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the Pathologist

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Case of the Month

This lymph node biopsy was taken from a 32-year-old female patient with enlarged, tender cervical lymph nodes of three weeks' duration. Serologic tests for autoimmune diseases were negative.

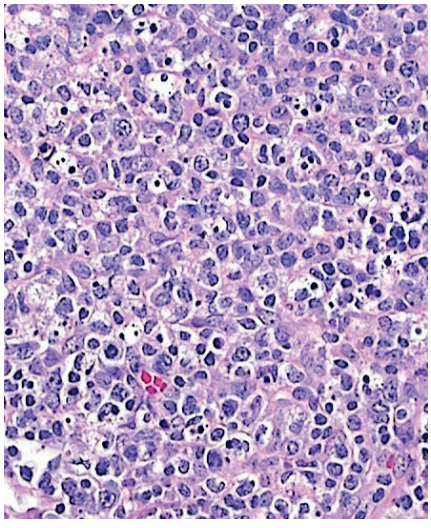
What is the most likely diagnosis?

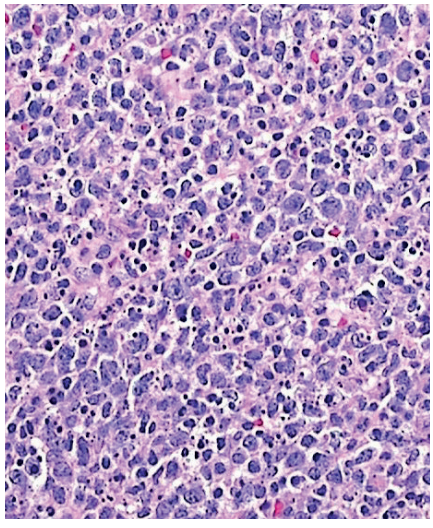
A *Toxoplasma* lymphadenitis

B Bacterial lymphadenitis

C High-grade lymphoma

D Kikuchi-Fujimoto disease





To register your guess, please go to http://tp.txp.to/1017/case-of-the-month
We will reveal the answer in next month's issue!

Answer to last issue's Case of the Month…

A: PKD1

This liver contains numerous cysts, typical of polycystic liver disease (PCLD). PCLD can be inherited as an autosomal dominant or autosomal recessive disorder. The Online Mendelian Inheritance in Man (OMIM) database lists some 50 gene mutations linked to the disease (1).

Most gene mutations found in patients with PCLD involve *PKD1* or *PKD2* (polycystin-1 and polycystin-2), the underlying molecular defect of autosomal dominant polycystic kidney disease. Polycystin mutations are found in approximately one in 800 people. Mutations in *PKD1* are approximately five to six times more common than mutations in *PKD2*. As adults, most or all people with such mutations will develop polycystic kidney disease, and some of them will also have polycystic liver disease. It is thus safe to conclude that most PCLD patients will also have polycystic kidney disease.

Mutations in the other genes listed here are much less common causes of PCLD. Changes to *PRKCSH, SEC63* and *LRP5* account for 20 percent of autosomal dominant PCLD, a very rare disease with an incidence of 1:100,000. The genetic basis of the remaining 80 percent of autosomal dominant PCLD remains unknown (1).

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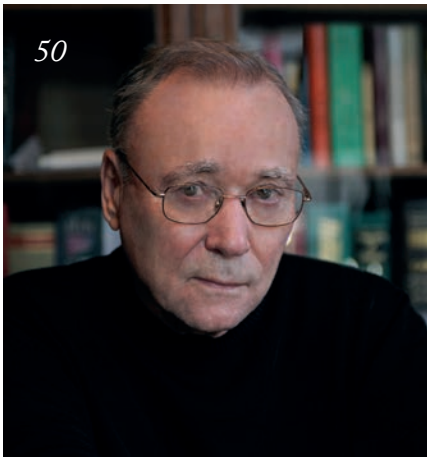

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the Pathologist

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Opening Pandora's Patient Portal

The debate over patient access to medical records rages on

The receptionist at my doctor's office assured me there was nothing to worry about. "All of the tests came back normal," she said.

"Great!" May I have a copy of the results, please?"

"Well, we don't normally do that…"

One possible reason for her reluctance came to light a couple of days later, when I picked up the printouts and discovered that few of the results were, in fact, "normal!" Because of my persistence, I was able to check them myself and then discuss and address the issues at my next appointment. But what if I hadn't been allowed to see my records? What could have happened to my health over the long term if those test results – clearly marked by the laboratory as out of the normal ranges – had dropped off the radar for an indeterminate amount of time?

It's a question I have seen hotly debated at conferences and on social media recently: should patients be given access to their own medical records? Proponents of open access feel that patients have a right to the information, and that it can serve as an additional layer of protection against potential error – as in my case. Those who disagree argue that there are dangers involved in giving non-experts unfettered access to specialist information: it can be confusing or difficult to interpret, may require a change to standard reporting language or format, and risks distressing patients who don't fully understand the implications of the data.

If these discussion points sound familiar, it may be because they're the same ones often used to debate the pros and cons of direct-to-consumer genetic testing. It's not a question of whether or not patients have a right to the information; rather, how can they exercise that right while avoiding its potential pitfalls?

There's no right or wrong answer. Some pathologists tackle the problem by holding "office hours" in which patients can visit them to view and discuss their results (1), thus sidestepping the problem of unnecessary anxiety arising from inexpert interpretation. Others provide a brief summary of the report in layman-friendly language. Another approach is to educate patients on their disease in general terms, so that they're better equipped to understand any reports they may read going forward. And sometimes, the reports may not be for the benefit of the patient alone – see Kamran Mirza's description this month of the relief he felt because he was able to read his mother's pathology report (page 42).

What do you think? Should patients have open access to their medical records – and, if so, what role can pathologists play in helping to prevent the inevitable misunderstandings?

Michael Schubert
Editor

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Upfront

Reporting on research, innovations, policies and personalities that are shaping pathology today.

Do you want to share some interesting research or an issue that will impact pathology?

Email: edit@thepathologist.com

All Eyes on Biomarkers

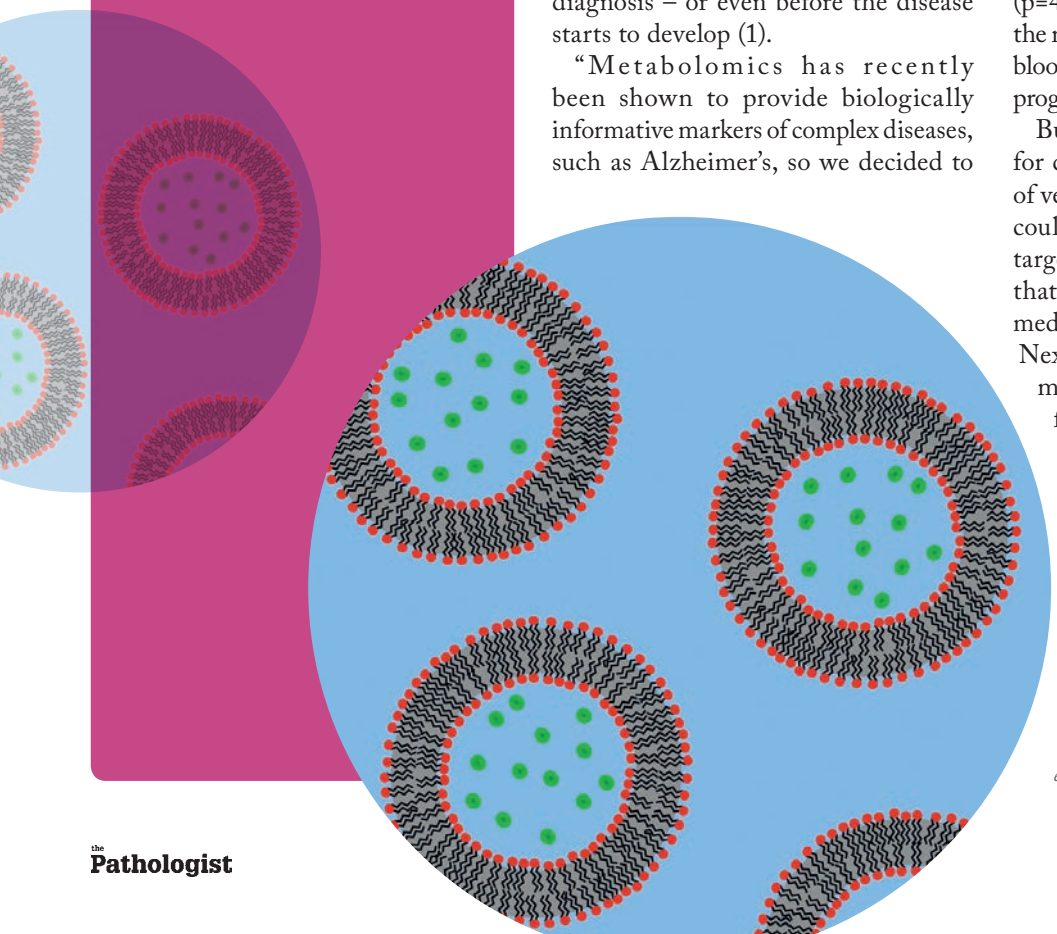
Could AMD be diagnosed through blood plasma analysis?

It's a classic "chicken and egg" scenario. When retinal diseases like age-related macular degeneration (AMD) strike, early diagnosis and intervention give the best prognosis and visual outcomes. But in reality, retinal disease cannot be diagnosed until structural changes are seen, and some patients only present at ophthalmology clinics when the visual symptoms – and the underlying pathology – are at an advanced stage. Now, a team from Massachusetts Eye and Ear Hospital, Boston, USA, are proposing that metabolomics analysis might hold the key to identifying those at risk during the early stages of disease diagnosis – or even before the disease starts to develop (1).

"Metabolomics has recently been shown to provide biologically informative markers of complex diseases, such as Alzheimer's, so we decided to research the role of metabolomics in AMD to find biomarkers for diagnosis and prognosis in this disease," says Deeba Husain, co-senior author on the corresponding paper (1).

In the study, the team took blood plasma samples from 90 patients with AMD (30 each with early, intermediate and late stage disease) and from 120 patients with normal macular health. The samples were analyzed using ultra high-performance liquid chromatography coupled to tandem mass spectrometry. They found that a total of 87 metabolites, mostly from glycerophospholipid metabolism, differed significantly between patients with AMD and the controls. Of these, 48 were significantly different across the different stages of AMD. "We were surprised to find that glycerophospholipid metabolism specifically seems to have a strong association with AMD – this pathway was highly enriched among the significant metabolites ($p=4.7 \times 10^{-9}$)," says Husain, who believes the results could form the basis of the first blood biomarker for early diagnosis and prognosis of AMD.

But their results aren't just important for diagnosing disease. "The detection of very significant metabolite pathways could lead to finding a new druggable target for treatment," says Husain. "And that could lay the path for personalized medicine in the management of AMD." Next steps for the team include a large multicenter study to validate their findings, as well as a long-term follow-up study to better define the role of glycerophospholipid metabolism in disease progression. *RS*



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Analysis at Your Fingertips?

Paper-spray MS provides a quick and accurate method for detecting cocaine use

Drug testing usually involves the collection and storage of blood and urine. These are potential biohazards that need to be properly stored and transported, and in a forensic setting, a chain of custody must also be established. But what if drug testing were as easy as taking someone's fingerprints?

Using paper-spray mass spectrometry, a team led by scientists from the University of Surrey, UK, has created a method for testing for drug use in fingerprints. "There are a few publications in the literature that report the detection of drugs of abuse in fingerprint samples. However, these methods rely on extensive sample preparation steps as well as lengthy analysis time. But our method is both quick and sensitive and can use samples that are not hazardous – and easier to collect than blood and urine", says Catia Costa, first author of the study and a researcher at the University of Surrey's Ion Beam Centre.

The fingerprint sample is collected on a piece of chromatography paper, then a solvent is added and a voltage applied (see Figure 1). This process extracts traces of cocaine and substances produced when cocaine is metabolized (benzoylecgonine and methylecgonine). Analysis of 239 fingerprints from patients at a drug rehabilitation center and a control group of people not known to be drug users yielded a 99 percent true positive rate, and a false positive rate of 2.5 percent – even when study participants had washed their hands with soap before having their prints taken (1). And since the ridges of the fingerprint are visually established as part of the procedure, the identity of the donor can be identified.

The technique is adaptable to other substances that might be of interest in a medical or legal setting, such as prescription drugs, and explosives. The team now plan to look into commercializing the test, and assessing which fields would find it most valuable – as well as continuing to work on bringing analysis time down, with the ultimate aim of creating a 30-second method. *RM*

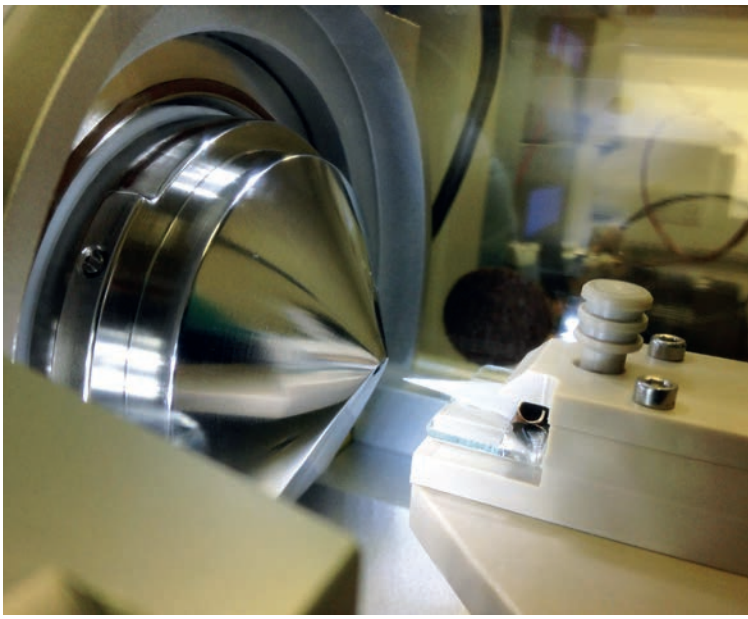

Figure 1. The mass spectrometer used to process the fingerprint sample on chromatography paper (white triangle).

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Superior Separations

Burcu Gumuscu explains an exciting method of DNA fractionation

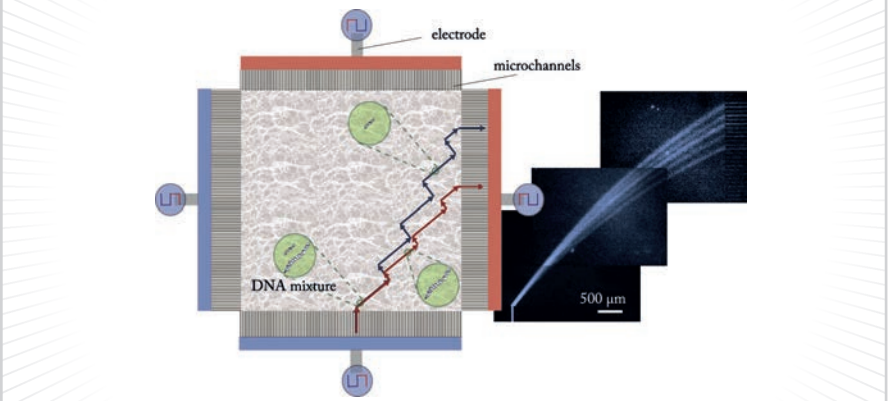

Figure 1

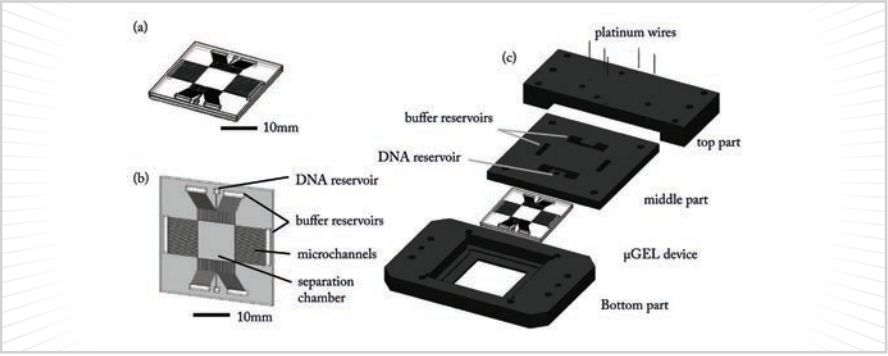

Figure 2

What inspired you to develop a new DNA separation technique?

