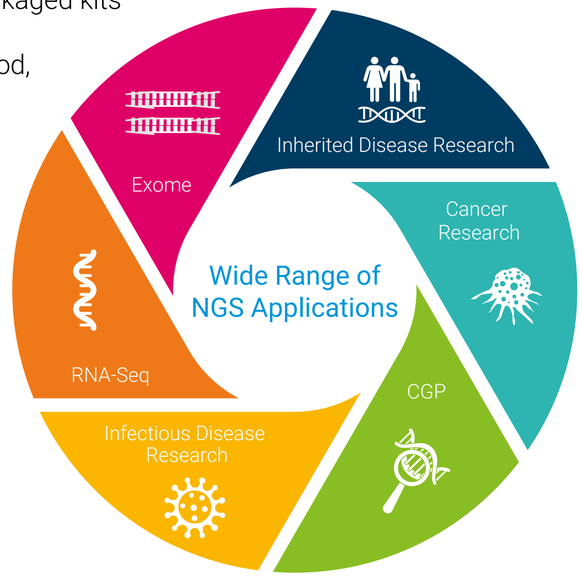

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The 60 Percent Diagnostic Gap in M/E

What earlier mNGS testing could mean for labs and clinicians

In meningitis/encephalitis (M/E) diagnostics, patients undergo scores of tests as clinicians try to eliminate each potential cause. Despite this, the cause of more than half of M/E cases is never identified.

Here, we speak with Steve Miller, Chief Medical Officer at Delve Bio, who was involved in a recently published study into the earlier use of comprehensive metagenomic next-generation sequencing (mNGS) for improving M/E diagnostics.

Why is M/E so difficult to diagnose quickly, even in well-resourced hospitals?
M/E sits at the intersection of infectious disease, neurology, and immunology. Patients typically present with nonspecific symptoms, and the differential includes bacterial, viral, fungal, parasitic, neoplastic, and autoimmune causes.

Most current diagnostics are hypothesis-driven. Culture, pathogen-specific PCR, and serologic testing each evaluate a limited portion of possible etiologies. If the correct cause is not suspected early, it may not be tested for. Even in well-resourced centers, more than 60 percent of cases remain without a confirmed diagnosis after comprehensive evaluation.

In a typical CNS infection workup, where do you see the biggest diagnostic delays?
Delays usually result from the stepwise nature of testing rather than any single slow assay.

Clinicians must prioritize tests from a limited cerebrospinal fluid (CSF) volume. In pediatrics, there may be only one opportunity for lumbar puncture, requiring decisions about which studies to run first: HSV PCR, a multiplex syndromic panel, cryptococcal antigen, tuberculosis, fungal studies, or autoimmune markers? If initial testing is unrevealing, additional assays are ordered sequentially, often as send-outs. Each step adds time, logistics, and cost, extending the path to diagnosis.

What did your study reveal about the earlier use of mNGS in the M/E diagnostic work-up?
Our study, published in *Open Forum Infectious Diseases*, evaluated what might occur if a broad mNGS assay with a 48-hour turnaround time were used earlier in the diagnostic pathway rather than as a last-line test. The cohort included patients with confirmed infectious M/E, as well as a separate group with autoimmune encephalitis.

The modeled analysis found that earlier use of the assay would reduce the number of microbiologic tests ordered per patient and shorten time to diagnosis. In patients with infectious etiologies, the projected reduction in additional microbiologic testing exceeded 60 percent. In patients ultimately diagnosed with autoimmune encephalitis, where no pathogen is identified, the reduction was greater than 90 percent.

Steve Miller

"Earlier clarification of etiology may influence hospital length of stay, antimicrobial use, and timing of immunotherapy. From a laboratory perspective, earlier comprehensive testing may reduce repeat lumbar punctures and downstream send-out assays."

Time to diagnosis was also shortened. In the infectious cohort, earlier mNGS testing was associated with a modeled reduction of seven days. In patients with autoimmune encephalitis, time to diagnosis was reduced by approximately 10 days by more rapidly excluding infectious causes.