The idea of introducing a new separation mechanism (1) to the mature field of gel electrophoresis excited me. During the research process, I focused on the design, functionality, and originality of the concept. Functional requirements have become the most prominent aspect for me, because things like economic value and societal embedding come into play when products are ready for commercialization. This technology has great potential to be commercialized, because it offers better functionality than existing technologies. For example, it can help diagnose genetic disorders using a much smaller sample than current devices.From the beginning, my project was about using the "continuous flow separation" method of fractionating DNA – so I focused on developing 2D separation matrices.

What are the challenges in separating DNA?

Standard gel electrophoresis is simple, versatile, and reproducible; however, it suffers from long processing times (many hours or even days). Trends toward next generation sequencing motivate the replacement of standard gel electrophoresis with microchip-based systems, which could provide efficient platforms to minimize the processing time and optimize DNA fractionation. To increase sample throughput and facilitate sample recovery, I feel that continuous flow separation is the way forward.

An ideal sieving matrix should have simple design and fabrication steps, yet provide high-resolution, high-throughput separation. That's why I opted for gel-based devices. People had originally considered continuous flow separation in such devices impossible, but I showed that it could work.

Could you share more details about the chip?

The new chip can separate DNA fragments within minutes, in high resolution, and even purifies the fragments by removing contaminant salts.

In the classic DNA electrophoresis approach, an electric field is applied in one direction in a gel matrix and DNA fragments with different base pair numbers are separated in that direction. Performing continuous injection and separation at the same time in a single device can reduce the overall experimental time. In our microchip, different DNA fragments follow different trajectories in the gel; smaller fragments move faster than large ones in the low field (separating them), whereas both move equally fast in the high field (resulting in rapid transport through the chip).

The device itself is made of glass and has a 1 cm^2 agarose-filled separation chamber with microchannels on the sides to properly direct the electric fields (see Figure 1). Next to that are a DNA reservoir and electrodes for applying the fields (see Figure 2). The chip is cheap and relatively easy to produce – and it is versatile as well; the type and concentration of gel and the electric fields can be adjusted to the application.

What can the device offer in the clinic?

The microchip would be of broad interest for next generation sequencing and clinical diagnostics, as it requires less effort and expense than current devices to produce similar performance and much faster separation times. For instance, the detection of pathogenic diseases by sorting pathogenic nucleic acids is of great interest in the clinic, as is the identification and analysis of biomolecules to diagnose genetic diseases. Our device can help by reducing the amount of sample needed to obtain results, and by speeding up the time to results; it can also be integrated into a more complex analysis system. In fact, its applications can even be extended to protein gel electrophoresis, simply by replacing the agarose gel with a polyacrylamide gel.

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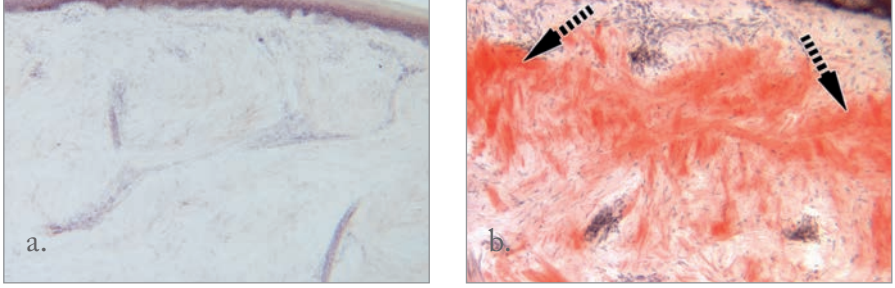

Image credit: Michael Polydefkis

Figure 1. a. A healthy skin biopsy; b. a skin biopsy from a patient with amyloidosis, with amyloid clumps in red.

Better Biopsies for Amyloidosis?

A more rapid alternative for familial amyloid polyneuropathy diagnosis

Transthyretin-mediated amyloidosis (TTR) is an inherited condition that causes familial amyloid polyneuropathy (FAP), a disease that often proves fatal within just a decade. TTR-FAP is usually diagnosed by sural nerve biopsy and genetic testing – but it is a highly invasive procedure, and distribution of the amyloid aggregates needed to make a diagnosis can be patchy. And that's why correct early diagnosis of the condition is uncommon (1).

A group of Johns Hopkins physicians have developed a modified approach using a skin punch biopsy, with the aim of creating a faster and relatively less invasive method of diagnosis. In a study of 30 FAP mutation carriers, 40 controls, and two patients with non-inherited amyloidosis, distal leg skin punch tissue samples stained with Congo red had 70 percent sensitivity and 100 percent specificity in diagnosing TTR-FAP (see Figure 1). The team also found that higher levels of amyloid aggregate were associated with loss of nerve fibers – which could lead to a new method of estimating disease severity and monitoring progression. They hope that, with a potential method for providing diagnostic and prognostic information less invasively, clinical trials of therapies could advance more quickly.

The current method has only been tested on the most common FAP-causing TTR mutations, but the team hope that their work can be built upon. "If further studies confirm and extend what we have found, we may use the skin biopsy as a biomarker for disease severity. And we will be able to diagnose more patients sooner," said Michael Polydefkis, professor of neurology and senior author of the study. "The good news is that drug companies are using our skin biopsy technique in ongoing clinical trials to monitor treatment success. (2)" *RM*

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In My View

In this opinion section, experts from across the world share a single strongly held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of laboratory medicine. They can be up to 600 words in length and written in the first person.

Contact the editors at edit@thepathologist.com

Laboratory Lessons in Conflict Resolution

What can we do when our clinical colleagues believe they have a laboratory problem?

By Glenda Wright, Pathology Resident, Department of Pathology and Laboratory Medicine, College of Medicine, University of Saskatchewan, Saskatoon, Canada

Laboratory practice is usually centered on generating quality results for clinical decision-making. Ethical issues are infrequently encountered and often overlooked. It is true that some of the more common ethical considerations – autopsy consent, retention and use of biological material, and medical error disclosure, for example (1) – are recognized and addressed during laboratory training programs; however, training rarely covers issues encountered during routine laboratory testing, including conflicts with clinical staff, inappropriate test utilization, tests ordered on patient specimens without consent, or the use of clinical specimens for legal or forensic purposes (2). The knowledge and skills required to resolve these issues are difficult to teach – especially as problems with far-reaching ethical issues seem to present to laboratory staff only sporadically.

Our laboratory recently faced a problem regarding test turnaround times for a secure youth detox and stabilization program. The program involves arrest, medical assessment, laboratory testing, and detention in a secure facility for troubled youths at risk of serious harm. Many services, groups, and individuals are involved in this sensitive process, including police, psychiatry, addiction services, child services, emergency departments, hospital security, courthouse staff, and the youths' family – all of whom obviously have very different, yet equally valid, perspectives on the program's benefits and challenges.

"Problems with far-reaching ethical issues seem to present to laboratory staff only sporadically."

One delay that was singled out involved the reporting of rapid urine drug screens from the hospital laboratory. Clinical staff considered these tests essential for decision-making, but felt they weren't receiving results in time – a laboratory problem. To help address this concern, we began to participate in the multidisciplinary team meetings, a setting where so many disciplines and perspectives are represented that conflicts are inevitable. Fortunately, we were able to use our laboratory expertise to ensure that forensic-quality lab methods and test results were part of the solution. The forensic-quality drug tests were not rapidly available, but the quality of those results was important for both the patients and our medical team –

so we consistently ordered them, as well as more rapid testing to minimize the time that patients and security personnel spent in the emergency department.

As a pathology trainee, this hands-on experience was a valuable way to identify ethical issues, acknowledge and address our colleagues' concerns, and see conflict resolution in practice. Having previously worked both in family and emergency medicine, as well as in laboratory practice, I have seen both sides of the clinical-laboratory divide. As laboratory practitioners, I feel it is essential for us to bridge the divide by leaving the confines of the laboratory, joining discussions with our clinical colleagues, and helping shape health care in our facilities. The communication skills to successfully collaborate with multiple disciplines require practice and experience – so, in my opinion, trainees should be involved in these sometimes difficult situations as part of training programs to help them develop the necessary skills and promote collaboration, positive interdisciplinary relationships and leadership (3).

Our work with the youth program also reminded me and my colleagues that our primary focus should be patient care; many of the patients involved have significant challenges. As laboratory professionals, we need to be cognizant of our role as directors of laboratory services, prompting the right tests at the right times. We do not serve patients by simply cancelling tests, or by passing the obligations on to our clinical colleagues. We can play an important role in health advocacy by bridging the gap between clinical and laboratory expertise – but only if we are prepared to develop successful communication and teamwork outside our laboratories. With easy access to results through electronic medical records, easy access to information via "Dr. Google," and the increasing adoption of point-of-care testing, the argument for better communication and teamwork will only grow.

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Tackling Cancer Myths

It's time to accept that ovarian cancer doesn't exist – and to address the knowledge gap in pelvic cancers

By Mark Boguski, Chief Medical Officer, Inspirata, Inc.

It's been known for almost 10 years that the term "ovarian cancer" is a misnomer. Almost all of these diseases begin somewhere other than the ovary – most usually in the fallopian tubes, meaning that ovarian cancer as a defined entity doesn't really exist. So why aren't most doctors, let alone our patients, aware of this?

When I first began studying this problem, my colleagues and I assumed the knowledge gap was between doctors and patients. But on further inspection, we realized that many doctors were also unaware of the true nature of "ovarian cancer." Why? Because many of the discoveries about its true origins were made by pathologists. Once prophylactic removal of the tubes and ovaries became commonplace in people who carry the *BRCA1* or *BRCA2* mutations, pathologists (notably Robert Kurman of the Johns Hopkins University School of Medicine) were able to study the specimens and gain further insight. They found that all of the cancers, or pre-cancers, started in the fallopian tubes or other pelvic organs, but not in the ovaries.

The information was published and is available in the pathology literature, but most gynecologists and oncologists aren't reading it. Our increasing specialization is compartmentalizing and fragmenting medical knowledge – and creating a barrier between the different specialties. It's only by stepping back and looking at the bigger picture that we can spot some of the truly remarkable things that the general public (and sometimes the medical community at large) simply aren't aware of. Even though the pelvic cancer discovery goes back to 2007, only since 2017 has the American Society for Gynecologic Oncology begun to acknowledge that a paradigm shift has occurred. But they're still using the term "ovarian cancer"…

I believe the name change will happen, but it will occur gradually as medical practice changes. There's so much history here; we've been calling these diseases "ovarian cancer" for generations. I think, in a way, it is hard for us to admit we were wrong about something so fundamental. But if we really want to be accurate in

our diagnoses, and practice precision medicine, we need to start calling things what they really are – and let go of traditional terms that modern science has rendered inaccurate.

"Our increasing specialization is compartmentalizing and fragmenting medical knowledge – and creating a barrier between the specialties."

Progress is being made: my colleagues and I are now using all of the tools at our disposal to bring about change. We communicate with the public via articles, television appearances, and Twitter, Facebook and other social media. We've also recently published a book: "Reimagining Women's Cancers." The word is now beginning to spread: to prevent ovarian cancer, some women may only have to have their fallopian tubes removed, leaving their ovaries intact and preventing premature menopause along with all of the associated comorbidities.

There are several small clinical trials now underway studying the comparative effects of removing the ovaries and fallopian tubes, versus just the tubes (1–3) – and to enhance enrolment, more doctors and patients need to know about them. Eventually, studies like these could lead to a completely new way of diagnosing and screening for "ovarian cancer" – one that is grounded more solidly in the latest science, resulting in better understanding of pelvic diseases, and better outcomes for patients.

It doesn't stop there – by looking at the bigger picture and not confining ourselves to disciplinary boundaries, we will be able to make connections between different fields of medicine and glean information that isn't yet taught in medical school – gaining insights that have the potential to transform medicine.

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On the Hunt for Laboratory Errors

The prize? Total information management…

By Mario Plebani, Professor of Clinical Biochemistry and Clinical Molecular Biology and President of the School of Medicine and Surgery, University of Padua, Italy

A hugely important goal in modern medicine is the improvement of patient safety. Although the maxim "primum non nocere" – a central element in any medical practice – dates back many centuries, the publication of the US Institute of Medicine (IOM) report on diagnostic error has shone a spotlight on the need to reduce risk in clinical practice by minimizing medical error (1). The data collected on medical errors – and especially on their prevention – has not only led to an ever-growing awareness, but has also kicked off initiatives aimed at reducing the error rate, with a particular focus on those correlated with adverse events for patients.

We have definitely seen improvements in patient outcomes from initiatives aimed at reducing procedural errors, or those in drug administration and dose. But what of diagnostic errors? In recent years, evidence has been collected on these types of medical mistakes, their severity, and the need to improve the diagnostic process as an essential prerequisite for ensuring better health and economic outcomes. Over the past few weeks, WHO has intensified its focus on this topic, launching a global initiative to slash medical error by 50 percent over the next five years. The project, along with the IoM report, is the result of many recent studies aimed at documenting the severity of the diagnostic error problem. It highlights the need for programs to understand the nature of the errors and develop

effective interventions, thus increasing the safety of the diagnostic path.

Many medical professionals may be surprised to learn that diagnostic error is now the most common type of medical mistake in clinical practice, at least in the United States and other developed countries. It is also the type of error most closely associated with mortality and morbidity; according to a recent estimation, diagnostic error mortality in American hospitals ranges from 40,000 to 80,000 cases per year (2). And to add insult to (literal) injury, it is additionally the most significant cause of unnecessary expenditure and liability reimbursement for those hospitals.

"We have definitely seen improvements in patient outcomes from initiatives aimed at reducing procedural errors."

Notably, most laboratory errors are not the result of analytical test inaccuracy; rather, they are a reflection of problems with test selection, sample collection and manual handling, and appropriate interpretation and use of results. The latter issue in particular is responsible for the 5 to 8 percent of laboratory errors associated with the greatest risk of harm to the patient. (3)

The vulnerability of the pre- and post-analytical phases is now universally acknowledged. The analytical phase, in contrast, has improved significantly in recent decades – primarily because of better training and increased use of quality indicators, but the introduction of automation and information technology has also helped. For example, my laboratory – the Clinical Laboratory of the University of Padua – has adopted a solution (Inpeco SA's ProTube) to ensure that patient data, samples and test results are all correctly matched. And it has contributed to a significant decrease in error in this phase of testing. The development of a harmonized model of quality indicators – in this case, for misidentification errors – creates opportunities to identify and correct the processes most exposed to the risk of error at each stage of the testing pathway (4).

The challenge? To move from analytical data management to "total sample management," and to achieve a total laboratory information management solution that ensures traceability, security, and the appropriate interpretation and use of laboratory data.

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A PATHOLOGIST'S PIERIAN SPRING

With the rise of newer, better liquid biopsy technologies, pathologists are finding a wealth of knowledge hidden in the blood

A WIN-WIN SITUATION

Liquid biopsy technology is ready – but are we?

By Joydeep Goswami

In 2014, the clinical sequencing team and our pharmaceutical partners began a collaboration focused on increasing the use of next-generation sequencing (NGS) in the clinical domain. We saw an obvious unmet need, especially in oncology, and we believed that NGS could help patients find the right therapy faster. NGS includes tissue (solid tumor biopsy) sequencing, of course, but liquid biopsy has the potential to become the laboratory professionals' assay of choice for several reasons: it's less invasive than a typical solid tissue biopsy, hence potentially cheaper for patients and healthcare providers, and can be used when obtaining a solid tissue biopsy is not feasible due to location of tumor or patient health.

The liquid biopsy sample is usually blood, but it can be any fluid containing genetic material; the source of the DNA or RNA is irrelevant after sample preparation. Of course, nucleic acids – though currently the main focus of liquid biopsy studies – aren't the end of the story; such tests have the ability to examine not only DNA and RNA, but also proteins, exosomes, and entire circulating tumor cells.

A continuum of diagnosis

I think of liquid biopsy as a continuum for diagnosis. Initially, its main applications will be in disease recurrence monitoring. Patients undergoing cancer treatment are checked every six to nine months (on average) to ascertain the status of their disease – initially via CT scan and then, if necessary, by solid tumor biopsy. Liquid biopsy can improve on the process in several ways: by reducing wait times; by increasing sensitivity over imaging alone; and by giving doctors more in-depth information – has the tumor mutated and, if so, how? Are there additional resistance genes, and how should the course of treatment be altered to counter these new

mutations? By monitoring the cells and DNA that tumors shed, liquid biopsy may help assess much earlier whether the patient is acquiring a resistance mutation. This is life-changing for patients whose health is severely compromised and who might not be able to withstand invasive procedures – or delays in transitioning to more effective treatments.

The next application on the continuum of liquid biopsy is its use as a potential replacement for solid tumor biopsies – even in the initial detection and analysis of cancer. If a patient walks in with a diagnosis of lung cancer, you want to understand the molecular nature of the tumor. But is a solid tumor biopsy the best choice? Not only are such procedures potentially dangerous, but they can also be resource-intensive, requiring the time of a radiation oncologist and costing tens of thousands of dollars. Instead, could you completely bypass solid tumor biopsy and go directly to liquid biopsies? This is a possibility, and many major academic centers are moving in that direction. Of course, there's still a lot of science to be done. We have to investigate whether the results of the two biopsies are comparable. Neither one is "right" or "wrong," but they do have different advantages; liquid biopsies, for instance, may potentially be better at detecting the polyclonal nature of a tumor because they capture a comprehensive DNA sample rather than accessing specific sites.

As people get more comfortable with liquid biopsies, I think there will eventually be a shift away from tissue biopsies and toward circulating tumor material. After that, the possibilities are almost limitless. Liquid biopsy could allow us to move from "disease management" to "health management". This could be in the form of a simple, annual blood test potentially allowing "at risk" (for example, due to family history, known genetic factors, or habits such as smoking) but otherwise healthy patients to check regularly for the appearance of cancer and work with their physicians to decide on the right choice of action. The best chances for beating cancer are provided by early detection. With survival rates many times higher for those diagnosed with stage I or II cancer versus stage III or IV, I see liquid biopsy as an eventual game-changer.

Pushing boundaries

At the moment, we are trying to extend the limit of detection of circulating tumor DNA and RNA in liquid biopsy research. We're down to a 0.1 percent limit with high sensitivity and specificity, using only a single tube of blood from which we extract either DNA or RNA. Because we can do both, we can now analyze gene fusions, single nucleotide polymorphisms and copy number variants in the tumor's genetic material. We obviously still want to increase sensitivity, and I think that's the direction the field will take. As it stands today, the technology provides valuable information that could be extremely useful to help manage a fatal disease where late detection is a particularly big problem.

There are two main areas where liquid biopsy should advance. The first is in simplifying assays. Ideally, the physician or the pathologist should be able to focus on just the genes of greatest interest for a particular patient. That should make the tests faster and reduce workflow complexity, so that liquid biopsy can be truly democratized. These solutions should to be easy, push-button assessments that any doctor can provide for any patient.

The second area of focus is improving the proposed course of action a clinician should take once the presence of a potential oncogenic mutation is detected – these could span the range from doing nothing (wait and watch) to immediate action in terms of additional testing or treatment. Some of these improvements are more within the realm of companies focused on the technology itself; others will require a concerted effort from physicians and pathologists as we push the boundaries of science in both detection and treatment.

Let's consider the real reason we should want to implement these tests. The time and cost savings are important, of course, but the main goal should be a better patient outcome. Whether you're a scientist, a pathologist, or a clinician, we all have to remember that our work begins and ends with the patient. I think some of the early adopters – pathologists and then oncologists – are starting to see the benefits of being able to monitor disease in a controlled and quantitative manner.

Clearing the last hurdles

Liquid biopsy in general has been very well received in the pathology community – it's clear that there is real interest, but there are several steps that need to be taken for it to reach its full potential. Scientific research needs to continue in terms of determining how best to use the results of liquid biopsy in the overall continuum of diagnosis and treatment. Solving the economic problem is important too. In the United States, for instance, we need to figure out the reimbursement criteria before we can expect to see widespread adoption. Pathologists and laboratories need to know that neither they nor the patient will end up footing the bill for a simple, life- and cost-saving test. Ultimately, liquid biopsy could have its largest impact if it's used as a regular screening tool for at-risk individuals – an important way of managing not only disease, but also the health of the population at large. For that, insurance companies, health professionals and governments need to come together to understand the real potential of liquid biopsy.

Discussions about the technology focus mostly on DNA and RNA of the tumor. At some point, we will also want to look at other markers, including protein markers, in the same assay to get a more holistic picture of the cancer. Expanding liquid biopsy into these areas will allow physicians to obtain a better understanding of the cancer including how the immune system is responding to the tumor, which will further inform treatment decisions. I think all those elements – expanded assays, tailored testing, increased sensitivity, and more accessible technology – will combine to make sure that every pathologist can use liquid biopsy, and every patient can benefit from it.

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BATTLING LUNG CANCER IN THE TOBACCO BELT

When speed is of the essence, liquid biopsy can help oncologists make life or death decisions

By Paul Walker

I direct the lung cancer program at East Carolina University, which is not only in the Bible Belt but also the Tobacco Belt. And that means we see a lot of lung cancer – on average, 8–10 new cases every week. Clearly, we need to make good treatment decisions fast. For the past two years, we've been using liquid biopsy – specifically, droplet digital PCR (ddPCR), which lets us zero in on the actionable mutations in lung cancer (*EGFR, ALK, ROS1, BRAF*, and *KRAS*). Because ddPCR looks only at the "hotspots," it has a quick turnaround time – about three days. The speed dramatically affects decision-making – to the point where I would describe it as the first step toward truly personalized oncology.

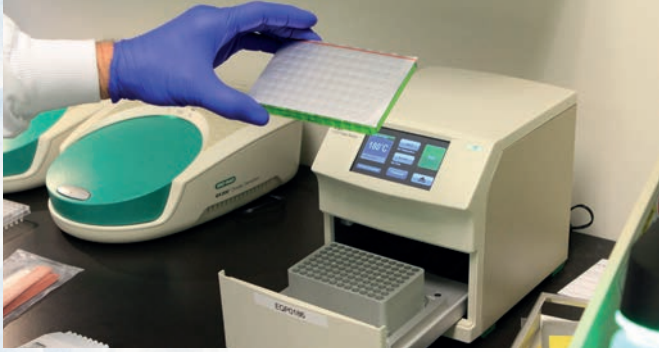
We can now identify a cancer's mutations and respond with appropriate therapy without the issues inherent in solid tissue biopsy – not only the delays, but also the intratumoral heterogeneity that is often missed when sampling only one or a few tumor sites. In fact, liquid biopsy has a particular advantage in that it captures the most aggressive clone; because it is the site of greatest apoptosis, it sheds the most cell-free DNA (cfDNA). When we began using ddPCR for cancer testing, we began to see mutations we never expected – and, in some cases, they completely changed our treatment decisions. And that's when we began to ask, "How did we ever make decisions without liquid biopsy?"

Breaking barriers and learning lessons

Liquid biopsy surely has great advantages, but we also face integration hurdles. One huge stumbling block is that the technology is hard to understand for those in other fields. Medical oncologists think biologically and therapeutically, and not from a technology standpoint, so there's been a lot of confusion. We have 12 medical oncology fellows at our institution, and I try to teach them that there are really two types of liquid biopsy. There are the ones that are going to give you information on actionable and/or druggable hotspot mutations – and the more limited the search you make, the quicker you get results. Whereas the other option – next generation sequencing (NGS) – can be endless: it can be 92 genes, or 582, or even the whole exome. You can get a lot of information, but you're not going to act on 99 percent of it. I think a lot of medical oncologists have used NGS and received a 12-page report and thought, "this is never going to be helpful!" By using the right method, you can get useful information that will help guide treatment.

Liquid biopsy is already teaching us a great deal. Cancer is a lot more genetically diverse than we suspected. You're not "supposed" to have co-occurring *EGFR* and *KRAS* mutations, but we've seen them. You're not "supposed" to have an *EGFR* T790M mutation in squamous cell lung cancer, but we've seen that. We've learned that not all lung cancers respond to platinum – for instance, those with *ALK* mutations, or *KRAS* G12C. These are aggressive cancers that require rapid response, and understanding their genetic landscapes gives us the ability to use the best treatment right from the start. And when you consider a variety of liquid biopsies – that is, not only those from blood, but also those from urine, cerebrospinal fluid, or any other source – you capture an even broader range of genetic alterations, because not every compartment has the same mutations.

A good example of liquid biopsy's utility is "chemoimmune" therapy (pemetrexed, carboplatin and pembrolizumab) for non-squamous cell lung cancer. It's a promising approach – provided



the tumor doesn't have an *EGFR* or *ALK* mutation. With ddPCR, we know within three days whether or not those mutations are present in our fluid of choice, which lets us determine the best treatment option for a rapid and durable response. For me, that really crystallized the benefits of having a quicker test – and it helps in conversations with patients, too, because we can reassure them that the results of the treatment will really be worth the downsides.

Tailoring treatments

I view solid tissue and liquid biopsy as complementary techniques – each makes up for the other's failings. When biopsying tumors, you may not always get sufficient tissue, especially in lung cancer. Liquid biopsy material is easy to access, so you can perform deeper analyses. In our program, we perform both the "hotspot" actionable mutation test and NGS (of tissue samples); the latter takes longer, but leaves us with information we can continue to use as precision medicine and immunotherapy evolve.

The United States has an additional, unique problem with tissue biopsies. If a hospitalized patient is under Medicare – as most over the age of 65 are – the material can't be sent out for 14 days. That means you have to wait until either two weeks have passed or the patient is discharged, and then the same amount of time again even for initial results. That's a full month of delays in providing treatment, and not every lung cancer patient has that time to spare. The problem is only exacerbated when the disease develops treatment resistance; re-biopsying a lung tumor is a difficult process at the best of times.

On the other hand, tissue provides us with a wealth of information that becomes increasingly valuable. From a pathology standpoint, I know the most important lung cancer question has always been, "Is it non-small cell or small cell?" Once upon a time, that was all the pathologist had to do. Now, with an adenocarcinoma, there's this never-ending algorithm – CK7, CK20, TTF1, p40, p63… to the point where the tissue sample is exhausted on tests with very little impact on therapy. We need to break away from that pattern of algorithms and focus on the data that will actually affect treatment decisions.

Historically, we clinical oncologists have referred to certain patients as "exceptional responders," because therapy has been highly effective even with advanced metastatic disease. Now, we're finding out the truth – they're not "exceptional responders," they've just received the right treatment appropriate to their particular disease.

It is, of course, possible to use a liquid sample for NGS – but, at the moment, tissue sequencing remains superior. Ultimately, I think liquid biopsy with cfDNA will replace other monitoring methods. For instance, patients won't need regular imaging after surgery. If the cfDNA no longer contains the cancer-driving mutation, I'd say there's at least a 90 percent likelihood that the cancer will not return. However, if you see the mutation beginning to recirculate, you know something is brewing – so you can alter the treatment accordingly.

Enhancing and extending lives

As a lung cancer specialist, I've also seen the impact that additional information can have on the experience of patients. Many people have seen family members and friends go through chemotherapy, and experience some really difficult side effects – and then die anyway. Being able to explain to patients that this is a targeted therapy with a high chance of extending their progression-free survival by several years is a radically different experience.

It's also helping to combat some of the stigma surrounding lung cancer – "You did this to yourself, because you smoked, and there's nothing much we can do." I think we still see this attitude even with some pulmonologists and oncologists. But people are living longer, and quantity and quality of life are being extended, which I think is helping to lessen the blame game and improve the outlook for our patients.

I've already seen some great results. One individual, a woman who was 70 but physiologically very youthful, had received radiation therapy for Hodgkin's disease, then later developed a widely metastatic lung cancer – it had spread to her pleura, adrenal glands, and bones. And her diagnostic tissue was just cytology

from the pleural fluid, so there was insufficient tissue to get a mutational analysis. But her liquid biopsy came back with a *KRAS* G21C mutation, which is not thought to be very platinum-sensitive. And so, although the standard therapy would have been a platinum doublet plus or minus bevacizumab or anti-VEGF-A, we just gave her a non-platinum doublet. The response was excellent. We repeated the liquid biopsy and found that the *KRAS* mutation was persisting, but also that there was an *EGFR* T790M mutation, which are thought to be mutually exclusive. So then we went on to immune checkpoint blockade, and transformed quality of life for the patient – she went from being breathless and in pain to enjoying a two-week African safari with her husband. Her cancer is under control, and she is still living a very full life.

Another woman, a mother to two young girls, had extensive brain metastasis – it was an *EGFR* exon 19 mutation, and she also had a T790M mutation, which is immunogenic (the other *EGFR*s are not). She went on to have immune therapy and, as of her last imaging, she is completely cancer free and able to live her life. Throughout her treatment, liquid biopsy told us exactly what was going on, and helped us to choose the treatment with the best chance of success.

As a medical oncologist, every patient visit is truly a life and death situation. It's so important to be able to look your patient in the eye and tell them what the best treatment decision is, based on all the information you have, and to know you did everything possible for them. Liquid biopsy is helping us to do that.

A fluid future

If you are looking to implement liquid biopsy in your own practice, I have two pieces of advice. First, you need to make sure your colleagues understand what this information can do for them. In our own multidisciplinary team, we have four thoracic surgeons, two radiation oncologists, and a dedicated pulmonologist; I make sure everyone sees the results we're getting, so that they understand we're ordering these tests for a reason – and they are impacting therapy. Your team members must understand why they need this information.

Second, there is the question of in-house versus partnering with another organization to do the testing. Some pathologists would rather do these tests in-house, but I would argue that partnering with industry is also a good solution. After all, the technology moves so fast and becomes outdated so quickly. In our practice, we chose to play to our strengths – we are innovative and aggressive with our clinical treatment, but we didn't want to duplicate the tools we can access by partnering with a company who can provide the information for us. I think a lot of pathologists resist the idea of giving up their tissue for testing elsewhere – but liquid biopsy solves that issue.