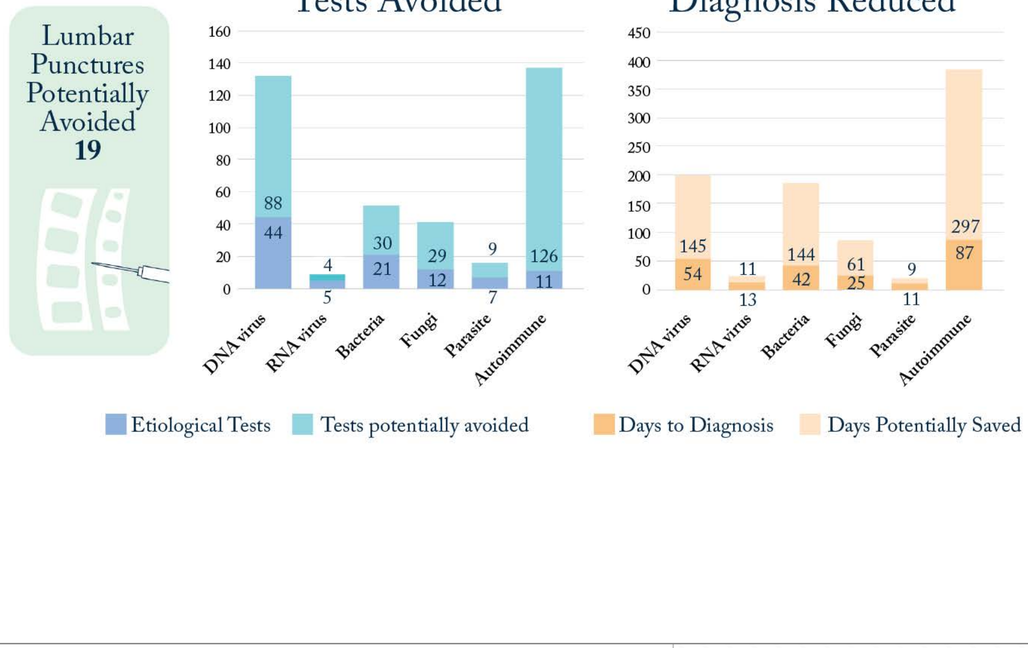
Earlier clarification of etiology may influence hospital length of stay, antimicrobial use, and timing of immunotherapy. From a laboratory perspective, earlier comprehensive testing may reduce repeat lumbar punctures and downstream send-out assays.

Where does mNGS fit alongside standard testing?
mNGS does not replace initial studies. CSF cell count, glucose and protein, Gram stain, and culture remain essential for immediate management decisions.

Broad molecular testing may be considered when the differential is wide, particularly in immunocompromised patients, severe or atypical presentations, culture-negative CSF with ongoing concern for infection, suspected rare pathogens, or when CSF volume is limited. It may also be useful when rapid exclusion of infection would alter management, such as in suspected autoimmune encephalitis.

Which patients or clinical scenarios benefit most from early mNGS testing?
Although individual institutions will define their own testing criteria and stewardship protocols, several clinical scenarios are frequently discussed for early use of mNGS:

- Immunocompromised patients, who are at risk for a broad range of opportunistic pathogens not fully covered by standard panels.
- Severe or atypical neurologic presentations, where infectious and autoimmune causes may overlap.
- Culture- or PCR-negative CSF with persistent concern for infection, when routine studies are unrevealing.
- Suspected rare or travel-related infections outside the scope of conventional panels.



- Limited CSF volume, particularly in pediatrics, where a single comprehensive assay may reduce the need for repeat procedures.

Earlier mNGS may also be considered when rapid exclusion of infection would alter management, such as before initiating immunosuppressive therapy.

What are the most important considerations for the interpretation of mNGS results?
Interpretation of mNGS results requires close collaboration among laboratory, neurology, and infectious disease teams. Because the assay is highly sensitive, contamination control is essential. Strict pre-analytic handling and appropriate negative controls help prevent background signals from being misinterpreted as infection.

Results must be evaluated in quantitative and clinical context. Low read counts of common commensal organisms – especially in blood-contaminated CSF – may not indicate true disease, whereas high read depth and broad genome coverage consistent with the patient's presentation are more likely to be clinically meaningful.

Unexpected or low-abundance findings may warrant confirmation with targeted PCR, serology, or culture when management decisions depend on the result. Ultimately, mNGS interpretation extends the laboratory's role by integrating sequencing data with clinical and microbiologic information to provide a clear, actionable report.

How can earlier mNGS results support antimicrobial stewardship?
Earlier comprehensive pathogen detection may help narrow empiric therapy when a causative organism is identified. Conversely, a negative result – interpreted within clinical context – may support de-escalation of prolonged broad-spectrum antimicrobials and consideration of noninfectious etiologies.

Looking ahead, what are the next practical steps for broader adoption?
From a systems perspective, the emphasis is shifting from analytic validity alone to practical integration of mNGS into diagnostic pathways.

First, validation and performance data are essential, and should focus on consistent sensitivity and specificity across pathogen types and specimen conditions. Equally, standardized reporting and clinician education are critical to ensure results are interpreted accurately and within clinical context.

Integration of mNGS into the workflow requires clear ordering criteria within standard systems to support appropriate use. The workflow must ensure that turnaround time is reliable and fast enough to inform treatment decisions.

Finally, reimbursement and utilization governance should also be considered, with institutional policies helping define when mNGS is most appropriate in patient care.

Cohort	Percent of tests potentially saved	Reduction in days to diagnosis per patient
Infectious Meningitis, Encephalitis	**64%**	**6.9**
Autoimmune encephalitis	**92%**	**10.2**

Industry Insights: Pride in Precision Medicine

Rita Shaknovich, Chief Medical Officer at Agilent Technologies, shares her insights into automation, access, and analytics

What is your background in molecular diagnostics?
During medical school, I quickly realized that a propensity to faint in the operating theater was not conducive to a career as a surgeon! Later, during my PhD in molecular biology, I recognized that scientific curiosity was actually my main career motivator – which led me to train as a pathologist and a physician scientist.