Liquid biopsy will lead to big changes in how we monitor cancer status in patients. We're already seeing this in chronic myeloid leukemia using *BCR-ABL* quantitative PCR, and there is more and more data emerging showing that after colorectal cancer surgery, cfDNA can tell us which patients are going to see cancer recurrence (1, 2). Such information can guide the decision to treat with adjuvant chemotherapy and give us an immediate answer to that crucial question – "did the surgeon get it all?"

For patients who don't have a diagnosed cancer, liquid biopsy is also poised to change how we monitor health. I always tell our fellows that they ought to go through an MRI at least once – no one enjoys spending 45 minutes in a tube! If we can screen patients using a blood draw, we can save them from X-rays and MRIs.

I suspect things are going to move much more quickly than expected – last year, liquid biopsies were validated at ASCO, and this year they've extended to diagnostic, prognostic, surveillance, resistance pathways and recurrence across many tumor types (3 – 6). I think this area is going to expand rapidly, and it won't be long before many of our questions are being answered with a simple blood test. Looking a couple of steps ahead, we know that cell-free DNA also appears in urine, so one day you may be able to simply provide a urine sample to find out if you're cancer-free or not. The technology is already here – and the possibilities for the future are amazing.

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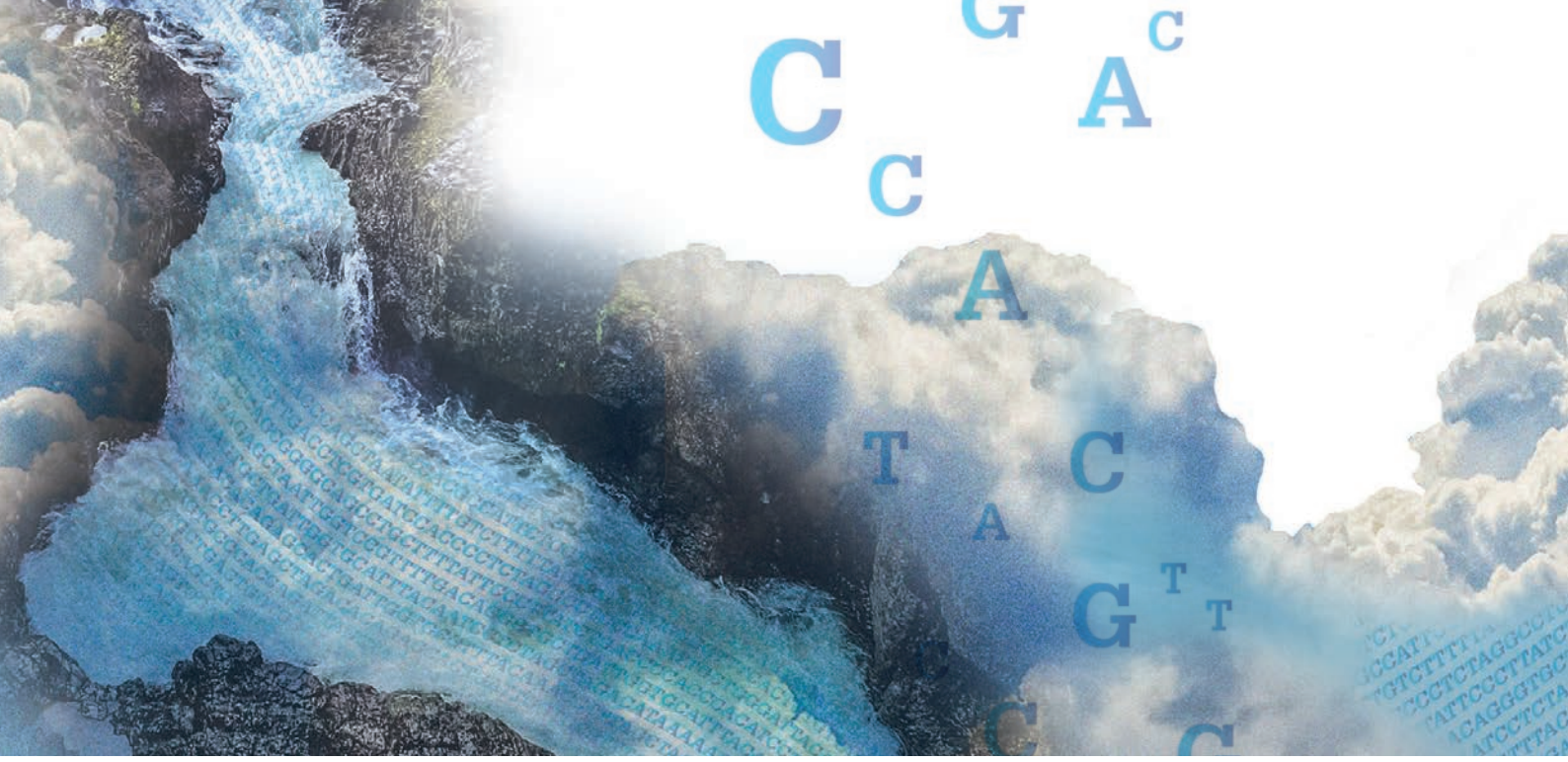

A DROP IN THE OCEAN

Applying droplet digital PCR to liquid biopsy: advances, applications, limitations and caveats

By George Karlin-Neumann

Molecular biologists have recognized for decades that when both diseased and healthy cells die, they slough their contents – including DNA – into the blood stream, and that the genetic variation present in these cells might be discernible in circulating cell-free DNA (cfDNA) found in blood plasma. The community theorized that it might be possible to monitor the status of solid tissues through a minimally invasive blood draw – a "liquid biopsy."

And indeed, liquid biopsy can detect many types of genetic variations, ranging from single nucleotide mutations to amplifications (or deletions) of entire genes. But, in a typical blood sample, such mutations are usually present in a tiny fraction of the detected cfDNA (the majority of cfDNA being derived from the patient's healthy cells), presenting a classic "needle in a haystack" problem.

Real-time limitations

Real-time PCR (rtPCR) – the basis of the oldest commonly used qualitative or semi-quantitative liquid biopsy tests – is too blunt a method for some applications; for example, detecting biomarkers of fetal trisomy in a mother's blood. Why? Because it is limited in its ability to precisely measure the slight change in fetal chromosome copy number when compared with the background of healthy maternal cfDNA. Similarly, rtPCR struggles to assess whether a single base mutation from a tumor, which often occurs at very low frequencies, is present in a patient's plasma either before or after treatment.

Facing very low concentrations of target species (perhaps only a few copies per milliliter of blood) creates a need for highly sensitive, specific, and precise tools. It is critical to have a platform that can discriminate single-nucleotide mutations, whether they're found in cfDNA fragments, miRNAs or other RNA types, from an abundant background of highly similar wildtype sequences. In other cases, it is crucial to have the capability to detect small changes in the copy number of a key gene target. Laboratories need robust technology with high-reproducibility. And to be adopted widely, the technology must enable rapid turnaround, be cost-effective, and be compatible with low and high throughput needs. To meet these needs, a new solution is emerging.

Digitizing nanoscale reactions

Droplet digital PCR (ddPCR) can measure samples with much higher precision and sensitivity than conventional rtPCR. Reactions can be "digitized" by subdividing a PCR reaction into nanoliter-sized compartments, or partitions, where the sample in each partition is separately amplified. The "positive" partitions containing the specific target(s) being assayed generate a strong fluorescent signal, and the "negative" partitions emit only weak fluorescence. The platform then counts the number and fraction of positive fluorescent partitions to determine the concentration of the target sequence in the sample.

The concept behind ddPCR was developed in the early 1990s but, back then, it was not easy to perform nor cost-effective. Researchers initially used the wells of PCR plates as individual partitions, and later, they used pre-formed nanofluidic

chambers in microfluidic arrays. The technique was then further developed to overcome these limitations and provide a high throughput, affordable and scalable platform that delivers absolute concentrations of DNA or RNA targets with high precision and sensitivity, without the need for a standard curve.

The key to modern ddPCR technology is the reliable generation of uniform, nano-sized droplets from the input sample, which required an inexpensive, disposable microfluidic chip. The droplets need to be stable enough to be transferred from the chip to a PCR plate for thermocycling, and then to be autosampled and microfluidically transported within the dedicated droplet reader.

Instrument control and analysis software was also needed to perform quality control on the droplet fluorescence signals in two wavelengths (corresponding to the emission of the two different dyes used). The resulting software, QuantaSoft, can reliably identify droplets and assign them to a particular category: double negative (droplets with a signal from neither of the dyes), single positive droplets, or double positive droplets (those containing a strong signal from both dyes).

> "The key to modern ddPCR technology is the reliable generation of uniform, nano-sized droplets from the input sample."

Additionally, the software was developed to calculate the concentration of detected target species, and to display the results in various types of plots and charts showing concentrations, ratios and numbers of accepted droplets per sample. Lastly, it was necessary to develop a variety of ddPCR reaction master mixes for different applications and assay design strategies for producing either Taqman probe assays or EvaGreen probeless assays that function well in droplets.

The road to adoption

Although clinical adoption of ddPCR for liquid biopsy of solid tumors is still in its early stages, about half a dozen molecular diagnostics labs are offering such tests for both liquid biopsy and tissue genotyping. So far, it has garnered a positive reception (1, 2).

Several academic centers have adopted the technology, including the Dana Farber Cancer Institute (DFCI), MD Anderson Cancer Center, and the Olivia Newton-John Cancer Research Institute. Some independent molecular testing labs have adopted the technology as well, including Biodesix Inc., which largely serves physicians at community care centers. Biodesix developed and validated a ddPCR-based test panel for non-small cell lung cancer (NSCLC) mutations, which turns around results in about 33 hours of receiving patient blood samples.

Pathologists at the DFCI/Brigham and Women's Hospital Pathology Lab are using ddPCR liquid biopsy testing to measure EGFR-sensitizing and resistance mutations in lung cancer patients to rapidly identify those who are eligible for tyrosine kinase inhibitor therapy. Nowadays, patients who do not have access to ddPCR technology must wait an average of two weeks (if the patient is newly diagnosed) or four weeks (if the patient has relapsed) while their tissue samples undergo next-generation sequencing, if the samples are available at all.

Currently in the labs that have adopted it, ddPCR is mainly serving NSCLC, melanoma and thyroid cancer patients. And importantly, the institutions using ddPCR have found success obtaining reimbursement for these tests. The approach to testing is ideal in two scenarios: i) when the physician needs to identify the potential presence of a recurrent hotspot mutation in a cancer patient quickly and inexpensively to make a treatment decision and ii) when a physician wants to monitor a patient's disease progression or response to treatment over time.

These types of monitoring investigations are informing the evaluation of patient responses to immunotherapies such as anti-PD1 (for example, in melanoma and lung cancer). ddPCR could also potentially help physicians make "real-time" adjustments to a patient's therapy based on changes in tumor DNA levels in the plasma; however, we must demonstrate ddPCR's clinical utility for this purpose. The approach is less effective for genotyping a cancer where there are numerous potential disease drivers, and when these mutations occur at low frequency in the population.

Future growth of ddPCR in the clinic appears likely, as scientists have discovered a number of actionable markers for new indications (resistance to anti-hormone therapy, for example) in breast and prostate cancers that can be readily tested. Further, numerous groups are running clinical trials that incorporate ddPCR, demonstrating its potential use in clinical decision-making (3–5).

A word of caution

Despite the positive results we've seen so far, I would alert laboratory professionals to the risks of "magical thinking." Even though digital PCR is a marvelous tool that can deliver absolute

Liquid Biopsy in Practice

By Hestia Mellert, Director, Molecular Development at Biodesix, Inc

Right now, tissue biopsy remains the gold standard for diagnosis – the literature shows that the ability to detect tumor mutations by liquid biopsy increases with increased tumor burden and is therefore more difficult in early stage disease (1). That said, liquid biopsy is a complementary method with great promise. At the moment, liquid biopsy and ddPCR are being used to identify mutations either before treatment or in patients with resistance to a treatment. But the hope is that, as the technology is sensitive and involves a simple blood draw, it could be used to monitor tumors over time, even during therapy.

Currently, the feedback that we hear from pathologists is that they'll use liquid biopsy as an upfront test for rapid results. If they don't find any positives, they can go back to the drawing board and wait for the tissue test result. Liquid biopsy also offers an alternate testing route when confronted with a patient who has insufficient tissue; if only a small amount of tissue is available for testing, it gives the pathologist the option to reserve the tissue for histology by using liquid biopsy for molecular tests.

In one study of ddPCR technology (2), physicians noted that up to 30 percent of patients had treatment decisions made without guideline-recommended mutation tests completed. They also noted that the median turn-around time of tissue-based mutation results was 12 days for a new diagnosis, and 27 days for patients who had developed resistance to tyrosine kinase inhibitors. Using a liquid biopsy approach, results were obtained in as little as 33 hours, with the majority of results (95 percent) obtained within 72 hours. And of the 179 patients tested, around 20 percent of them had a variant result that informed treatment decisions – demonstrating that liquid biopsy has the power to change practice, if targeted to the right patients.

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counts of target molecules without reliance on a standard curve, it is still necessary to validate your assays to verify that they are giving accurate answers. Of course, a poorly-validated assay will not perform well, even in ddPCR. But even a well-designed assay occasionally doesn't reach its target efficiently, particularly for large DNA fragments that are multiple kilobases long. In these cases, assays may significantly undercount the number of target molecules present. Here, a pathologist should reduce the size of the template fragment – such as by restriction enzyme digestion – to alleviate this. However, it should be noted that this is only seen in a small minority of cases, and is therefore an infrequent problem.

Similarly, digital PCR does not remove the need for reference standards or controls that can confirm that the testing run was successful and that the results can be trusted. In this regard, it may even be prudent to use spike-in controls to assess pre-analytical sources of noise, such as extraction efficiency, and unusual inhibitors present in a sample, such as another drug in the patient's blood.

In addition, pathologists should be careful about using the terms "precision" and "accuracy." Even the best scientists are prone to conflating these two terms and assuming if a result is reproducible, that it is accurate. But even if the results are reproducible, the assay needs to be validated before its results can be trusted in terms of accuracy.

Ask the right questions

It is very important for a physician or scientist to understand exactly what question he or she is trying to answer, and to choose a technological approach that will let them collect the right information. If a researcher is looking to discover new cancer biomarkers, he or she may wish to broadly profile a patient's DNA variations using NGS. But if a physician is trying to determine the optimal treatment for his or her patient, particularly after the patient's tumor has been profiled, it may be more expeditious and economically sustainable to use a more focused approach – in which case, ddPCR is an excellent tool.

George Karlin-Neumann is the Director of Scientific Affairs at Bio-Rad Laboratories' Digital Biology Center.

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THE MYSTERY DISEASE

Liquid biopsy can reveal the characteristics of otherwise inscrutable cancers

By Shumei Kato and Razelle Kurzrock

Cancer is always a diagnostic and therapeutic challenge – but far more so when the origin of the primary tumor is unknown. By definition, carcinoma of unknown primary (CUP) is metastatic, and without knowing where the disease began, oncologists are often at a loss to suggest the most appropriate treatment. Although the current standard of care is a platinum-based combination therapy, the approach is often less effective than treatments for known cancers, buying patients only a few short months of life. In liquid biopsy, though, a research team from the University of California San Diego's Center for Personalized Cancer Therapy, has found a potential answer – sequencing the DNA shed by the mystery tumors (1).

> "Liquid biopsy [...] is a valuable tool, but pathologists should take a clear-sighted approach to interpreting the results."

Why turn to liquid biopsy for CUP?

Patients with CUP generally have a poor prognosis, with a median survival of only six to eight months with standard-of-care approaches. We decided to investigate blood-derived circulating tumor DNA (ctDNA) to better understand the biology of CUP.

When patients are suspected of having CUP, the diagnostic team makes an effort to figure out in which organ the disease originated. Patients may undergo additional evaluations including esophagogastroduodenoscopy, colonoscopy, mammogram and CT imaging to find the primary cancer, as well as more high-tech approaches like a microarray-based assay used to predict the origin of a cancer. However, even with this extensive testing, the putative primary origin is only assigned in about one quarter of patients with CUP.

How does liquid biopsy for CUP work?

Liquid biopsy is used not to diagnose CUP, but to understand the molecular makeup of the cancer – which, in the eyes of many laboratory medicine professionals, is as valuable a "diagnosis" as any other. In our work, we currently employ a commercial assay that analyzes up to 73 genes at a time. The test isolates the tumor DNA shed by the cancer into the bloodstream and then applies next generation sequencing to assess the relevant genomic alterations. The implications of such a test for patients with CUP are clear – but its utility doesn't end there.

Nowadays, for instance, many clinical trials with targeted therapies require patients to have certain genomic markers, many of which a simple blood test can identify. It's true, of course, that there are other ways of testing for such markers; we could potentially use archival tissue or even obtain a new biopsy – but tissue-based approaches come with a host of challenges. A single sample cannot reflect tumor heterogeneity at multiple metastatic sites; archival tissue cannot reveal any changes that might have occurred as a result of therapy; new biopsies carry the risks inherent in any invasive medical procedure. Nevertheless, biomarker testing is vital – so blood becomes the new "go-to" method.

Of course, we still believe that testing for genomic markers in tissue is equally important. Blood-derived ctDNA has its own disadvantages – for instance, sensitivity is low, so liquid biopsy may not detect as many genes as tissue sequencing. In our clinic, we consider the results of tissue and blood-derived ctDNA sequencing complementary to each other. At the moment, neither reigns supreme – but nor can either one be omitted entirely.

What are your top tips for liquid biopsy?

Liquid biopsy to assess molecular alterations in blood-derived ctDNA is a valuable tool, but pathologists should take a clear-sighted approach to interpreting the results. Differences between ctDNA and tissue sequencing, for instance, should not be a de facto cause for concern regarding the technical validity of the tests; there are valid biological reasons to expect some variation between ctDNA and tissue-based genomic tests – even when the blood and tissue samples are obtained on the same day. Consider the two assays complementary to one another, as we do, rather than relying on either one in isolation, or panicking when the results are not identical.

At this moment, liquid biopsy with ctDNA can potentially be used to identify actionable genetic targets. In fact, testing ctDNA for *EGFR* mutations in patients with non-small cell lung cancer is FDA approved (2) – and as the technology improves, we expect ctDNA panels to expand to the point where they can test several hundred genes simultaneously.

ctDNA is an important addition to our armamentarium to better understand the underlying abnormalities driving cancer in our patients. Along with other routine tests, ctDNA is set to become a vital tool to guide treatment and selection of clinical trials for patients.

What's next for the field?

As our understanding of genomics grows, we will probably learn more about the utility of variations of unknown significance (VUS) in the future. Right now, we do not know their functional significance – and, in general, we give most of our attention to characterized variants when deciding on therapy. We have recently shown, though, that the number of alterations in ctDNA can predict a patient's response to immunotherapy in a manner similar to that of tumor mutational burden in tissue. In other words, patients with hypermutated ctDNA are more successfully treated with checkpoint inhibitor immunotherapy. When we count alterations in ctDNA to assess the hypermutated state, we include VUS – so even without understanding their effects, we still find value in detecting and enumerating such mutations. To that end, it's vital for pathologists and laboratory medicine professionals to become experienced and knowledgeable in the genomics field.

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THE CHAIN REACTION

A new type of PCR can lower the cost and complexity of liquid biopsy

By Hanlee Ji

When it comes to liquid biopsy, not all tests are equal. In fact, many suffer from challenges, such as low sensitivity, expensive equipment requirements, or the need for error-prone enhancement steps like pre-amplification. Can these obstacles be overcome? Certainly – and a new polymerase chain reaction technique known as single-color digital PCR can help. The assay requires very little blood, carries a price tag much lower than sophisticated sequencing methods, and bypasses the pre-amplification step and its concomitant risk of error. It's possible that this technique, and others like it, may offer a new avenue for lower-cost, higher-accuracy liquid biopsy.

Why develop a new type of liquid biopsy?

Current methods of liquid biopsy, such as next generation sequencing and probe-based digital PCR, can be time-consuming and costly to optimize and run. Single-color digital PCR, which is essentially a standard PCR reaction, is cheap, simple to optimize and implement, and can be completed in a matter of hours after sample collection.

Tracking a patient's tumor burden is a critically important part of their medical management. Given the high cost and complexity of imaging studies, these scans are performed at intervals of months at a time – or even longer. The clear need for a faster, more frequent means of monitoring inspired us to use digital PCR to create a simple, low-cost method of monitoring cancer growth and spread so that it can be implemented at every visit.

How does single-color digital PCR work?

Digital PCR involves partitioning one or several DNA molecules into individual micro-volume droplet reactions that each generate a specific PCR amplicon product. Single-color digital PCR uses double-strand DNA binding dye, which incorporates into amplicon products and produces a fluorescent signal. We use this signal to distinguish between droplets that contain the amplicon of interest and those that are empty. By counting positive droplets, we can easily count the number of molecules that contain the target DNA, even if those molecules are incredibly rare within the sample. This approach enables users to design standard PCR primers for any target of interest to obtain absolute quantification in a matter of hours.

Because this blood-based test is exquisitely sensitive, capable of detecting tiny amounts of cancer DNA, it is possible to apply the technology to a wide range of patients. We require only a fraction of a tube of blood to check for tumor DNA, and the test can be completed in a matter of hours. Most importantly, the test can be "personalized" to detect mutations unique to any individual cancer. As a result, this molecular test can be universally applied to monitor cancer in any patient, regardless of the type of tumor.

Any surprises along the way?

One of the most surprising results we have encountered is the variability in concentration of cell-free DNA across samples. It can often be a two- to 100-fold difference! We find dramatic differences in cell-free DNA between different patients, as well as between individual patient time points. This speaks to the importance of developing highly sensitive tests capable of handling low-input amounts while still providing informative results.

How will this change routine laboratory testing?

The ideal clinical test would be low-cost, high-performance, delivered within 12 hours, and require only limited amounts of blood. Single-color digital PCR addresses many of these points.

There are only a handful of blood tests for monitoring patients' tumors at the moment, and those are limited to only a few types of cancers. Because there are so few liquid biopsy options, nearly all cancer patients require whole-body imaging, such as CT scans, to ascertain the presence and growth of their disease. Our new liquid biopsy method will enable laboratory medical professionals to monitor disease status without the need for complex, invasive, or time-consuming tests. Instead, a single tube of blood – or less – is sufficient to check for tumor DNA molecules. And it's so simple that any laboratory professional can prepare and perform the analysis without extensive training.

What are your top tips for liquid biopsy?

As the liquid biopsy research space expands, it is important to continue developing tools and technologies that are customizable, sensitive, and low-cost. These features are critical in generating real-time information that can affect patient care and medical treatment.

As a practicing medical oncologist, I see many patient scenarios where liquid biopsy would be informative for the patient. Such rapid, robust tests could become commonplace in clinical practice in a matter of years – and will definitely be the go-to for cancer testing in the future.

Hanlee Ji is Associate Professor of Medicine (Oncology) at Stanford University, Stanford, USA.

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In Practice

Technologies and techniques
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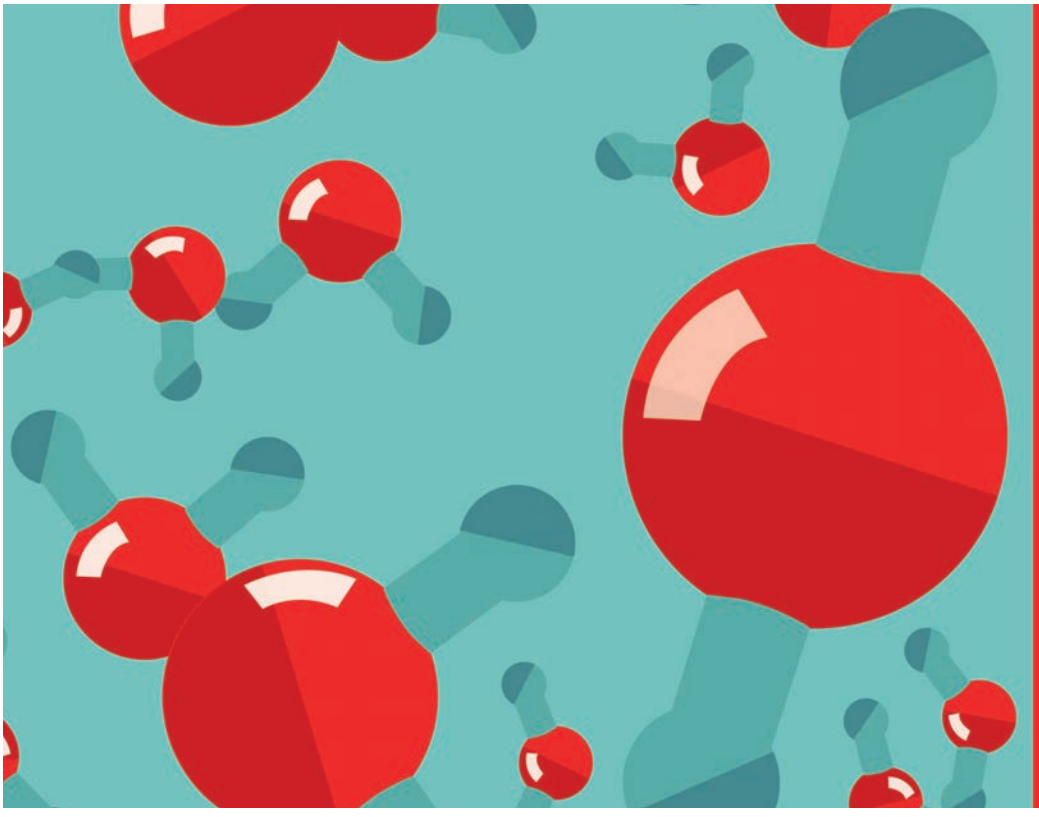
Molecules Against Microbes
When testing for infection – especially in cases of potential drug resistance – we often require results that are fast, sensitive and specific. Could molecular diagnostics help?

Molecules Against Microbes

As molecular diagnostic methods evolve, so does our approach to microbial testing – with important implications for the quandary of antimicrobial resistance

By Sherry Dunbar and Gunjot Rana



We are witnessing a time of rapid change in the world of microbial testing. In recent years, molecular diagnostic tools have emerged from their previous niche use to become the gold standard for more and more conditions. Our need for the particular advantages molecular assays bring – which include fast results, and high sensitivity and specificity – is more pressing than ever, as we strive to overcome a range of diagnostic challenges. And most pressing of all is the growing crisis of antibiotic resistance.

Several developments are underway that will shape microbial testing for years to come. From determining targets to performing tests in the lab or at the patient's bedside, the entire process of microbial assays is undergoing a shift that should dramatically enhance care and outcomes for patients.

Antimicrobial stewardship programs are working to identify resistance markers in microbes to guide drug selection for the best chance of success. Improvements in molecular testing for microbes will help doctors ensure that the right treatment gets to the right patient at the right time, and inappropriate treatments are never prescribed – which benefits both individual patients, and public health. If this can be achieved, it would be a major step towards addressing antibiotic resistance. But how close are we? Here, we take a look at some of the most important trends affecting microbial testing today.

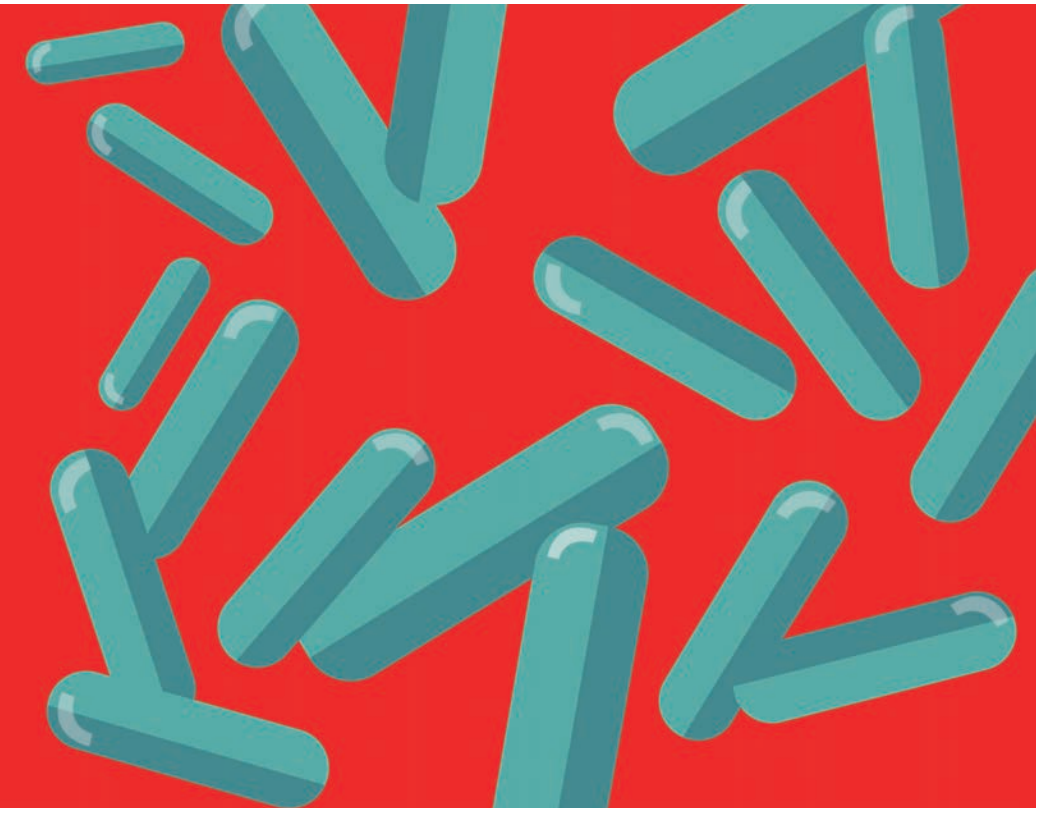
At a Glance

- *The danger of antibiotic resistance is only growing over time – and we need faster, more accurate diagnostic options to help prevent it*
- *Molecular diagnostics could have the greatest impact, providing quick answers with high accuracy, and also allowing for more complex testing*
- *Automation and multiplexing options are also improving diagnostic testing, saving lab teams time and minimizing the risk of human error*
- *Molecular testing advances will translate into benefits for patients – and offer labs versatility to meet a range of testing needs*

"Molecular assays can shave many hours or even whole days off of the timeline compared to more traditional diagnostic processes."

Rapid testing

Molecular diagnostics offer a significant benefit to clinical labs and the physicians they serve: a much shorter turnaround time to generate results. Molecular assays can shave many hours or even whole days off of



the timeline compared to more traditional diagnostic processes, such as culture-based tests. Typical process for culture is that the specimen is received and inoculated onto a variety of culture media based on the typical pathogens that would be expected for that specimen and infection type. Then the inoculated media are incubated and checked visually on daily intervals to see if there is any growth.