I specialized as a hematopathologist and molecular diagnostician, which coincided with a period of rapid advances in our understanding of the human genome that really inspired my work. My focus shifted more and more towards translational medicine – developing molecular diagnostic tools – that naturally led me from academia to industry.

I was lucky to work for some very transformative companies, focusing on cutting-edge cancer screening, and liquid biopsy testing. A natural progression led me to join Agilent – a global company with a large footprint in healthcare – and I'm super excited to continue advancing molecular diagnostics.

What do you think is missing from the molecular diagnostic toolkit?
The development of cutting-edge technologies in molecular diagnostics has led to incredibly sophisticated tools entering the lab. But we have now reached the stage where the assays are so complex, they can be difficult to implement. So I think the biggest challenge for the instrument manufacturers is simplification of lab workflows.

Our customers tell us that when they buy a new instrument, they want to be able to "plug and play." Operators need less hands-on time and more automation to relieve workflow burdens. Labs also need the instruments to monitor, maintain, and replenish themselves. In the complex landscape of next-generation sequencing (NGS), manufacturers like Agilent are working hard to provide solutions that automate many steps of NGS library preparation.

The other challenge with NGS and other molecular technologies is the complexity of interpretation of the results. There is an urgent need for better tools to assist with analysis of the vast array of genomic variants, and help translate them into clinical action.

And, finally, I think we need to see more research dollars being spent on rare and chronic diseases. While cancer diagnostics are justifiably prioritized, other diseases with genetic origins are equally painful and burdensome. But I do sense that the healthcare industry is starting to shift in that direction, and broaden the lens to focus on overall health.

How do you think precision medicine can be made more equitable in terms of patient access?
Personally I think there is a need for decentralized solutions. Generally, only the large, specialist cancer centers have

Rita Shaknovich. Images for collage sourced from Adobe Stock.

sophisticated molecular diagnostic tools – the investment of capital and specialist staff is beyond the scope of most hospitals. But that leads to access problems for patients.

I do think it is the responsibility of the diagnostic industries to find ways to decentralize and democratize access to molecular diagnostics. We need to be asking: How can we create more regional solutions? How can the cost and complexity of DNA sequencing be reduced? Can we design mobile units to reach the more remote areas?

Then we need to look at the economics of healthcare. Complexities in reimbursement for molecular diagnostics can be a key impediment to their implementation. We need more government interventions to remove barriers to reimbursement of novel, cutting-edge diagnostics. And we need more pathologists engaging with governments and advocating the value of these technologies.

What's your vision for the future of precision medicine?
Returning to the challenge of simplifying the complex landscape of molecular diagnostics, I think artificial intelligence (AI) and machine learning tools will be transformative in precision medicine.

Our customers tell us that one of the main difficulties with adopting NGS is the translation of a multitude of data points into clinically beneficial action for the patient. I look forward to a future where the combination of molecular pathology experts using AI-augmented interpretive tools will make the technology more accessible. But to achieve it we need to be responsible stewards of the AI.

So my vision is simple: we will become better at delivering precision medicine through the aid of AI and other technologies, and, at the end, the patients will benefit.

To what extent do you think AI-augmented tools will increase the pace of adoption of precision medicine?
Enormously! Let's look at the example of companion diagnostics (CDx), which could benefit significantly from digital pathology and image analysis capabilities to identify eligible patients for targeted treatments. Interpretation of the results is complex and time-consuming, and CDx manufacturers typically spend a lot of time educating pathologists on biomarker identification, scoring systems, and so on.

However, if we integrate a rigorously trained and validated image analysis software into the pathologist's workflow, it speeds up the CDx workflow dramatically – especially where there are multiple biomarkers involved. If AI analytics are included as standard, CDx assays that used to be beyond the reach of resource-stretched labs suddenly become accessible.

Which emerging trends or innovations in precision medicine are you most excited about?
In the NGS space, long-read sequencing is a really important development that will continue to enhance the accuracy and sensitivity of the technology. I'm excited about its potential for unlocking further secrets of genetic disease.

I am also a keen advocate for liquid biopsy testing – it makes a huge difference to patients to have the option of a blood test in place of a painful tissue biopsy. The full potential of cell-free DNA diagnostics will only be unlocked when tests have the requisite sensitivity. But, when they do, the patient impact of liquid biopsies for both cancer and chronic disease diagnostics, will be huge.

The other exciting area involves multi-omics approaches for investigating multiple biomarkers. Now that we can perform DNA methylation testing along with proteomics and histone modifications analysis, for example, molecular diagnostics is growing even more powerful than before. There is an emerging trend among diagnostics companies towards combining multiple analytes to understand complex diseases – and I predict we will start seeing the fruits of those labors very soon.