For a molecular test, the specimen is either directly placed into the test device and the results are available in minutes to hours, or the specimen may be processed to extract and purify any nucleic acids present first and then the extracted nucleic acid placed into the molecular test, with results available within a few hours.

Several recent studies show the clinical results of getting answers more quickly (1–3). In general, these analyses demonstrate that rapid tests allow patients to be treated more quickly with the right therapy, which in turn leads to shorter hospital stays and reduced readmission rates. They also provide evidence that this approach lowers overall healthcare costs for these patients and the institutions serving them.

Superior target selection

Some molecular tests are designed to detect a single microbe, but an increasing number can identify several species in a single assay. These panel-based tests allow clinical labs to look for some likely culprits in parallel, avoiding the time-consuming sequential testing of individual microbes as each diagnostic comes back with negative results. Physicians can now choose syndromic molecular tests to look broadly across several candidates multiplexed into a single test, which in many cases makes it more straightforward to diagnose the source of an infection.

For certain situations, however, pre-selected target panels may be too broad. For instance, during flu season, it would likely not make sense to start with more than flu and respiratory syncytial virus testing for an otherwise healthy patient presenting with respiratory symptoms. If an immunocompromised patient came into the hospital with the same symptoms, physicians might decide to use a much broader range of targets to cover all the bases.

A testing method known as masking allows clinical labs to implement either approach without changing assays. In this protocol, the lab runs the same multiplexed test for each sample, choosing which targets to report, and the masked targets report no results. If the first round of testing yields no useful answers, additional targets from the panel can be unmasked and viewed. In a new variation of this known as flexible testing, the lab only pays the manufacturer for the targets it chooses to be reported. Such approaches help labs keep costs in check while delivering as much or as little testing as requested by the ordering physician.

Sample to answer

As molecular testing becomes more mainstream, developers are improving automation to allow clinical lab teams to run tests with minimal hands-on time. These so-called "sample to answer" platforms essentially allow users to load the patient's sample, choose the test, and walk away. The instruments handle everything else, from adding reagents at the right time to managing complex thermal cycling profiles. Results can often be monitored at a central command station, rather than instrument by instrument.

Although convenience is a major factor here – these machines allow technicians to run more tests at once – another important element is the reduced risk of error. Every manual intervention carries a small opportunity for mistakes; eliminating such opportunities increases the accuracy of results. In the coming years, lab teams can expect that even more elements of microbial testing will become automated for a truly streamlined workflow.

Centralization and decentralization

The trends on where testing is performed are also shifting. Many types of tests that were traditionally performed in a central or reference lab, such as *Clostridium difficile*, MRSA, and flu, are moving toward the point of care, perhaps in a small regional lab, or even close to the bedside. At the same time, new high-complexity testing, such as sequencing-based testing of oncology markers, is shifting to modern centralized labs that have the capacity and sophistication to manage them. Where any individual test occurs can depend on the size of the healthcare system, geography, patient demographics, test type, and more. Medical professionals have more flexibility than ever to decide whether they need a very simple test that can be performed near the patient, or a more complicated diagnostic that is handled by a central lab facility. This allows lab teams to respond more nimbly to shifting needs for speed versus complexity.

At the point of impact

Following the decentralization trend, point-of-care testing has enabled a number of advantages for treating patients, such as responding more quickly to hospital-associated infections. Getting rapid results from onsite labs has also been essential for understanding antimicrobial resistance profiles, allowing hospital staff to choose more targeted treatments, and quickly quarantine patients when necessary.

The same information feeds into antimicrobial stewardship programs, making a real difference in how patients are treated for MRSA, *C. diff*, norovirus, and many other infectious diseases. Antibiotic resistance has become a major public health threat, with some experts estimating that 700,000 people die each year from drug-resistant infections (4).

In light of this trend, it is no longer enough to identify the microbial source of an infection –already a tall order in some cases. Now, labs must also quickly detect markers of resistance to support therapy selection for optimal outcomes and reduce the misuse of antibiotics (5–9).

Looking Ahead

Though we cannot anticipate every change that will affect microbial testing in the next few years, we can safely predict that most advances will be developed to support recognized needs in clinical labs today: streamlined workflows and information systems, cost-effective and accurate tests, rapid generation of results, and ultimately, better outcomes.

In the near future, many of the developments explored above will continue to gain traction. Increasing flexibility for clinical lab teams – whether in assay design, platform capacity, cost options, or other areas – will serve as a driving force for innovation. Particularly for microbial testing, where demand changes dramatically by geographic region, season, and more, labs need as much versatility as possible to meet the needs of their patient populations.

Advances in microbial testing have already had a noticeable positive influence on patient care and outcomes. As newer, more flexible technologies are developed, molecular methods hold tremendous potential to improve human health.

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Meet the Winner

Richard Jähnke

Richard Jähnke from the Global Pharma Health Fund (GPHF) has received the 2017 Humanity in Science Award for "development and continuous improvement of GPHF Minilab™ (www.gphf.org), which represents a breakthrough for the rapid and inexpensive identification of substandard and falsified medicines in low- and middle income countries in Africa, Asia and Latin America".

Richard received his award at a special jubilee reception in Berlin, Germany on October 2, 2017 hosted by KNAUER to celebrate the company's 55th birthday this year. Richard's work will feature in an upcoming issue of The Analytical Scientist.

Could it be you in 2018?

Analytical science has been at the heart of many scientific breakthroughs that have helped to improve people's lives worldwide. And yet analytical scientists rarely receive fanfare for their humble but life-changing work. The Humanity in Science Award was launched to recognize and reward analytical scientists who are changing lives for the better.

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NextGen

Research advances
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A Halo Model
Multiple organizations partner to consolidate over 30 sites into a single, state-of-the-art hub laboratory – one of Europe's largest.

A Halo Model

The mission: to consolidate 30 separate sites into one of the largest pathology laboratories in Europe

How does a laboratory go from being just an idea to a functioning reality? Health Services Laboratories (HSL) – a partnership between the Doctors Laboratory, the Royal Free London NHS Foundation Trust and University College London Hospitals NHS Foundation Trust – conceived the idea of consolidating over 30 individual sites into one brand new, state-of-the-art pathology laboratory. Fast forward two years and the building, dubbed "The Halo," is nearly complete.

We spoke to Tim Herriman, Group Laboratory Director at HSL, who worked on the Halo concept, and to Paul Sharp, a member of the construction company who helped make it a reality.

At a Glance

- *How do you turn a 1970s office block in central London into a huge, state-of-the-art laboratory capable of housing the latest equipment?*
- *Two of the minds behind the Halo share the story behind a laboratory designed to consolidate 30 separate sites into one central building*
- *From ceiling height considerations to obtaining custom software, the project was a huge undertaking, spanning several years*
- *With plans to process more than 20 million samples every year, the finished structure will bring together the latest tech and a host of different specialties*

Bringing Together the Best and Brightest

Tim Herriman, Group Laboratory Director at HSL

What makes the Halo unique?

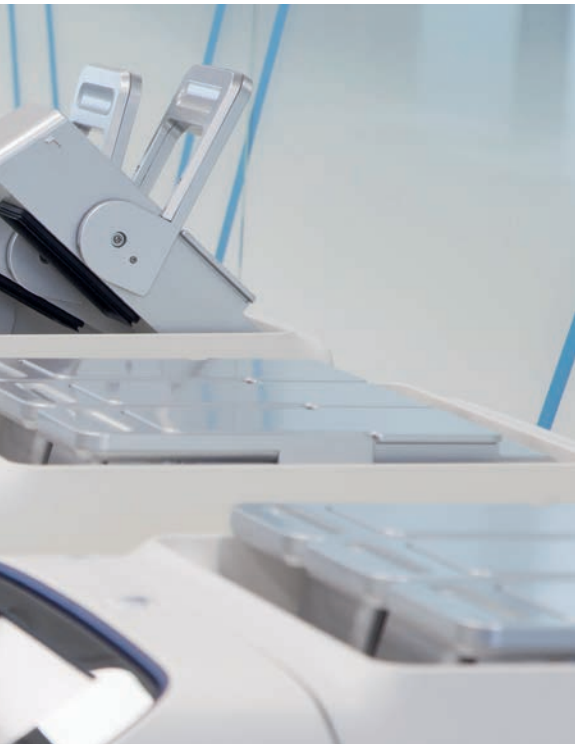
HSL is at the forefront of creating a "hub and spoke" system, in line with Lord Carter's recommendations for National Health Service (NHS) pathology services in England, and the Halo is our flagship laboratory. Its location, right in the heart of London's bioscience hub, also makes its stand out from the crowd. Being situated next door to world-class medical research institutions and hospitals, such as the Crick Institute, the Wellcome Trust and University College Hospital, reflects our ambition to provide an outstanding service. Our aim is to bring together the best facilities, latest technologies and brightest minds to deliver world-class diagnostic and pathology services.

What will The Halo include?

The Halo's 11 floors, when fully functional, will contain a range of specialist departments and disciplines. Broadly, these departments include blood sciences (flow cytometry, biochemistry, viral serology, protein electrophoresis, haematology and coagulation), infection sciences (microbiology, parasitology, mycology and virology), cytogenetics, and genetic and molecular testing. The molecular suites will combine molecular and genetic testing platforms for over 20 individual specialties.

What were the main considerations when designing the lab?

The Halo was designed with workflow, equipment utilization and clinical adjacencies, rather than discipline, in mind. For example, the building contains a dedicated molecular unit, combining



molecular and genetic testing platforms for specialties including hematology-oncology, hemophilia, virology, parasitology, microbiology, and noninvasive prenatal testing. Sharing the same equipment encourages greater interaction between formerly separate disciplines, concentrating a huge amount of expertise and allowing a range of scientists, doctors and molecular biologists to work side by side on new developments.

Ensuring that the lab has capacity for growth is another essential part of its design – we wanted it to be future-proof, not only in terms of physical structure, but also in its ability to deliver an efficient pathology service. The administrative floors of the lab have all been equipped with the right infrastructure to allow the lab to expand if needed, with as little disruption as possible.

The building also boasts a unique GLP track – the most complex tracking configuration in the world. Designing this sample reception delivery solution was central to the design of the Halo itself. It took a great deal of collaborative working and close understanding of laboratory workflows to design and create a suitable tracking system. Support from several custom software packages ensures the system can be as flexible and efficient as possible.

What about digital pathology?

We are looking into the feasibility of using digital pathology in a number of disciplines, including hematology (for blood films and remote bone marrow review) and histology. By liaising closely with other laboratories who use digital pathology routinely, we plan to gain more information on the systems available, and consider how they might best be incorporated into our current operations.

When will Halo be fully operational?

We are nearing the end of the transition. The lab will be operated by a cross-section of staff ranging from medical laboratory assistants and associate practitioners to biomedical scientists (trainee, specialist and senior), clinical scientists, and scientific leads for specific disciplines.

What will the path of a sample look like?

Samples will follow very simple rules depending on their clinical urgency. Urgent samples will be performed at local rapid response laboratories, usually based within hospitals themselves, to ensure a fast turnaround of results.

Specialist esoteric and non-urgent tests are centralized into our off-site hub laboratory – in this case, The Halo, which has several advantages. It enables a core network of specialists to work collaboratively on the most up-to-date methodologies and techniques, while also providing a center for training and retention of staff. The Halo is also able to analyze larger volumes of non-urgent work via highly automated systems – providing an efficient and effective service.

From Office to Laboratory

Paul Sharp, Divisional Director at ISG's Engineering Services business

The Halo lab is huge and technically demanding. How did you approach planning?

Even within a purpose-built structure, the 20-month project time would have been an ambitious target, given the technical complexity of delivering one of the largest pathology laboratories in Europe, including the largest sample tracking system ever installed in the northern hemisphere. But The Halo Building is in fact a 15-story former 1970s office block in central London, so this added an unprecedented level of complexity to the scheme – the structure was clearly never intended to be such a highly serviced clinical environment.

The key to the successful delivery of the project was identifying the critical elements and processes fundamental to the operation of the building, as well as understanding what existing infrastructure we were working with. Scoping the building's existing services infrastructure early in the process gave us the confidence to make important decisions quickly, as did the location and the construction implications of the laboratory's four vertical transportation systems – essentially, intelligent mini-lifts.

Another consideration was the lead in times and immoveable delivery windows for pieces of equipment, such as the Kiestra equipment on Level 3. Flexible programming of key areas of the building enabled us to accept deliveries of equipment that otherwise would have had serious implications for the program. Early identification of key pinch points was crucial to the efficiency of the build sequence and enabled us to lock down areas of the building once they were completed.

What design and logistical considerations did you have to keep in mind?

With strictly limited internal vertical transport options during the construction phase, a large hoist and scaffold gantry system was erected to service all floors, which enabled material and equipment deliveries to keep pace with the challenging construction program.

A separate hoist and scaffold platform was also installed to facilitate the installation of the specialist process equipment. The additional capacity enabled this important element of the works to overlap with the construction

program, ultimately reducing the overall program by several months

In the Containment Level 3 laboratory areas, we worked extensively with HSL and workflow specialists to ensure the design of the space worked for the end-user requirements. The functionality of the space was fundamental and ensuring the equipment and workflow procedures were known and understood at the early design stages was key to success.

Workflow design was key to the automated blood analysis GLP system on the first floor. All the medical process equipment was modelled in Revit and uploaded into the 3D construction model of the project. A series of collaborative workshops with the design team and clinical end users interrogated and planned the clinical work flow in 3D real space, rather than the traditional 2D method.

"We have dispelled the myth that a state-of-the-art laboratory cannot be housed within a pre-existing office building."

The sample transport system spans over five floors, so information on where things would be located, down to the millimeter, was needed – planning in 3D made this possible.

What is unique about this project?

We have dispelled the myth that a state-of-the-art laboratory cannot be housed within a pre-existing office building because of incompatible floor-to-ceiling heights. Typical laboratory buildings have a slab to slab height of 4.5–5 meters, whereas a typical office building is in the range of 3.5–4 m. The Halo Building's floor to ceiling height is 3.46 m and features a 500–600 mm ceiling void space, with laboratory bench services fed from the raised access floor below. So despite not being a custom-built space from the very outset, when fully operational, the Halo will operate 24/7 and process over 20 million samples a year, with the most advanced diagnostic technology in the world – a result to rival any custom-built building.

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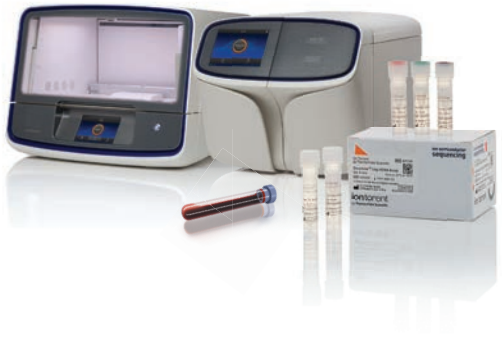

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Our Secret Language
In fast-paced modern medicine, the descriptive pathology report may seem redundant. But don't be fooled, says Kamran Mirza – it's more important than you think…

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Different laboratories use different tests and processes – so we need EQA-industry connections to ensure consistently high levels of quality and accuracy.

Our Secret Language

The role of the descriptive pathology report is often lost in the world of fast-paced diagnostics

By Kamran Mirza

My wife and I both work at tertiary care academic centers in the Chicago area – she is a pulmonary and critical care physician; I am a pathologist. Because of our proximity, it is quite common for patients to transfer back and forth between our hospitals. While I was still in training, she mentioned one evening over dinner that she had seen my name on a pathology report. *Extremely* exciting news. Patient privacy prevented us from discussing too many specifics, but I was itching to know what type of tumor it had been. Unfortunately, it turned out to be an autopsy report. The patient had transferred from her hospital to mine – *transferred to die*, I thought. I was the primary prosector, and she had received a courtesy report. *This was even more exciting.* I was very proud of my autopsy reports. I always made a special effort when putting the pieces of the autopsy puzzle together, so I felt that my summaries were quite good and I was eager to hear some praise. "So…?" I asked.

"You guys didn't find a cause of death," she replied casually, while passing the salad.

"But I'm sure I alluded to something in the description," I pressed.

"Oh, I just read the summary," she said, through a mouthful of food. "No one reads the entire report – you know that, right?"

"Of course, of course," I lied. "So where's the report?" I was hoping she had committed a huge HIPAA violation and brought it home for me to frame.

"I shredded it."

"Like my hopes and dreams," I muttered.

Yes, I exaggerate to make a point. I understood; she had patients crashing in the intensive care unit – bleeding, suffering from strokes… The pearls of my microscopic descriptions were of no value to her. But surely they would be of importance to my fellow pathology comrades… right?

I went on to become the hematopathology fellow at my institution. That one year taught me depths of pathology I hadn't even known existed. Sitting at the microscope, learning from the gurus with whom I was fortunate enough to train, was an exceptional experience – and an inspiring one. It made me want to be the best, so that I could show them all they had made a worthy investment in me. Our reports at the time were very long – three pages on average and works of art, each and every one. Pathologists from around the country would send us cases in consultation that were so difficult they considered them non-diagnosable; my mentors would solve the mysteries and send back detailed reports, revealing the secrets of the cases in their microscopic descriptions.

Were those reports appreciated? The diagnoses certainly were, but the reports noticeably less so. Every so often, we met pathologists from other hospitals – and they would jokingly make fun of the length of our reports. "Who has time for that?" "No one reads them" "What good is that

At a Glance

- *Descriptive reports are time-consuming and often go unread – but even so, they carry great value*
- *Not just physicians, but also patients, families and even legal experts may reference descriptive reports after the fact*
- *Not every case requires a descriptive report, but they can be useful for clarification, explanation, extrapolation, or even catharsis*
- *Even if not immediately referenced, descriptive reports retain their value – and one day, their creation will pay off*

description?" "Don't say more than you need to!" "The more you say, the more a lawyer will have to use against you!" "I would prefer a faster report to a longer one…" And the clinicians were no better. They would tell me, "No one reads this stuff, Kamran," "I can't even tell where the diagnosis is," or even, "Why don't you just say clinical correlation is recommended and get it over with?" All heartbreaking reactions to reports that I considered a labor of love.

I'll admit that writing the reports could be tiring. It was a busy service, and by the end of the umpteenth report of the day, I was exhausted. But when I began to lose my energy, I would pause and listen. From the open door of the small fellows' office, I could hear my mentors' hemepath counters pinging and their keyboards clicking away – and my energy would be renewed. I would read the reports churned out by these hemepath giants and be inspired by their greatness.

Two strikes

When it came time to start my practice, I was at a crossroads. What would my report look like? Of course it would be comprehensive in the sense that it would have all the essentials. But would it have something more? Would the contours of the malignant nuclei, the exact texture of the chromatin, or the shade of amphophilia in the cytoplasm be fondly described? Would every case, malignant and benign, usual and rare, all be given the same loving description – or not?

Two things helped guide my decision.

The first was a call that I had received while still in training. It was my mother, calling from her home thousands of miles away. A physician herself, she had been having some oddball symptoms that necessitated a biopsy. "Beta [son]," she said, "you're never really prepared to read the word carcinoma on your own report." I had two parallel reactions: that of a pathologist, and that of a son. It was a uterine disease, so as a pathologist I wanted to know everything about the remaining endometrium. Was this a polyp? Any secretory changes? As her son, I needed to know whether or not she was okay. I was far away, and it was hard. So I asked her to read me the whole report. Apparently, there were two parts to the specimen – a polyp and an endometrial scraping. From what I could tell, they had put both the polyp and the endometrium in a single cassette, and now I didn't know which part contained the carcinoma. There was no gross description saying what went where. No histologic grade was provided. Just "carcinoma." Nothing further to go on.

I took a deep breath and considered the future. Should my mother come to the United States for a hysterectomy? How would we deal with insurance? But she was adamant that she wanted to have the surgery at home. I suggested she go to the hospital associated with my medical school, a prestigious institution with a fantastic pathology department. She did, and the surgery went well – except for one small hiccup: they didn't find any carcinoma. *Wait, what? Surely there's a mistake.* I didn't trust this at all. I needed to see the report. Oddly enough, I would have been happier and more confident if they had found something and reported it; at least that way, I would have been sure that there was nothing left to hide.

And then I opened it: my mother's pathology report, an innocuous-looking attachment in an e-mail. I started reading, ready to shred it to pieces. What did I find? The gross description was impeccable. The inking (or painting, as they called it) was perfectly described. Sectioning seemed to have been done adequately and correctly. They hadn't found any tumor, so they had sampled the entire endometrium. Then I encountered a surprise: a detailed microscopic description. I wasn't used to seeing a long microscopic description for a case that was ultimately called benign. But as I read the words, I could visualize the slide in front of me. Descriptions of glandular epithelia with benign nuclei, emphasis on pertinent negatives, such as the lack of mitoses or necrosis; a magical prose brush building a normal-looking stroma around the glands; some chronic inflammation for added effect; reassuring notes stating that multiple sections had been examined; even a small fibroid satisfyingly described with "interlacing fascicles of closely packed cells with elongated nuclei and abundant eosinophilic cytoplasm."

"The pathologist had taken the time to write a report that, to many, was a waste of time – but to this patient's son, it meant the world."

As I finished reading this report, I truly felt that I didn't need to see the slides. I was completely reassured – no exaggeration. The pathologist had taken the time to write a report that, to many, was a waste of time, but to this patient's (pathologist) son, it meant the world. A secret language that relayed from one pathologist to another exactly what needed to be said.

Strike one.

Strike two occurred early on in my career at the scope with my residents. "How do you make sure you don't miss dysplasia in a lineage, Dr. Mirza?" followed by, "How are you sure you have thoroughly evaluated

"The ability to translate what you see under the microscope into prose is the essence of a superlative pathologist."

the aspirate smear?" and a host of similar questions about the adequacy of a marrow review. At first, I wasn't sure how to respond to these overwhelmed residents. Although the World Health Organization (WHO) classification for hematopoietic neoplasms sets out how to do the job perfectly, it is in itself an overwhelming read. When I thought about it, I realized that, as I review the slide, I write my microscopic description in my head. Every pathologist does it. As the scope moves, we look at patterns, architecture, features, outlines, contours, atypia, pleomorphism, changes, features, colors, textures…

As I look back on the early days of my career, that's exactly what my hemepath mentors were doing – and teaching me to do. So now, I encourage my residents and fellows to write out microscopic descriptions. They don't have to be very long. As pathologists, it is key to be able to express how you feel about cells (figuratively). Being able to say those things on paper goes a long way in aiding our ability as trainees. You only get your time in training once, so use it well. The microscopic description will go a long way.

As for practicing pathologists… I have learnt firsthand the beauty of the microscopic description. It truly is a secret language we can use to talk to other pathologists across the globe. It transcends regular conversation in ways that are difficult to explain in regular ol' English. Of course, not every GI biopsy can have an extensive microscopic description, nor can the day's seventeenth case of run of the mill myeloma, but there's always something that can be said. When prudently used, pathologic descriptions still have a role to play – even in this crazy world of fast-paced diagnostics and relative value units.

Painting with words

The ability to translate what you see under the microscope into prose is the essence of a superlative pathologist – so it is no surprise that the microscopic description has been referred to as "painting with words." As romantic as that sounds, let's face it: there is a place and time for descriptive reports. Running a busy GI service with over 150 biopsies a day? Not all of your reports will be descriptive. More importantly, not every report *should* be; unnecessary descriptions run the very real risk of confusing the reader.

So when are such reports appropriate and efficient? Do all pathology reports demand a descriptive aspect? There are no guidelines on what to include, or on how to write microscopic descriptions in a way that can be easily interpreted by clinicians – and there are certainly none on when such reports should be written. Published data suggests that pathologists tend to use certain phrases to indicate specific levels of diagnostic certainty (1), but such usage is not standardized, and this sort of individuality can be the source of immense confusion (2). The streamlining of reports and the existence of synoptic reporting for tumors has come into existence for a reason! So, first and foremost, there are legal communicative aspects of a report – by way of the final diagnosis and synoptic reporting – that should be crystal clear. It also needs to be clarified that a pathology report does not require a microscopic description to be a complete legal document. That said, allow me to share my thoughts on some scenarios where I believe descriptive analysis may be of value.

1. *The expert opinion.*
   By far the easiest example of a useful descriptive report is that of a second opinion, wherein the case has been sent to an expert for their thoughts. The description (different from the diagnosis or final interpretation) serves as the expert's explanation to the pathologist as to how the interpretation was made. One assumes that the need for an expert opinion arose in light of diagnostic ambiguity, and thus, clarification by way of a description is of utmost utility for the original pathologist or clinician.
2. *Clarifying a contentious diagnosis.*
   Along the same lines as above, some neoplasms or diagnoses don't "read the book." In such cases, descriptive reports explaining the specific features that led the pathologist to confidently arrive at a conclusion (despite ambiguous staining or morphologic features) are useful for other pathologists who may review follow-up biopsies or re-review the original material at a different institution. This information often has no place in the final diagnostic line – but that doesn't make it any less vital.
3. *Explaining suboptimal material.*
   Often, a biopsy is negative for malignancy – so when the final diagnosis of a 10 cm, radiologically worrisome mass is "acute inflammation," the descriptive report is the place to share with the reader that the submitted material may not be representative of the mass described in the patient's history. The old adage of "clinical correlation is recommended" may actually have a role to play in these cases. The descriptive report can

be a conduit to explain what the pathologist "feels" about the case without overstepping.

4. *Intelligent extrapolation of data.* Although I would never condone guessing of any kind in a report, the descriptive report can serve as the platform for extrapolation of histologic/cytologic findings in the context of an appropriate history – detailed morphologic assessment of a residual myelodysplastic syndrome, or the presence of only mature neuroblastoma. Such instances deserve recognition in the final diagnosis, but the details of what they look like belong in the descriptive report. For example, a description of findings could lead to the following: "Although no residual neoplasm is identified, the constellation of these findings are consistent with tumor bed."
5. *Catharsis.* Sometimes, I have worked hard to figure out a case – and maybe I just want to talk about it in the microscopic description. It's my report, so I will! There's no harm, as long as I make sure to include only relevant information. On many occasions, I have seen pathology gurus make statements like, "This is a difficult case to characterize," and the subsequent lines not only sum up their thoughts, but are also catharsis after days spent patiently working up the case.

This is by no means an exhaustive list of the appropriate times for a descriptive analysis on a pathology case – but, whatever your reasoning for such a report, they should never be either repetitive or confusing. There is no need to rehash what is already said in the final diagnosis; no one wants to read the same thing twice, and it gives us a bad rap. If anything, the descriptive report should clarify a diagnosis – not complicate it. A descriptive report should only be entertained if it facilitates clarity. To introduce differential diagnoses after determining a final answer, or to dilly-dally around non-committal morphologic features or stains, has never helped anyone.

Enduring value

I find that the best way to go about writing descriptive reports is to literally describe what you see. For cut sections (non-cytology), I find it helpful to start from the outside in. Does the lesion have a capsule? Is it a pseudo-capsule? Describe your cell(s) of interest and the pertinent background. Descriptions of nuclear and cytoplasmic features are basic, and commentary on special features such as specific "differentiation" or nuclear immaturity comes in handy. In some cases, starting with the lesion and working outward fits the case best. Are the cells discohesive but individually epithelioid? Do they have a hint of rhabdoid maturation or an intensely acidophilic nucleus? Offer the details of how unruly, vagabond cells break from the pack and infiltrate distant sites, disrespecting neighbors and causing mayhem on their journey. Often, the next paragraph goes through any immunophenotyping studies that were performed, and then a summation paragraph allows you to collect your thoughts and add anything you couldn't say in the final line. Be careful not to say anything that is not true, don't go out on a limb if you don't need to, and always remember – these are legal reports and can always be held against you in a court of law. That does sound like a little bit of a buzzkill, but if you have no reason to mention something, or to derive a conclusion, please don't!

By sharing with you some of the times when the microscopic description was important to me personally, I hope to underscore its value when appropriately executed. The stakeholders for this are not just other pathologists, but can include the patients and families themselves. In this age of "Googling" answers, with the abundant medical knowledge (correct or incorrect) at our patients' fingertips, it may not be a bad idea to consider the descriptive report's utility for informing patients – at least, those who want to know. And for archival purposes, some of these descriptions can help assist the eventual discovery of new morphologic correlations to different molecular alterations. As you can see, there are many good reasons to consider the descriptive report – but for me, they were most helpful when I was training. They helped me coordinate my skill and relayed to my mentors how I was progressing as a pathologist. I cannot stress enough the importance of these reports to my own trainees.

It's true that we will not be able to convince everyone to read these reports – but not everyone has to. When written appropriately, the value of such reports remains within them. I cannot guarantee when their value will be realized – perhaps the next day by the patient; perhaps the next week by a different pathologist; or perhaps a century from now by a medical archivist – but this I can say with certainty: your effort will not go to waste.

Kamran Mirza is Assistant Professor of Pathology at Loyola University Chicago Stritch School of Medicine, Maywood, USA. His wife is a Pulmonary and Critical Care specialist who does, in fact, read entire pathology reports adoringly.

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A Quality Undertaking

Connecting EQA and industry to ensure quality diagnostic testing

By Jacqueline Hall, Espen Walker, Colin Tristram, John Garratt and Beth Sheppard

Independent organizations around the world perform external quality assessment (EQA), or proficiency testing, to ensure that diagnostic laboratories produce valid and accurate test results. The need is clear – when using immunohistochemistry (IHC)-based diagnostics, for instance, it is critical to know (and be able to prove) that the staining results are accurate so that pathologists, physicians and other healthcare professionals can reliably use the tests to guide patient treatment.

Ensuring accuracy and precision in the EQA process is easier said than done. Different laboratories use different tests and procedures, resulting in variation across the field, but EQA schemes need to demonstrate accurate testing regardless of procedural variations within a laboratory or group. How is this accomplished?

- EQA schemes may provide universal standardized cell lines or tissue samples, and may involve centralized review of staining.
- EQA providers provide feedback and suggestions for improvement, for example on the reporting of test results.
- As EQA is a continuous process it provides ongoing learning opportunities and often the chance to participate in workshops setting best practice standards and guidelines.

The importance of EQA participation is underscored by the fact that accrediting agencies, such as the Internal Standardization Organization (ISO) and the College of American Pathologists (CAP) require it. In fact, in some jurisdictions, diagnostic laboratories must adhere to the standards established by their regional EQA agencies to receive reimbursement for any completed test. In the United States, for instance, laboratories who have difficulty passing EQA assessments are reported to the Centers for Medicare and Medicaid Services (CMS); in the United Kingdom, EQA participation is mandatory and laboratories that have persistently poor performance are reported to a committee who decides the appropriate course of action and investigation. In some jurisdictions, laboratories cannot even gain accreditation without EQA – in Canada, for example, testing requirements for crizotinib therapy mandate participation in an EQA scheme for predictive biomarkers associated with targeted therapies. High-quality, harmonized EQA activity is so vital that the World Health Organization (WHO) also recognizes its importance; in August of 2016, it released a document (1) entitled, "WHO manual for organizing a national external quality assessment program for health laboratories and other testing sites." The guideline contains principles for the governance and operation of EQA schemes designed to assist in considering all the necessary factors, establishing a program, and reaching best practice standards at every level of healthcare.

"Ensuring accuracy and precision in the EQA process is easier said than done."

Industry's helping hand

Standardization isn't easy. There are myriad factors to consider – establishing processes, streamlining operations, choosing references – and each one can have a knock-on impact on quality assurance and improvement.

Recognizing the inherent difficulties in standardization, EQA providers came together to form the International Quality Network for Pathology (2), a not-for-profit group that aims to encourage global harmonization by seeking areas for potential cooperation between EQAs providers, laboratories, diagnostic companies and patients (see Figure 1). IQN Path's goals are multiple:

1. To establish benchmarks and best practice for EQA.
2. To promote the use of EQA and to provide education on quality for diagnostic testing (including laboratory outreach, personnel training, and educational workshops).
3. To create a communication platform on EQA, allowing stakeholders to participate in working groups to

At a Glance

- *External quality assessment (EQA) ensures that diagnostic tests are reliable, accurate and translatable between laboratories*
- *Each laboratory's selection of tests and procedures is different, and not all participate in similar EQA schemes, which makes quality assurance challenging*
- *Organizations like the International Quality Network for Pathology can bring together companies, EQA providers, and end users for the education of all*
- *With education comes greater understanding and the ability to shape future product development to better meet pathologists' needs*

exchange expertise on new biomarker tests, upcoming assessments, and the challenges they encounter.
4. Professionally recognize best practice in EQA via the EQA Provider Scheme standards.
5. Develop internationally recognized standards, controls, tools and data resources (questionnaires, websites, templates and more) to support EQA providers and laboratories.
6. Facilitate data sharing by pooling anonymized EQA performance data information from multiple providers to identify areas of suboptimal testing and provide data-driven suggestions for quality improvements.

A recent example of a successful IQN Path project was the launch of a survey and pilot EQA on cell-free DNA (cfDNA) testing for lung and colorectal cancer. Testing cfDNA mutations to manage patients' treatment is a novel technology that has now entered the clinic. With any new form of testing used for patient management, we must ensure appropriately designed EQA. In this case, four providers came together under the IQN Path umbrella to establish best practice standards and design a pilot cfDNA EQA and consensus scoring system. A survey of 164 laboratories indicated that 17 percent already used cfDNA testing to inform patient treatment, and many more are preparing to use it. The pilot EQA organized under IQN Path offered a chance for the providers to discuss and design appropriate reference materials and scoring systems and harmonize their use. Any organization can access this type of support; the IQN-Path application process gives them the opportunity to request funding and support for projects that benefit the international pathology community at large, and allows the IQN Path Board to strategically support initiatives and to allocate resources appropriately.

Education in action

Cancer immunotherapy – and the many companion diagnostics that accompany it – have occupied a prominent place in recent scientific news. PD-L1 is normally associated with immune homeostasis, but overexpressed in many cancers, binding to surface markers on cytotoxic T lymphocytes and preventing the natural anti-tumor immune response. Tumors that overexpress PD-L1 are susceptible to treatment with immune checkpoint inhibitors – so knowledge of a cancer's PD-L1 status is key to selecting the most effective treatment.

Currently, there are four different FDA-approved clinical tests for PD-L1.

Figure 1. The stakeholders involved in biomarker testing quality, one area where EQA is rapidly evolving.

In addition, there are several antibody clones employed in laboratory-developed tests (LDTs) with commercially available cell line controls. The adoption of LDTs is driven by cost – they are usually far cheaper than commercial assays – but those savings are often offset by the cost of mandatory validation to ensure accuracy.

IQN Path is now launching a new project – a digital, educational self-assessment on PD-L1 readout. The online portal will feature images of PD-L1-stained cases with associated H&E slides, covering the four different FDA-approved kits, and provide a learning opportunity for pathologists to test their skills and receive feedback by comparing their results to the gold-standard scoring for each sample (established by an expert pathologist committee). Anyone can participate by creating a login via an EQA provider of their choice; there is no requirement for previous engagement with that provider, although we recommend that laboratories performing PD-L1 testing also join an EQA scheme covering the analytical phase of testing that includes the provision of physical samples for staining.

A forum for discussion

In February 2017, IQN Path staged an educational forum on FDA-approved PD-L1 assays, bringing EQA members together with industry professionals to share expertise. The meeting had four main goals:

- To acknowledge the complexity of the current diagnostic landscape of PD-L1;
- To inform EQA members about the available assays;
- To provide EQA members with training from industry experts on the specifics of those assays and their assessment;
- To give EQA providers the opportunity to ask questions, raise issues and provide feedback on recent and upcoming EQA rounds involving PD-L1 testing.

The meeting included EQA delegates from Europe, China and the United States who represented organizations across all fields of diagnostics. Topics included case reviews and assays, as well as good practice and potential pitfalls related to PD-L1 testing. The open forums were particularly useful, facilitating vigorous and productive discussion between EQA representatives and the drug and medical device delegates. As with all IQN Path meetings, this forum included diagnosticians as well as industry representatives, making it fertile ground for innovative thought and discussion.

Delegates left the meeting with two key messages: first, that industry and EQA agencies must work closely together to understand the practical aspects of assay implementation in clinical laboratories; and second, that training and education in assay interpretation are critical to properly assess the assay's performance.

Did it work?

Feedback from delegates indicated that the meeting was extremely useful, with an overall mean satisfaction score of 4.9 on a 5.0 scale – a clear indication of the demand for such training among EQA providers. The open forums in particular yielded vigorous and productive discussions between EQA representatives and the drug and medical device delegates. As a result, the providers ended up with a more nuanced understanding of the complexities involved in developing a fully validated assay, and learned about the staining patterns and scoring algorithms directly from the pathologists who had developed the assays.

It's not only the quality assurance professionals who benefit from such collaborations, though. The industry partners who co-hosted the event found themselves better able to understand the needs of EQA professionals, and gained a first-hand perspective of the quality assessment methods used by different

"EQA in diagnostic laboratories is critical to safeguarding the health and safety of patients."

organizations. In the future, this type of insight should allow industry professionals to assist EQA providers in setting up testing schemes – providing additional benefit for those providers, and for the laboratories who ultimately make use of external quality checks.

Of course, this is only one meeting – but it serves as a model to help open lines of communication between assay development companies and the pathologists who are implementing those assays in their laboratories. By sharing their goals and ideas, participants can help tailor future product development to better meet their laboratories' – and their patients' – needs.

The future of EQA

We need to investigate new ways of supporting EQA. Digital pathology and online assessments like the PD-L1 readout self-assessment project are good examples – although these digital tools should complement assessments of the analytical phase. Another is the identification of common requirements for reference samples between EQA providers, which we need to promote harmonization and develop shared best practices.

EQA in diagnostic laboratories is critical to safeguarding the health and safety of patients through best practices. Our educational forum was the first formal collaboration between IQN Path, a medical device company, and the EQA organizations representing regions across the globe. Organizations like IQN Path can link drug and medical device manufacturers, EQA scheme facilitators, end users and other interested parties together in a neutral environment. By bringing together like-minded and committed contributors to safely and openly discuss concerns and solutions, they can help diagnostic laboratories to deliver accurate, high-quality results in a timely fashion.

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Colin Tristram is Co-Founder and Director of HistoCyte Laboratories Ltd., Newcastle upon Tyne, United Kingdom.
Beth Sheppard is Senior Director of Market Access at Ventana Medical Systems, Inc. (Roche Tissue Diagnostics), Tucson, USA.

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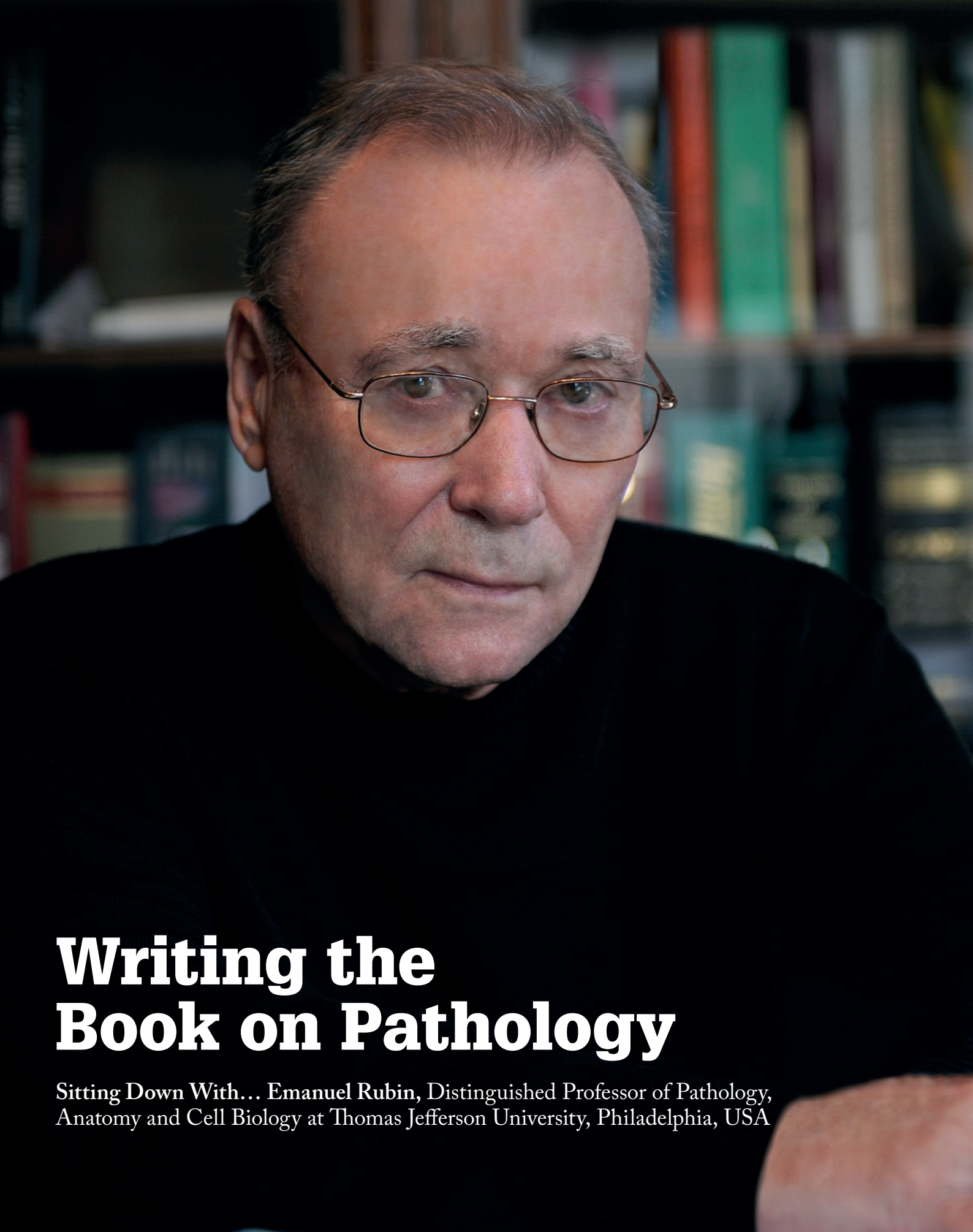

Writing the Book on Pathology

Sitting Down With… Emanuel Rubin, Distinguished Professor of Pathology, Anatomy and Cell Biology at Thomas Jefferson University, Philadelphia, USA

Where did your interest in pathology originate?

I began my medical career in internal medicine, but I soon realized that I was more suited to an intellectual discipline in which I could engage in both practice and research. Pathology turned out to be my cup of tea. To mix metaphors, I slipped into it like a well-tailored suit.

My mentor, Hans Popper, was an outstanding pathologist and an expert in liver diseases. My initial research projects were morphologic studies, demonstrating the spontaneous evolution of micronodular into macronodular cirrhosis and describing the etiology of primary biliary cirrhosis as a disease of bile ducts. When I began my pathology training, alcoholic cirrhosis was attributed to nutritional deficiencies. I was able to show that alcohol – independent of nutritional factors – was toxic and injured the liver directly. This concept has guided alcohol research ever since.

Since those days, practice and research in pathology have been revolutionized by technological advances. It's a different world.

How did you get involved in medical education?

When I became chairman of pathology departments – first at Mount Sinai in New York and later at Jefferson in Philadelphia – I also became responsible for the pathology courses at those institutions. I had always had an interest in medical education, so I quickly realized that the same deep thinking required for significant research could be applied to the transmission of knowledge to future generations.

An academic pathology department should not only provide outstanding services to patients and other health care professionals, but also excel in basic research and medical education. When I became Chairman of Pathology at Jefferson Medical College in 1986, I was fortunate to be surrounded by young, energetic collaborators who were also interested in medical education. It helped that the Dean, Joseph Gonella, was also the Chairman of the Department of Medical Education – which meant that he had a sincere interest in supporting our reforms and initiatives.

We informed our students that the pathology faculty was not there simply to instruct them by rote, but rather to help them learn. We emphasized that pathology was the bridge between science and clinical medicine, although the route might be long and difficult. In that context, we explained that they would be exposed to more than 6,000 new terms – a situation similar to learning two foreign languages. Like the relationship between Virgil and Dante in The Inferno, we would serve as their guides into a lifetime career focused on the distinct culture of medicine. The result? At Jefferson, the scores on the National Board examinations rose from the 30th percentile to the top five in the country.

My colleagues and I published quantitative studies of factors that influenced student performance in the pathology course. Mostly, we concluded that student attitudes were more important than the nature of the curriculum or the popularity of the faculty. I was quite friendly with Stan Robbins, so when these endeavors earned the 2018 Robbins Distinguished Educator Award, I was honored by the recognition afforded me in his name.

What do you think of new trends in medical school curricula?

The traditional curriculum comprises a series of departmental courses, beginning with basic science and pathology, and proceeding to medical specialties. The student is expected to combine this information and synthesize new knowledge as he or she transitions to clinical practice. The current fashion is an "integrated curriculum" in which all subjects are taught together in blocks devoted to organ systems. Compared with the traditional responsibilities of individual departments, the latter serves as a full employment program for assistant and associate deans, committees, coordinators, and secretaries. Despite the additional expense, personnel and time required for the integrated curriculum, I am unaware of any conclusive evidence that it results in a better graduate. Absent such evidence, the integrated curriculum can be viewed (by skeptics) as a species of human experiments carried out without informed consent or permission by a Human Subjects Committee.

Your textbook is foundational in the pathology field – how did it come about?

A representative of the Lippincott Publishing Company appeared in my office and told me that they were interested in publishing a new textbook of pathology – and that a number of academic pathologists had suggested I might be the one to undertake this task. I informed her that I saw no point in simply constructing a compendium of known facts; if, however, the publisher was willing to underwrite a new heuristic approach that used diagrammatic graphics and combined morphology with basic science, I might be interested. About a month later, she informed me that her superiors had approved my suggestion and that they would pay for a medical artist and the costs of reproduction. That was the beginning of Rubin's Pathology.

In the second edition, I received approval to substitute color photographs for black and white. These presentations were original at the time, but are now standard. The textbook has a worldwide distribution and has been translated into numerous languages. I have relinquished my administrative duties, but still contribute by writing and editing. I am dedicated to the proposition that students who master Rubin's Pathology should be able to integrate basic science and pathology with clinical medicine. We are currently working on the eighth edition – a task I consider a labor of love.

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